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(54) COMPOSITIONS AND METHODS FOR
EXPRESSING FACTOR IX

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(60) Provisional application No. 62/840,352, filed on Apr. 29, 2019, provisional application No. 62/829,621, filed on Apr. 4, 2019, provisional application No.

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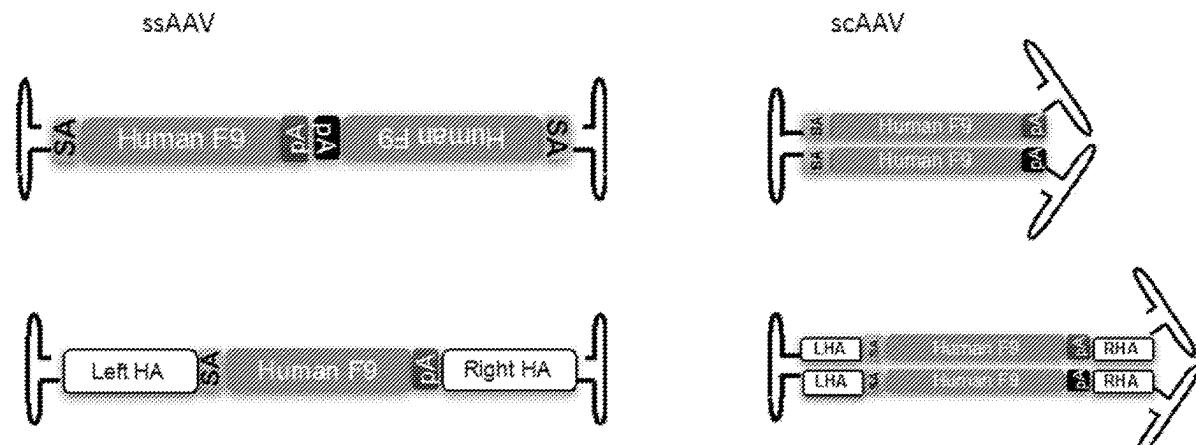
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C12N 15/90	(2006.01)
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(57)

ABSTRACT

Compositions and methods for expressing Factor IX in a host cell or a population of host cells are provided. Also provided are engineered host cells expressing Factor IX.

Specification includes a Sequence Listing.



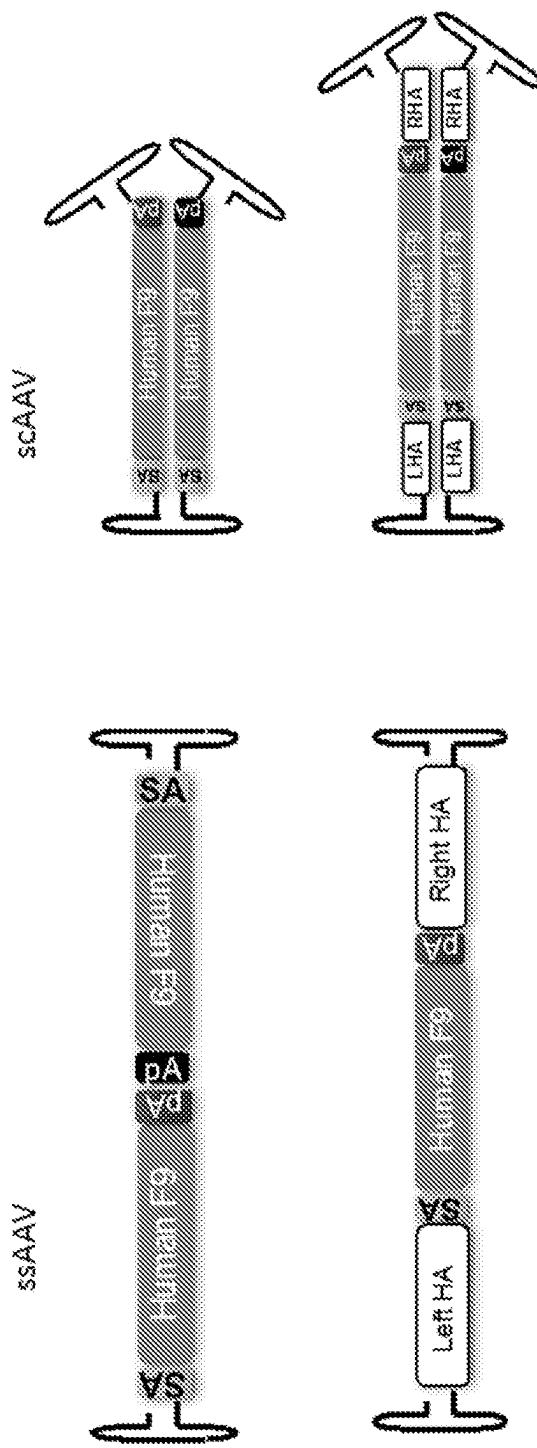


FIG. 1

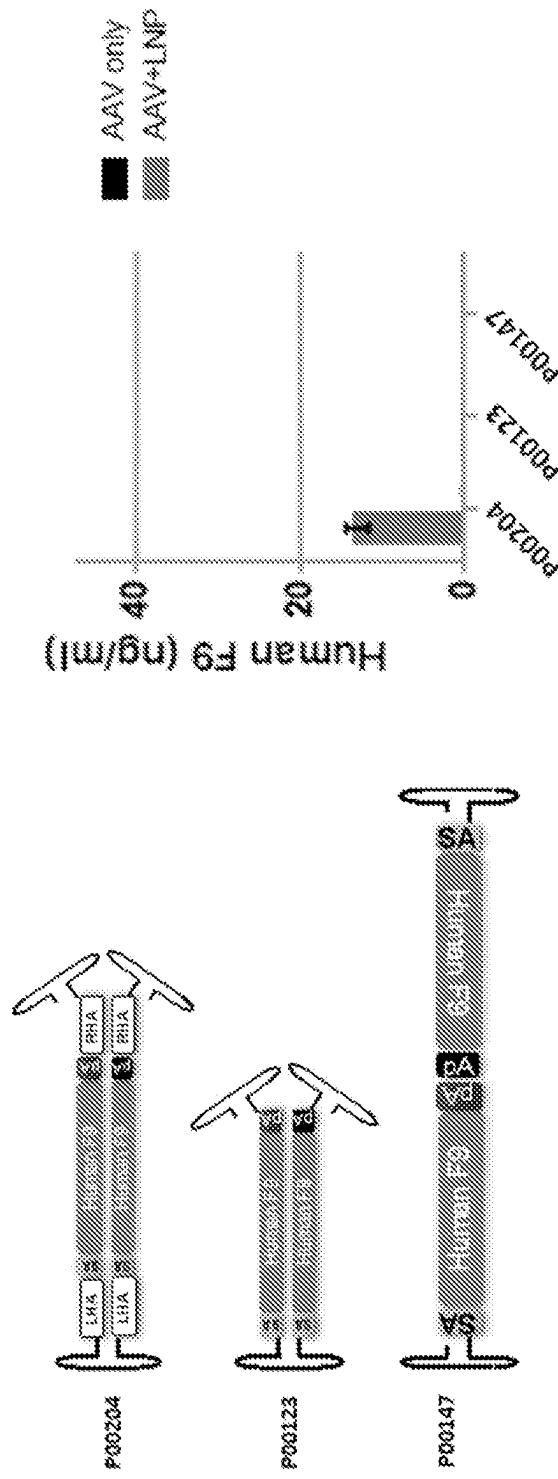


FIG. 2

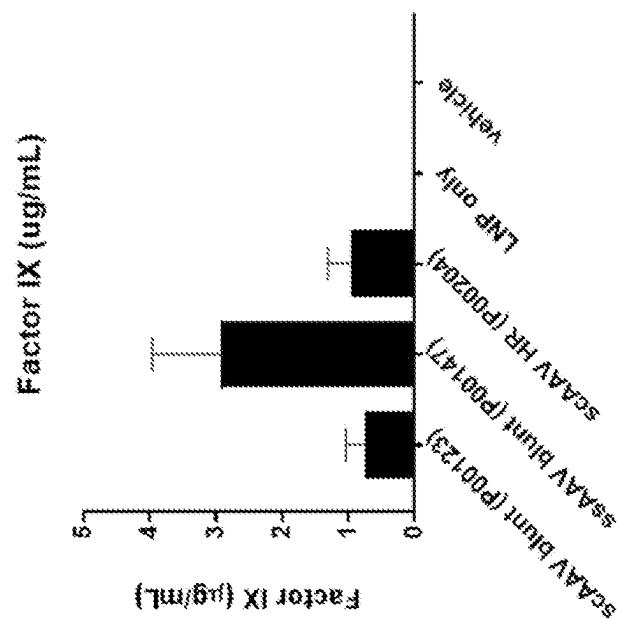


FIG. 3B

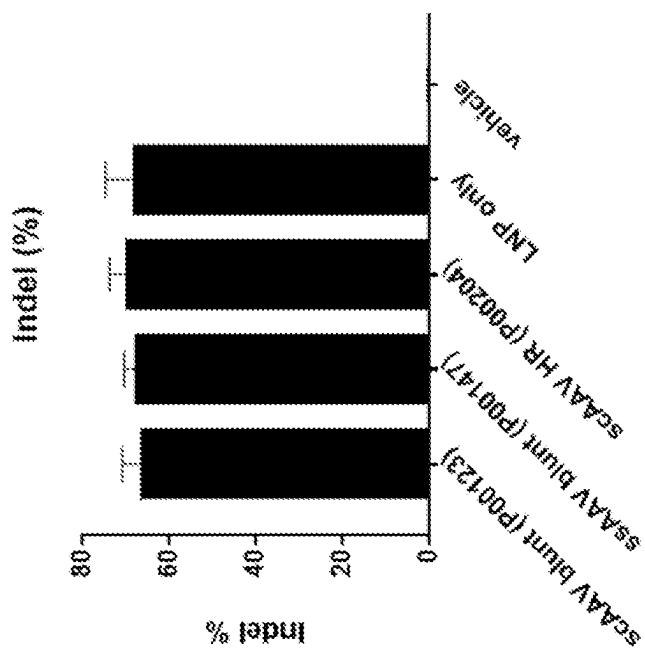
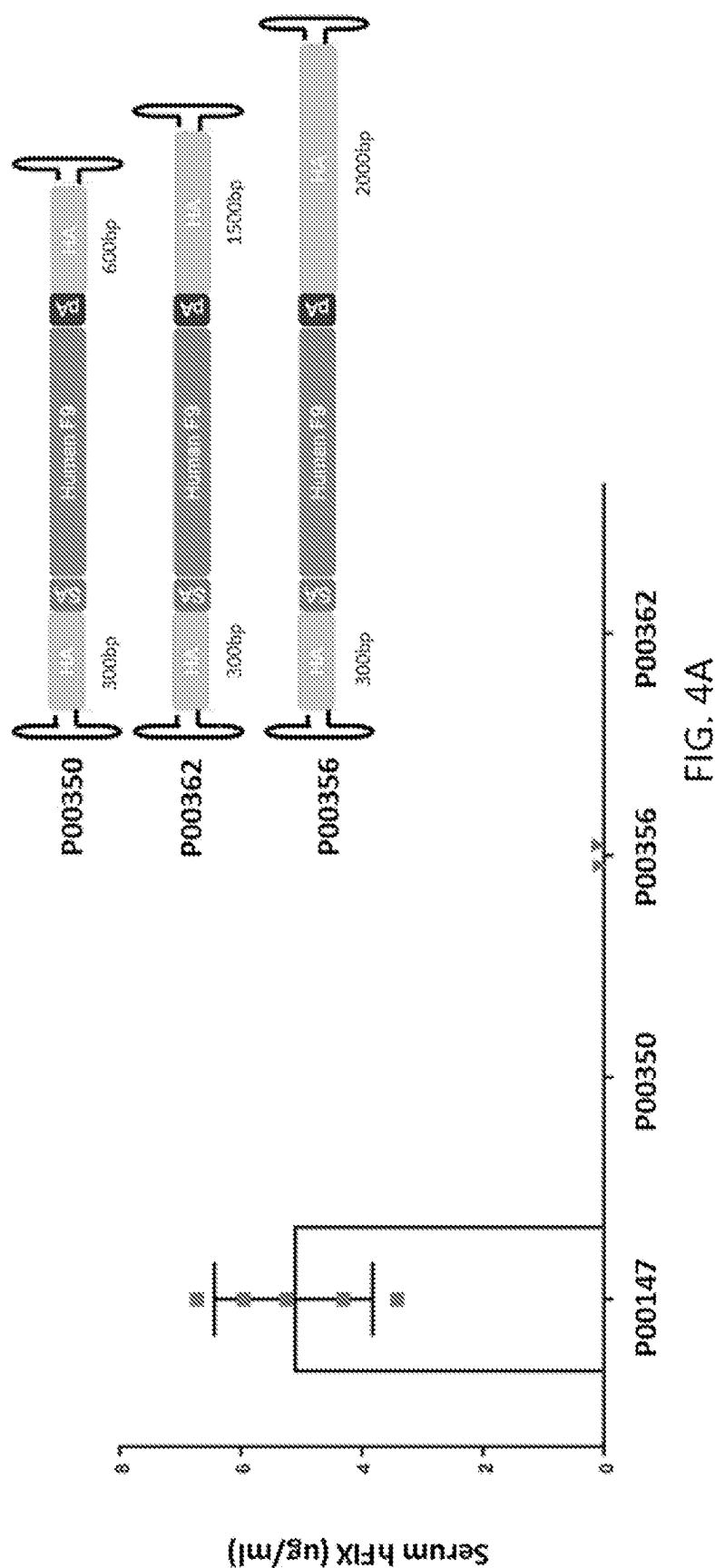


FIG. 3A



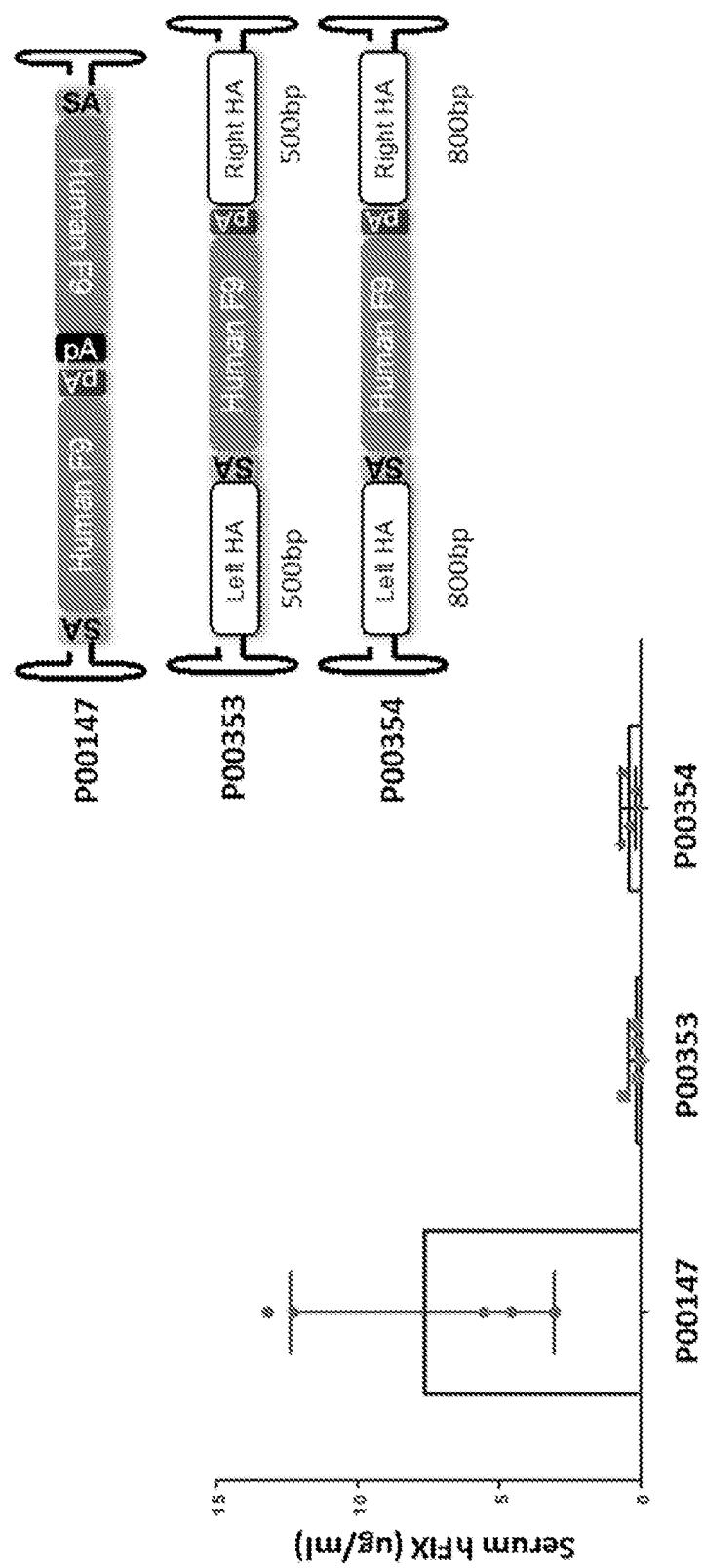


FIG. 4B

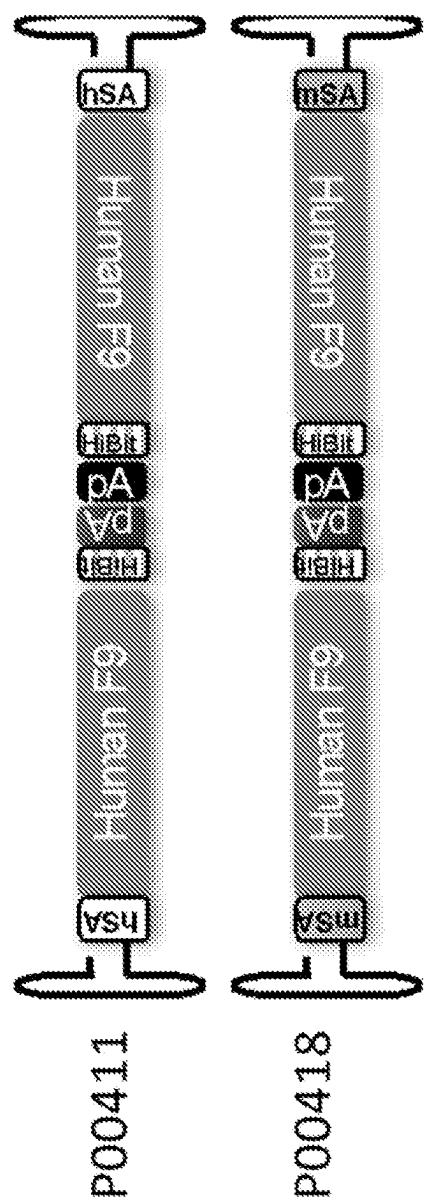
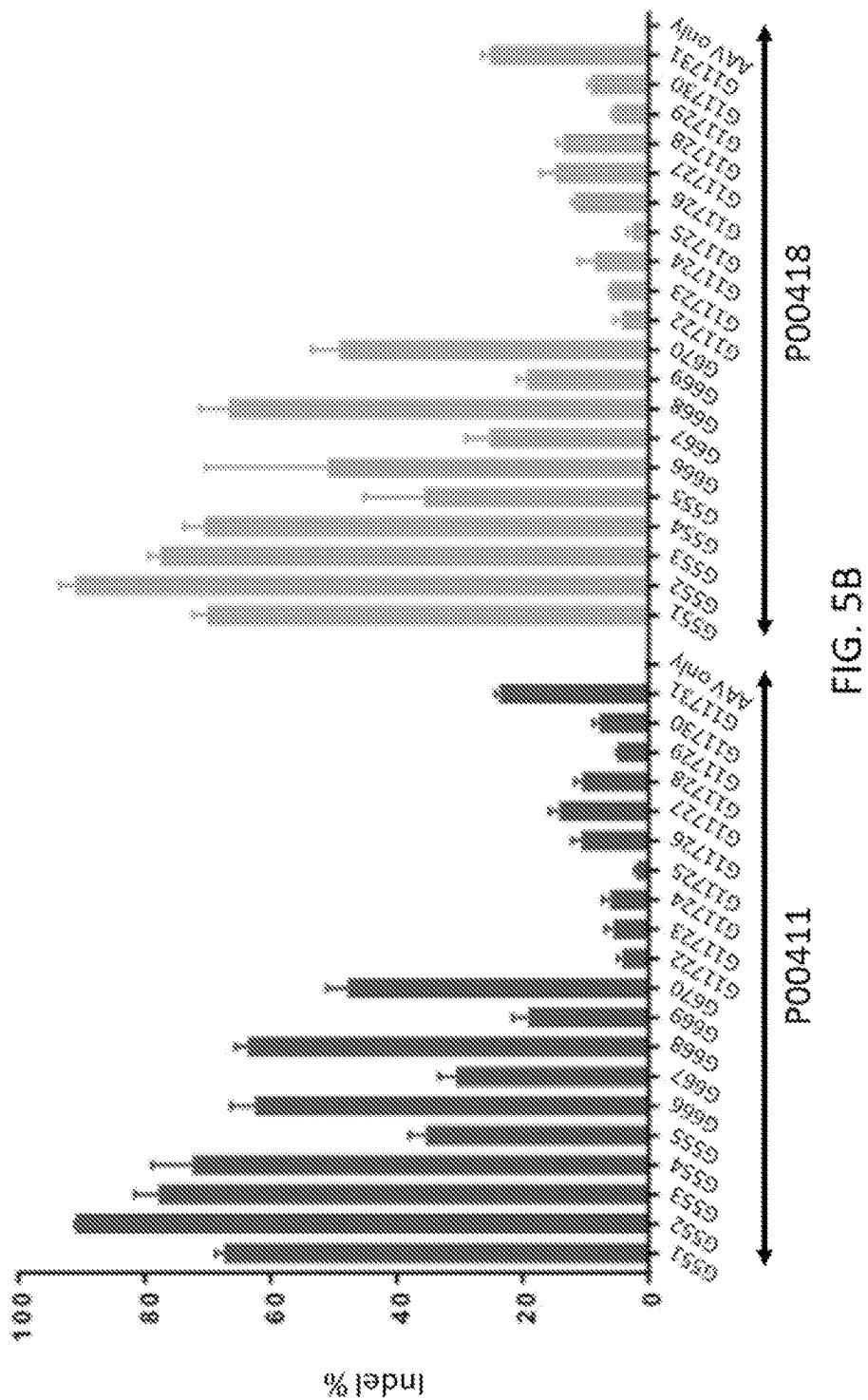
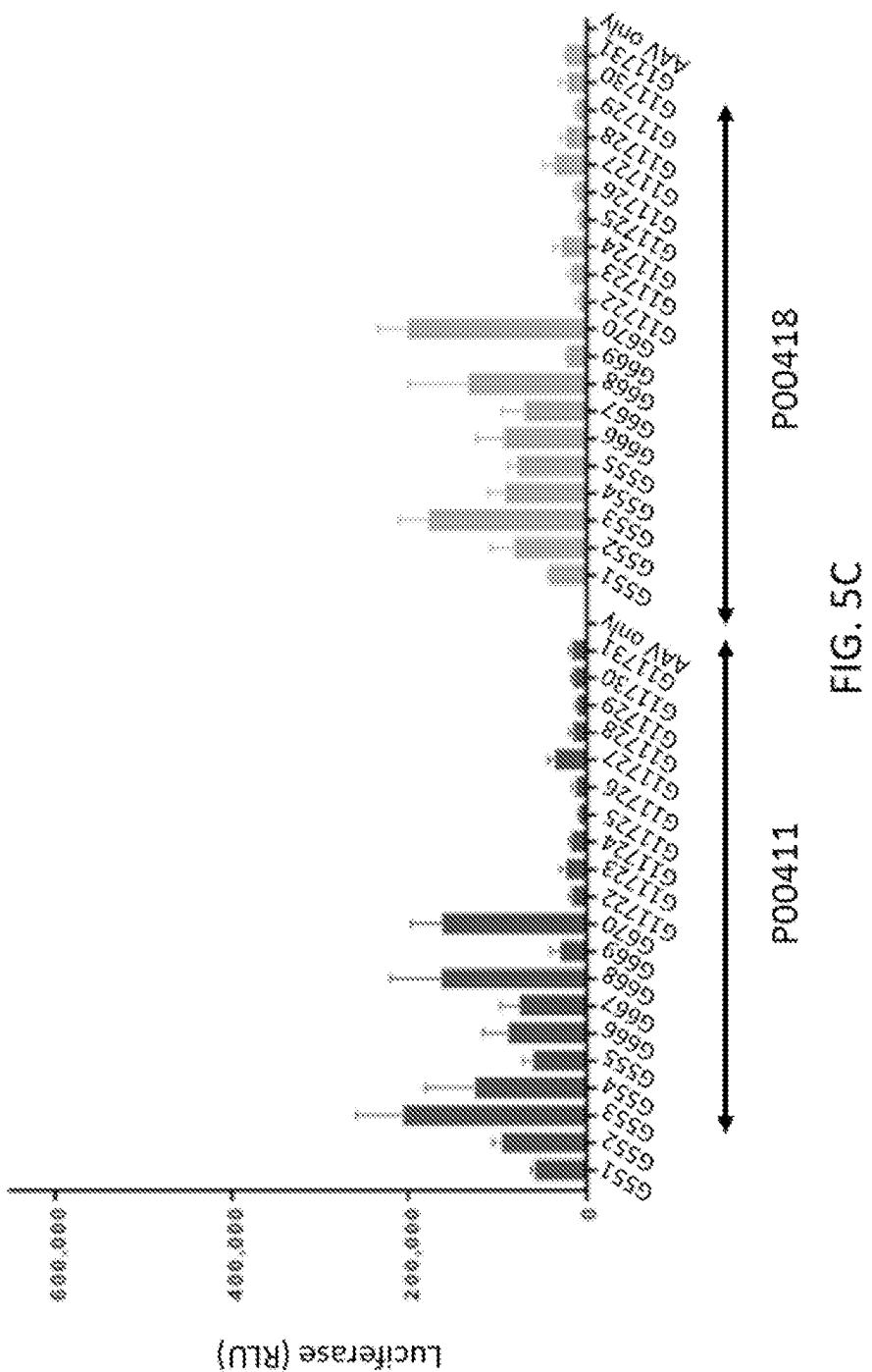


FIG. 5A





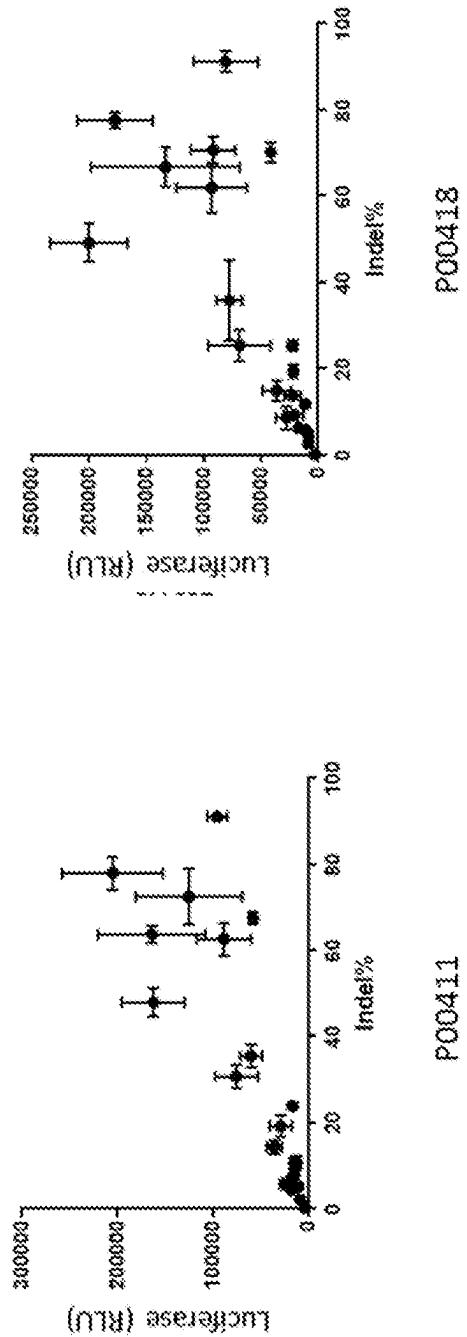


FIG. 5D

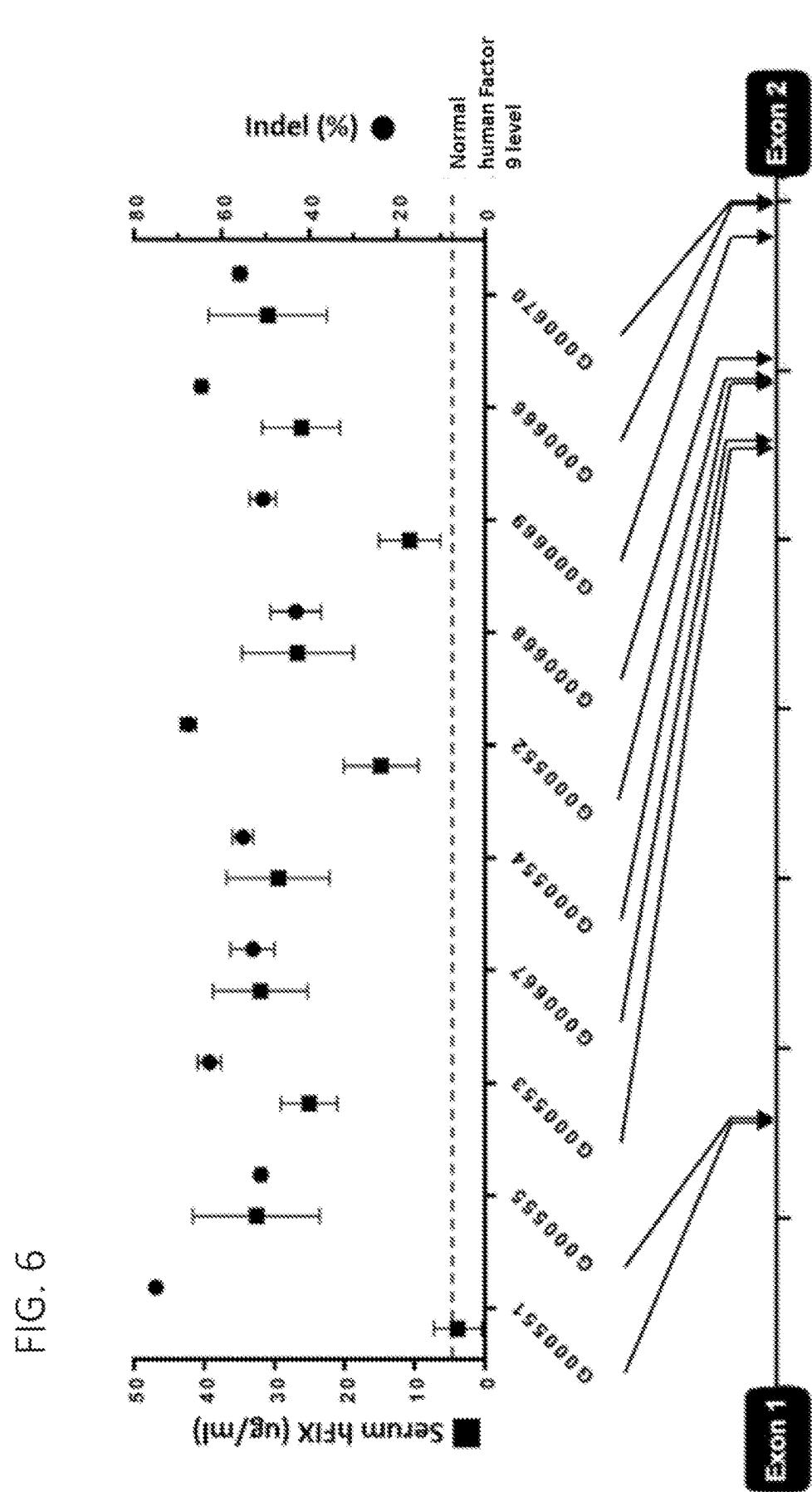
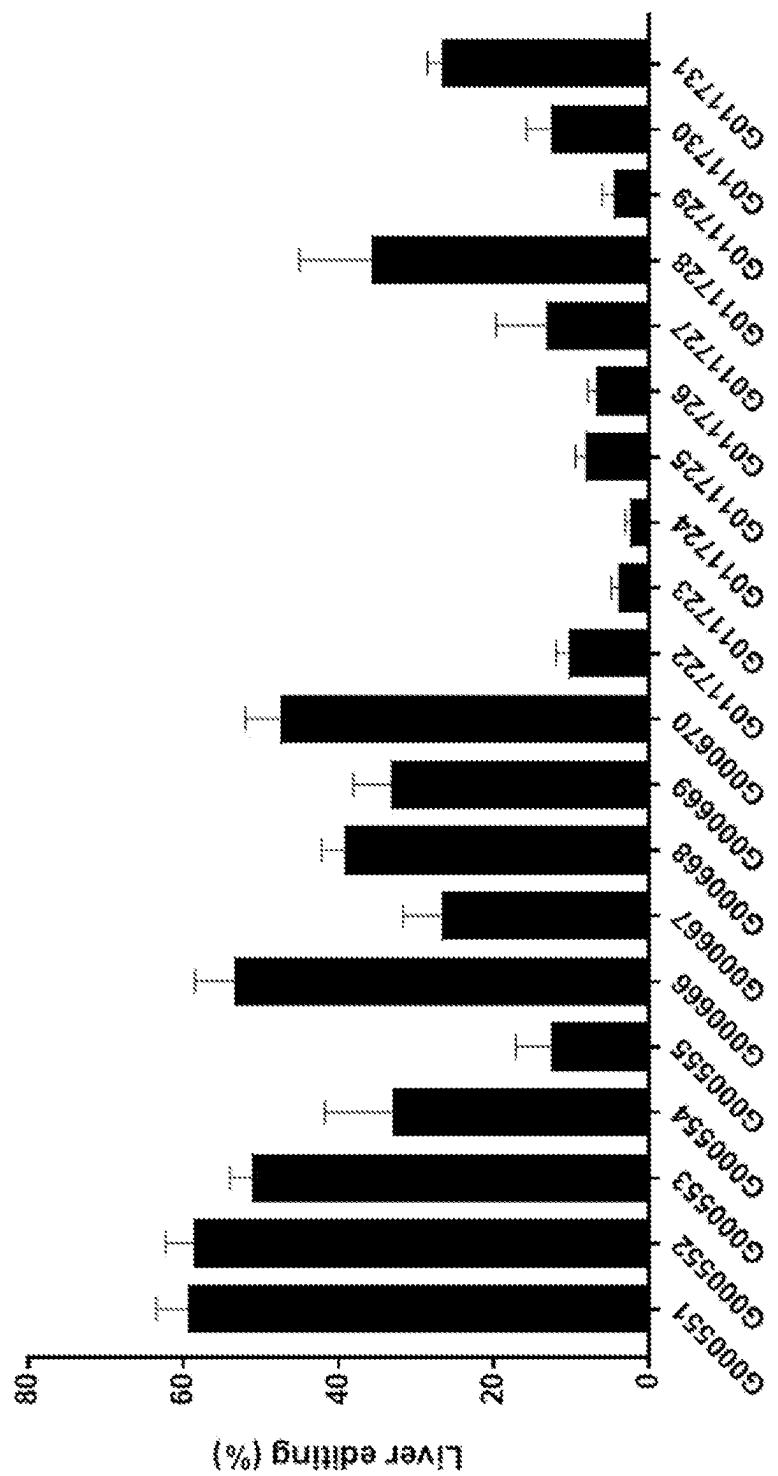


FIG. 6

FIG. 7A



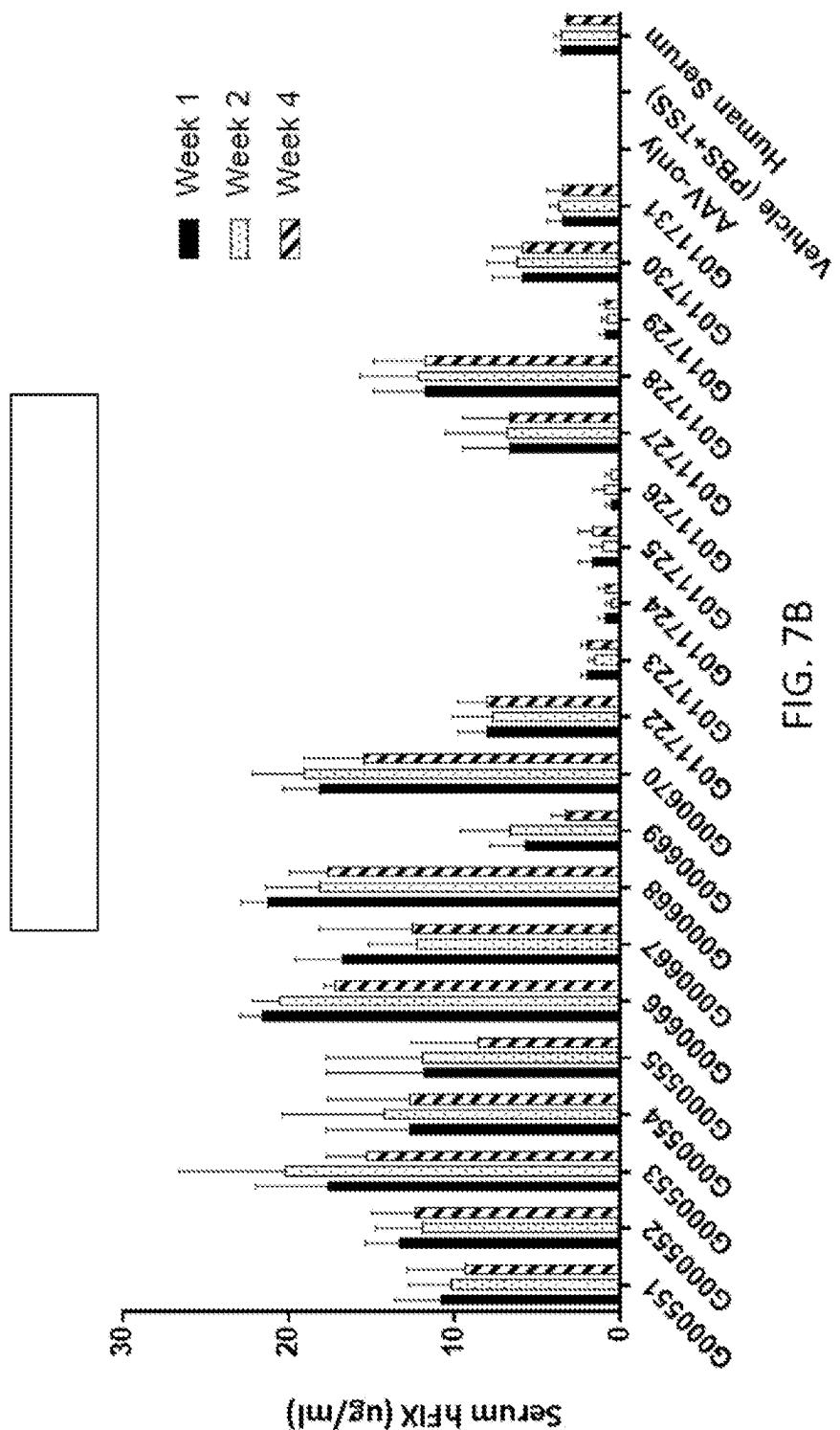


FIG. 7B

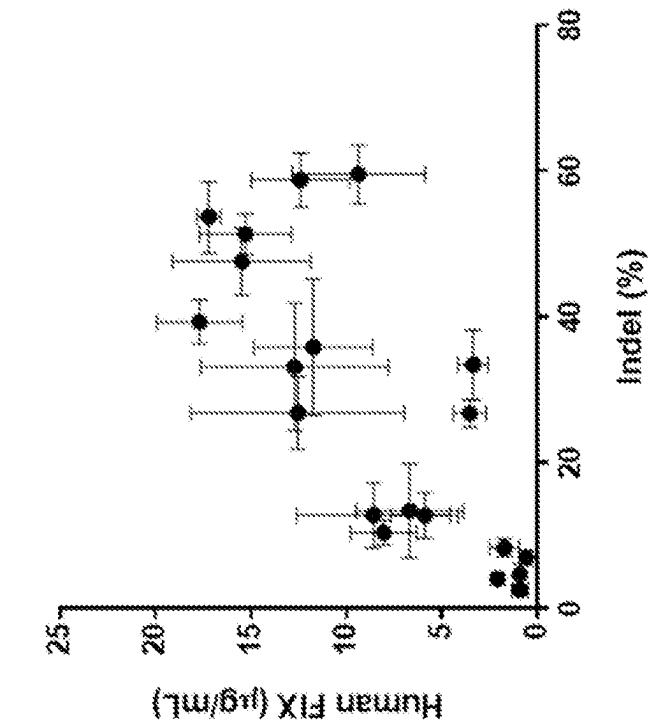


FIG. 7D

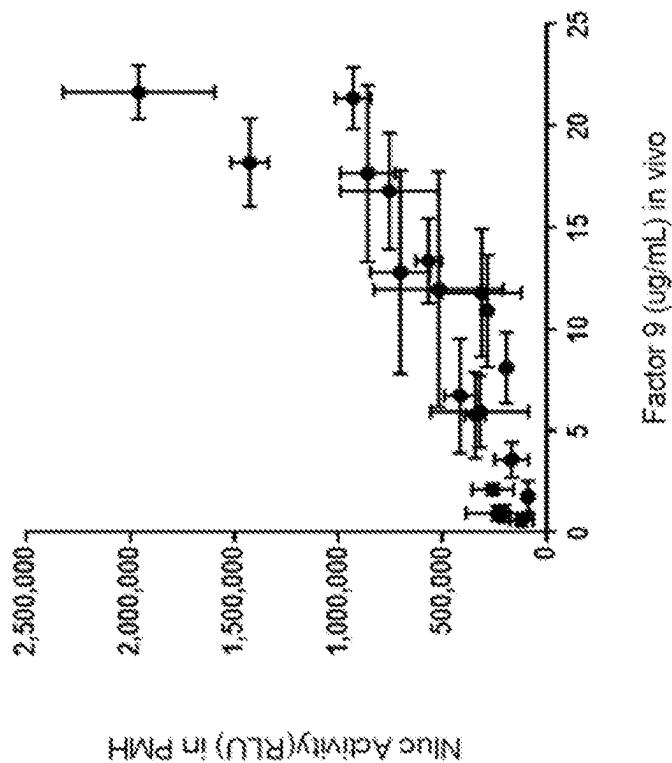


FIG. 7C

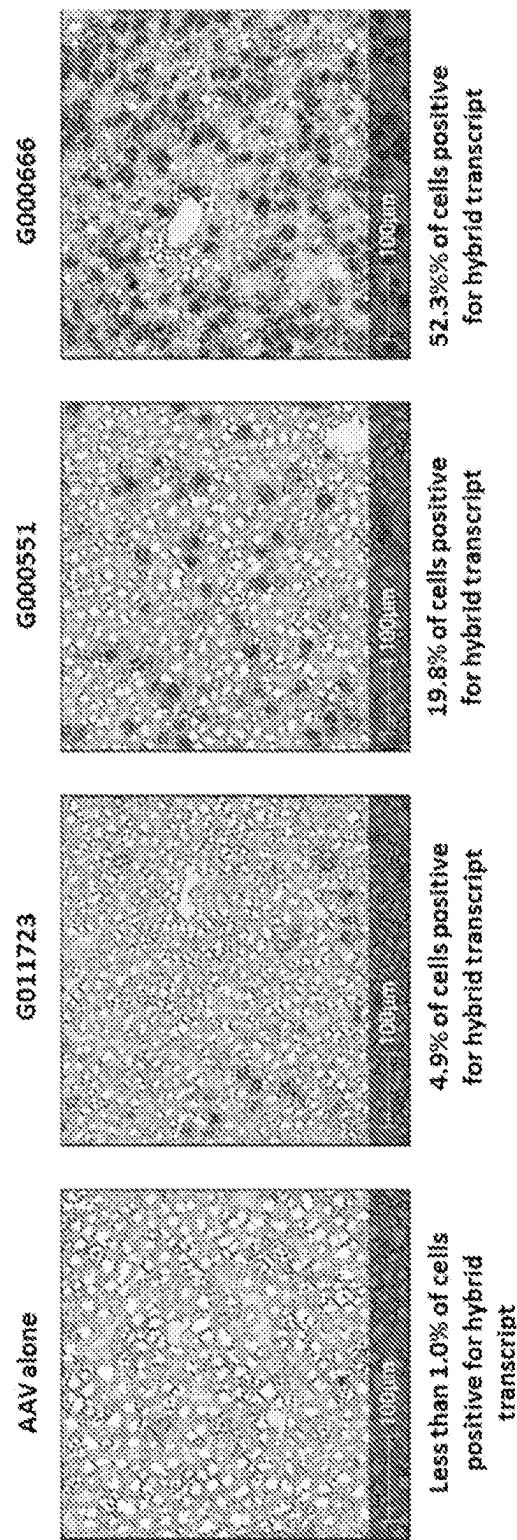
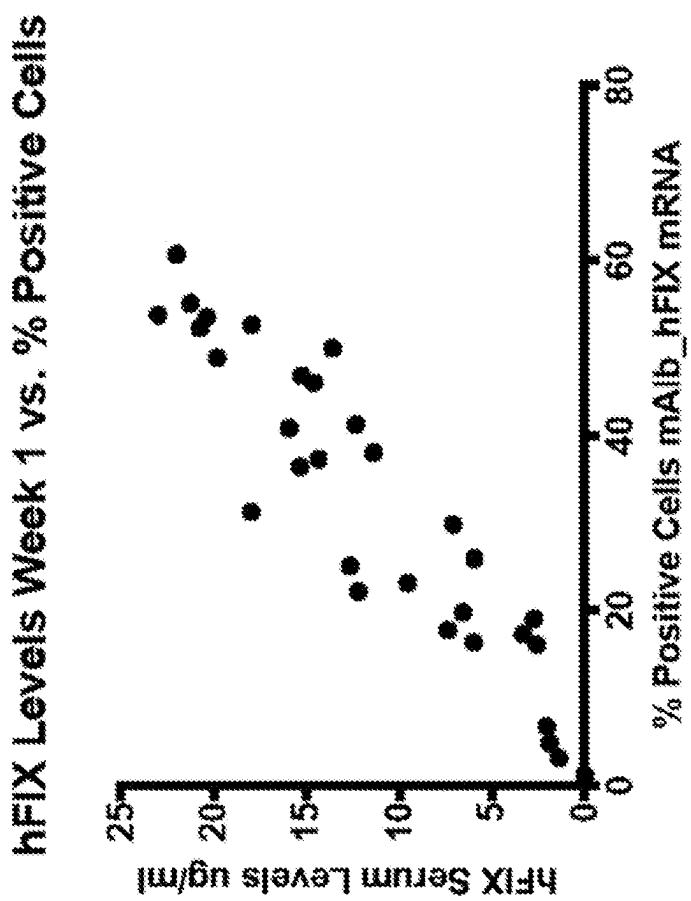


FIG. 8A

FIG. 8B



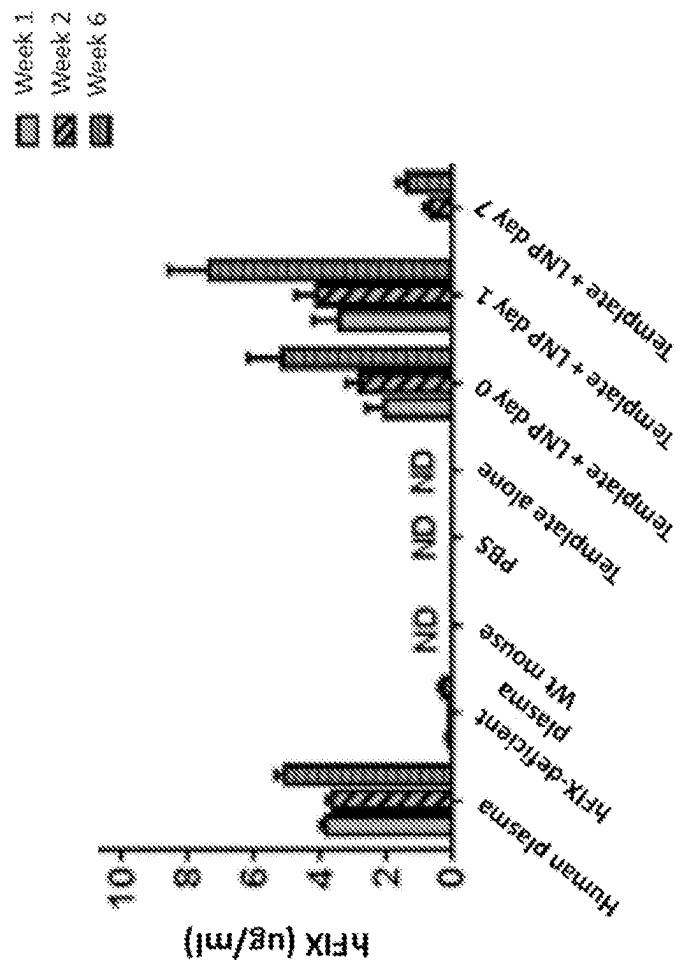
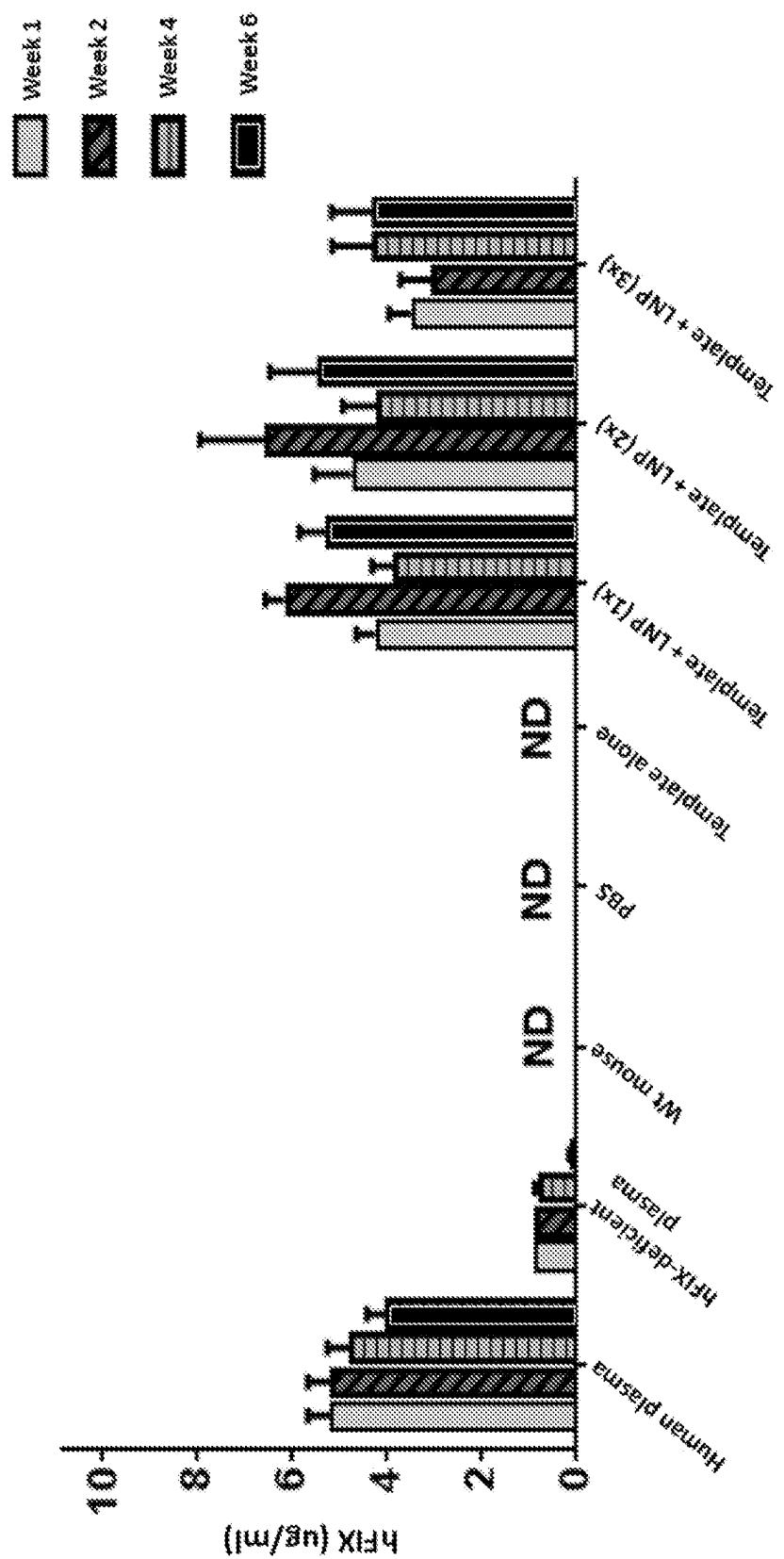
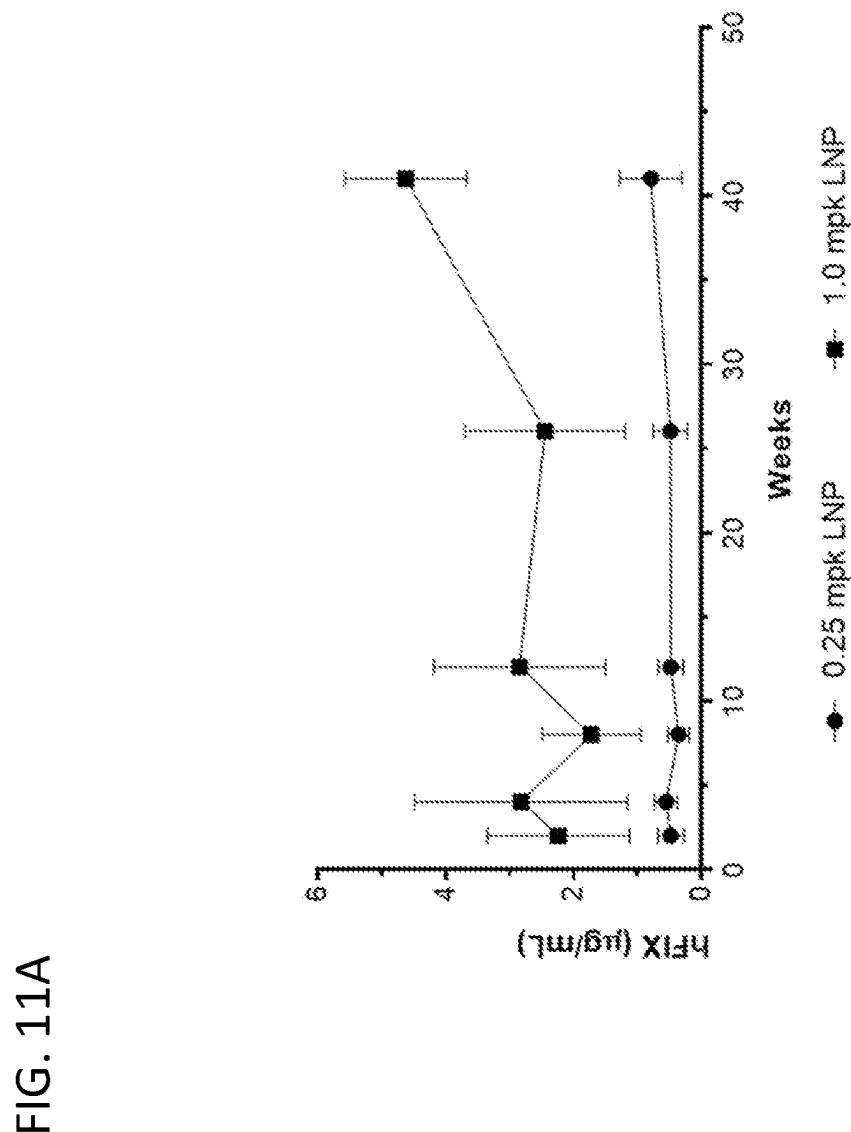


FIG. 9

FIG. 10





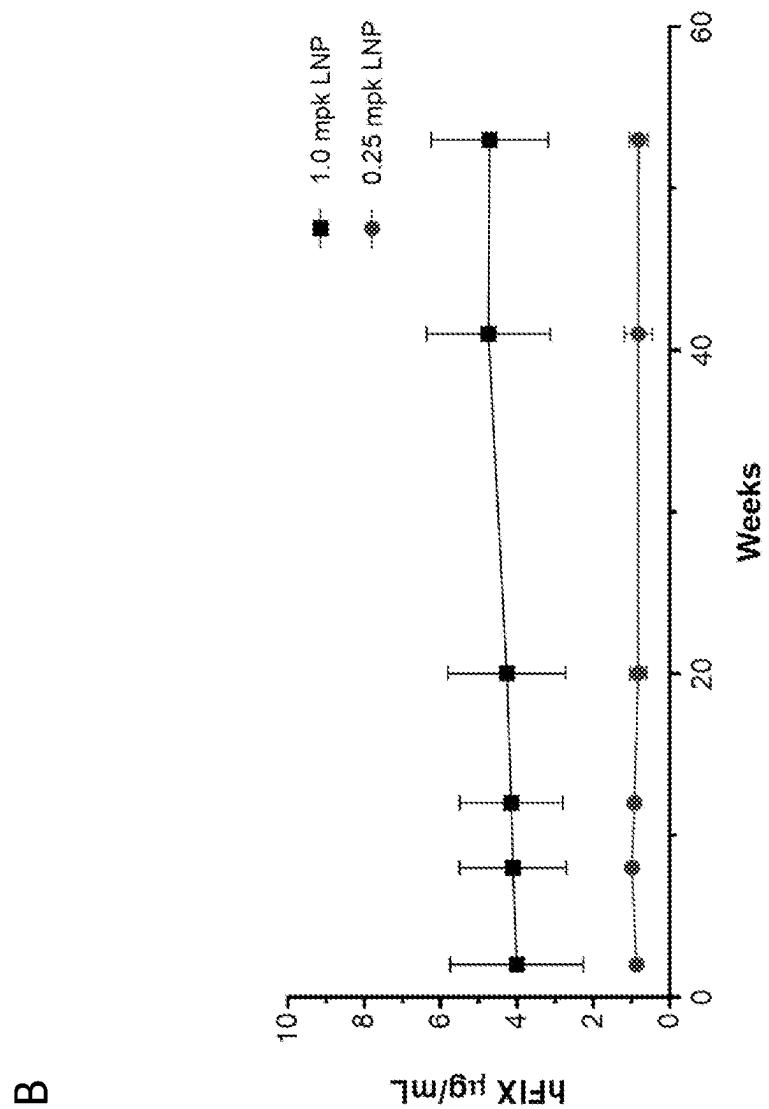
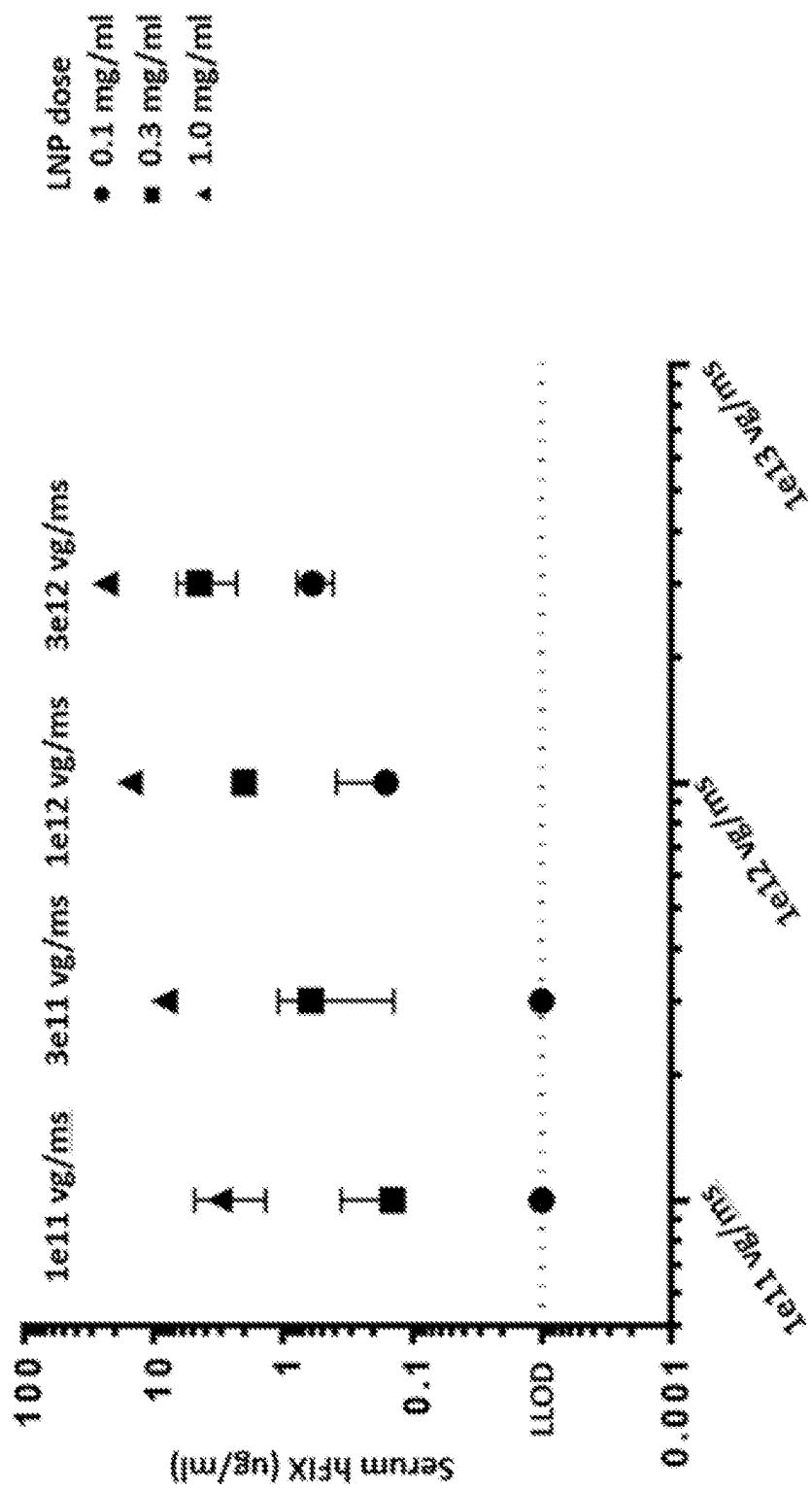
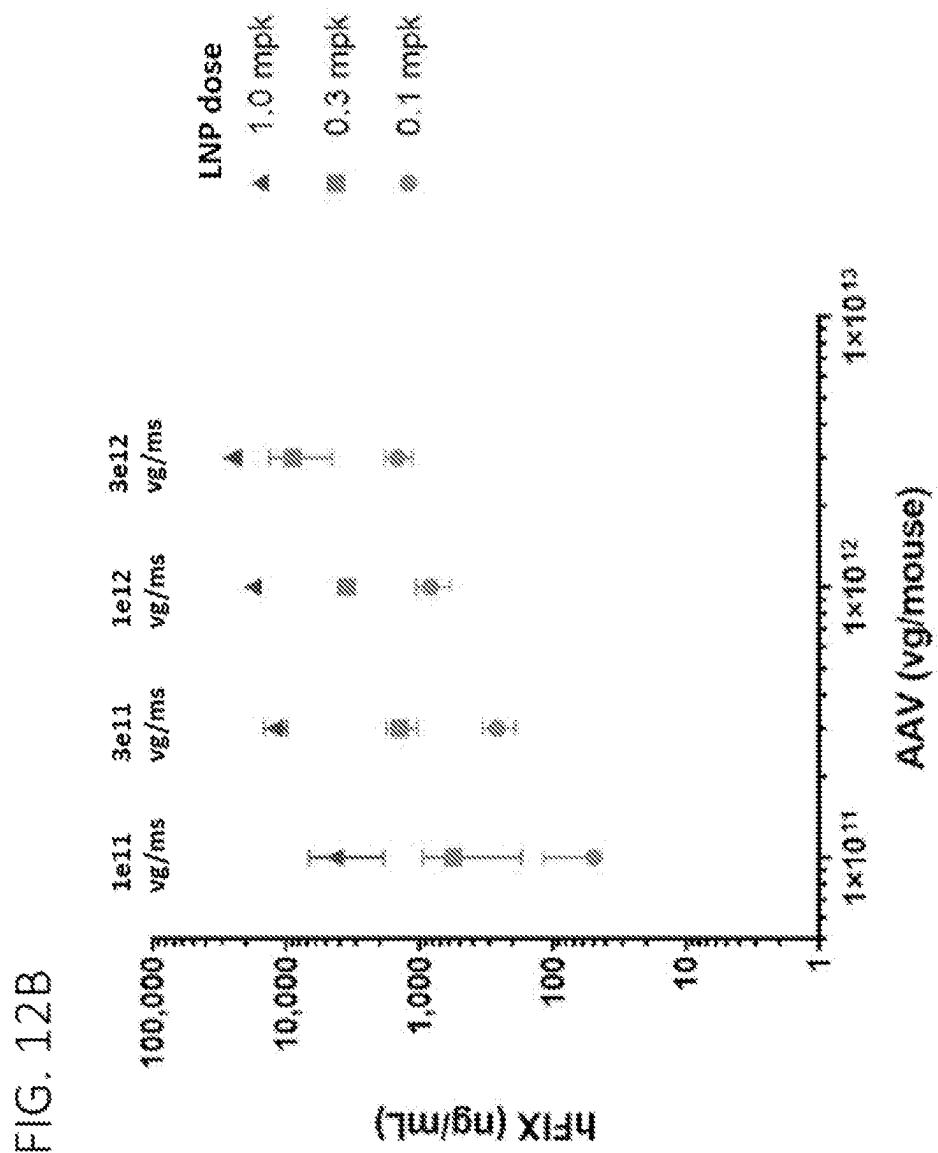
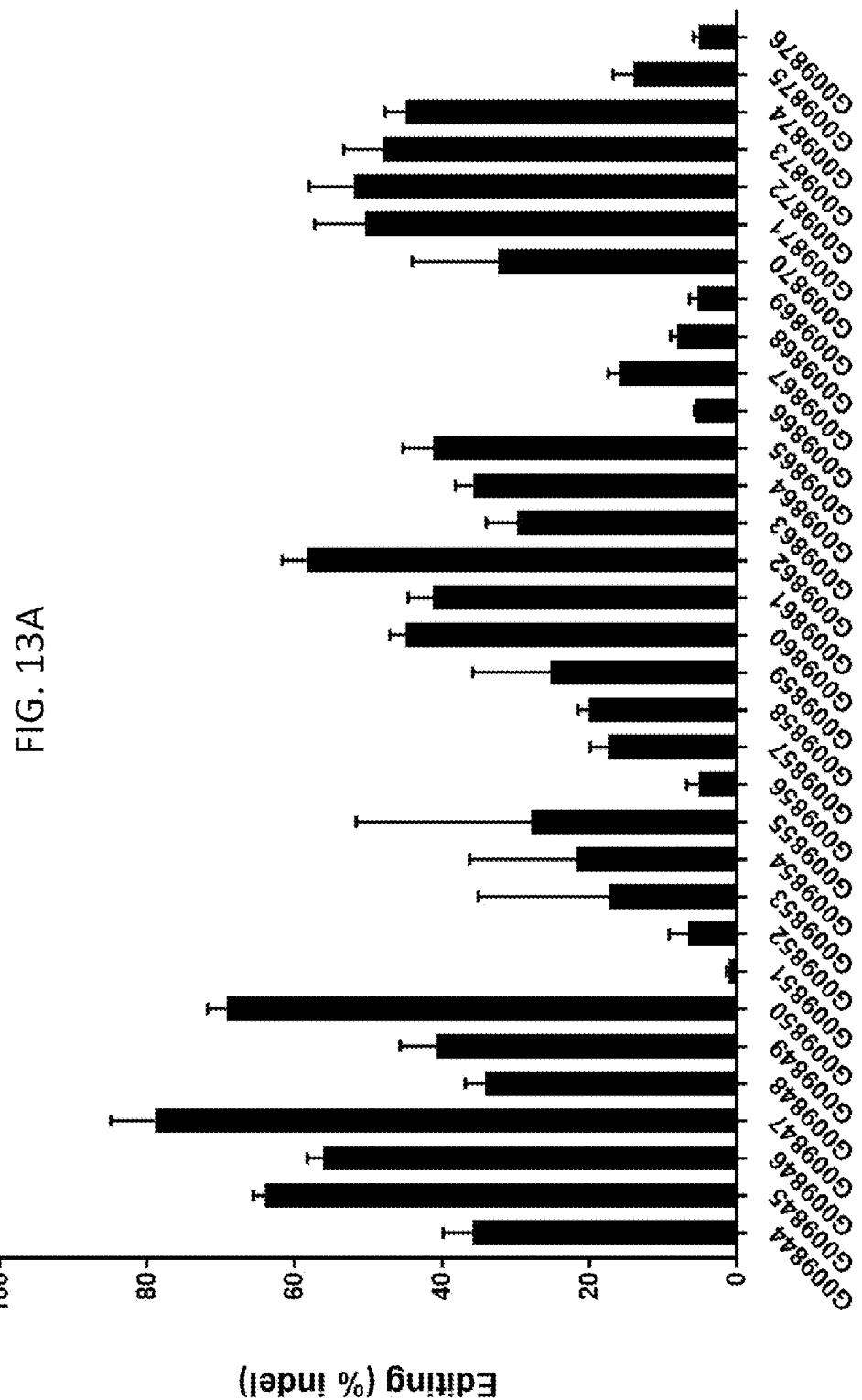


FIG. 11B

FIG. 12A







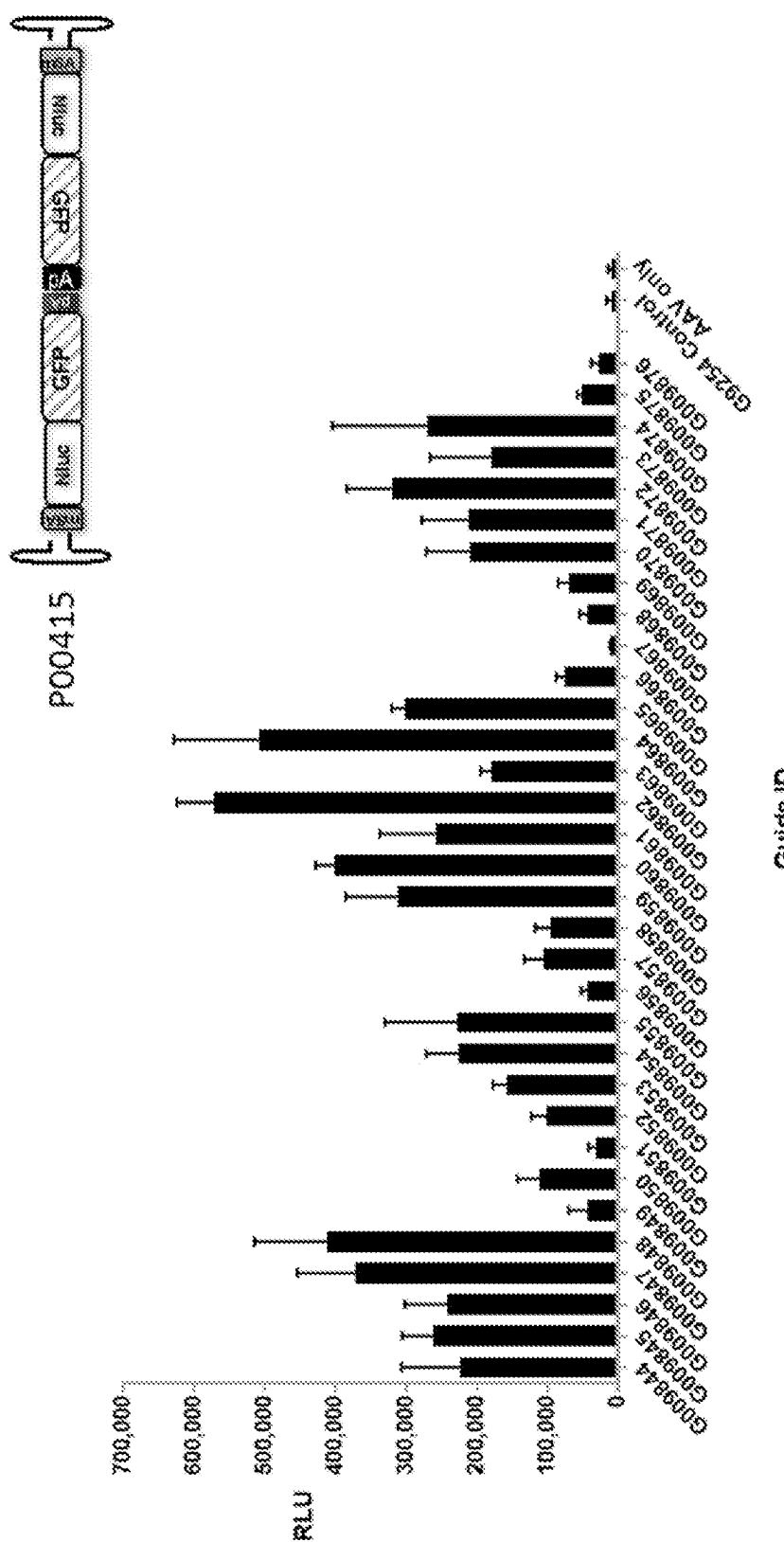


FIG. 13B

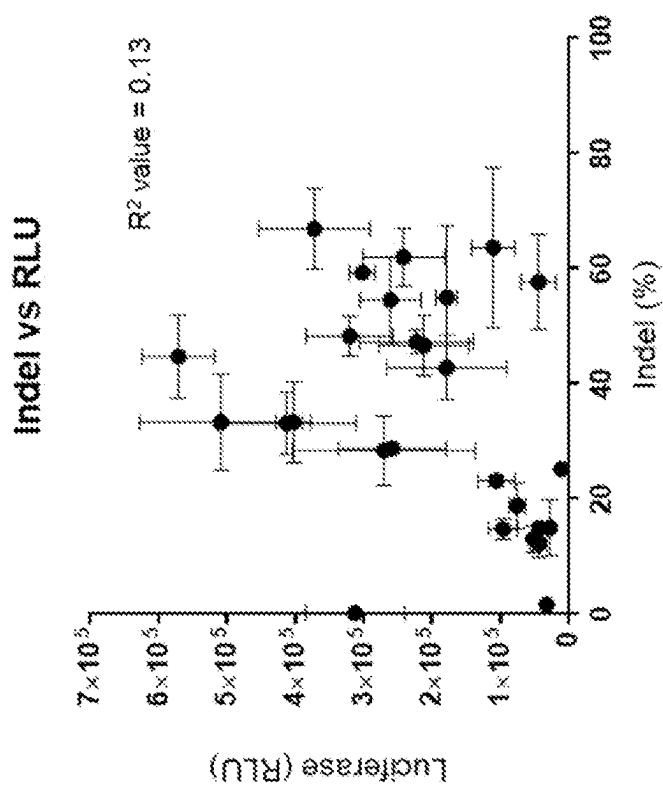


FIG. 13C

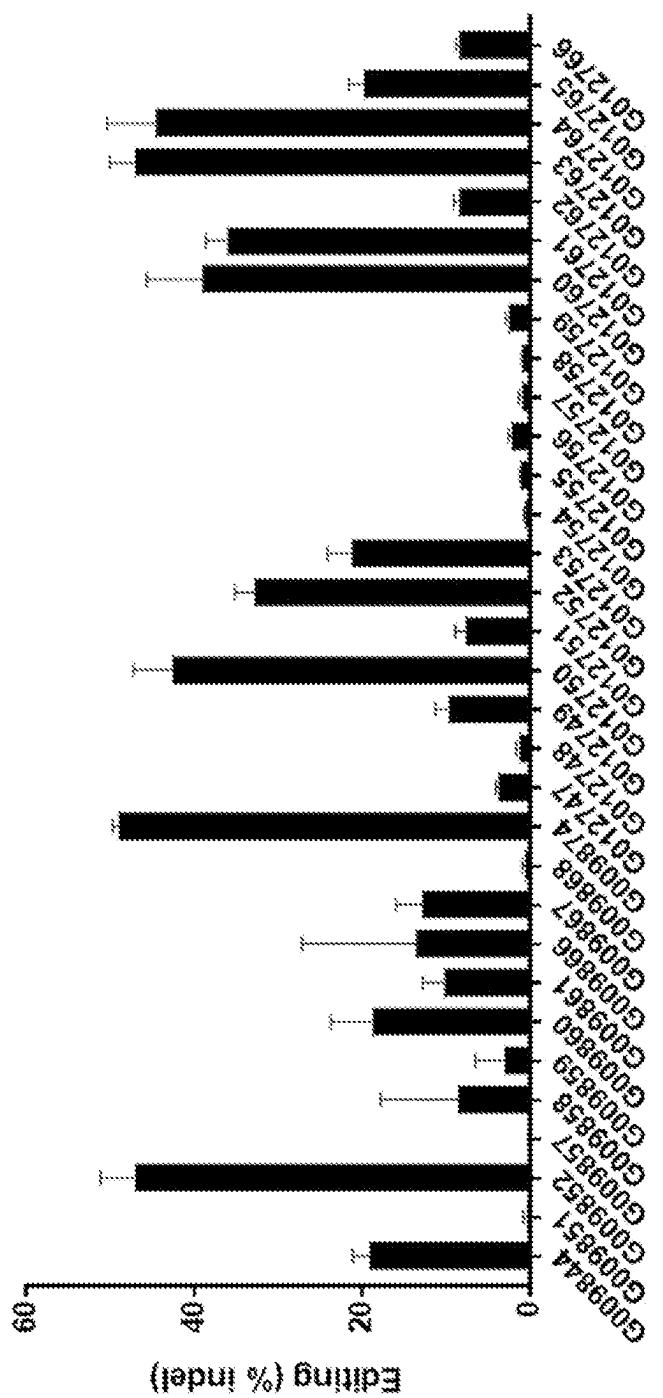
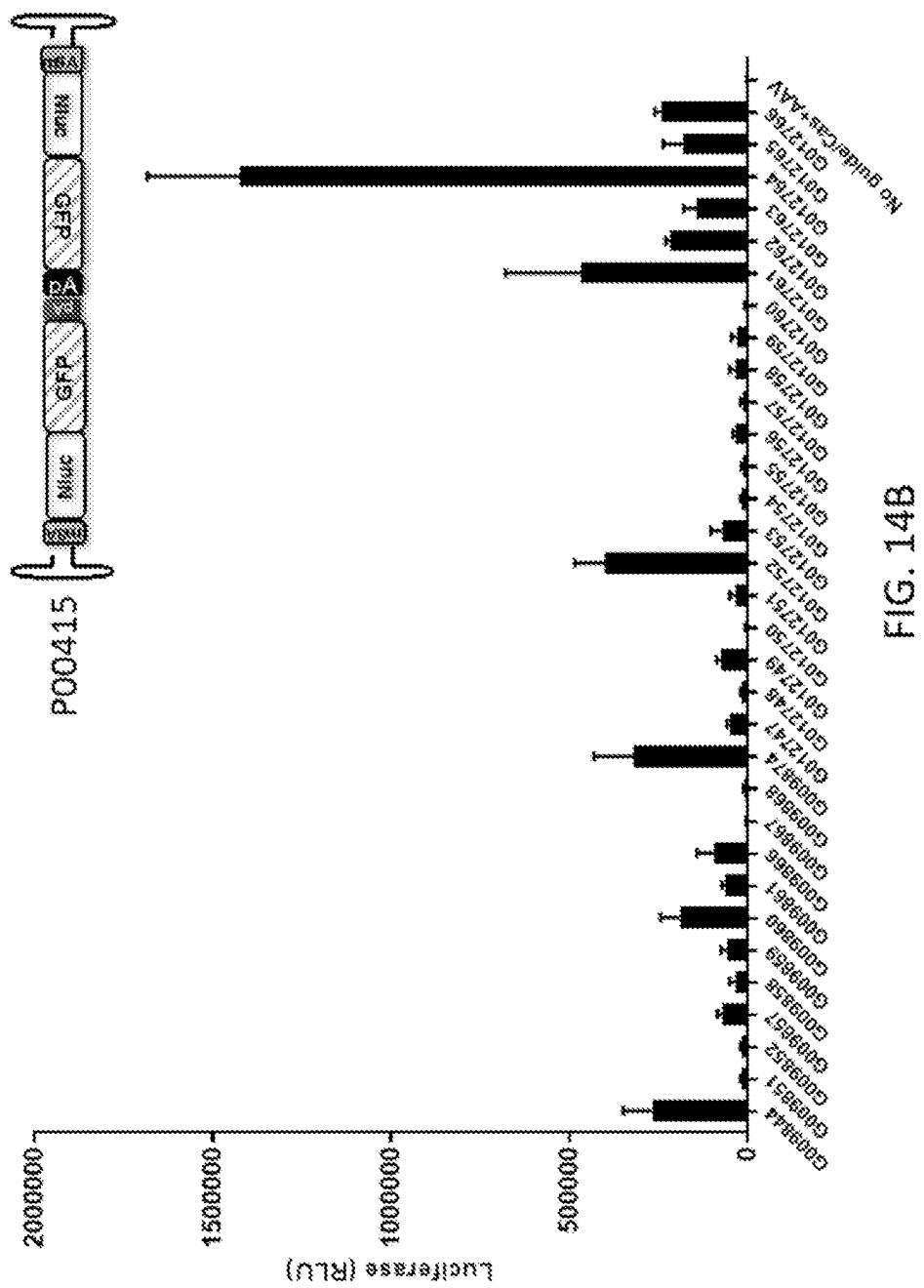


FIG. 14A



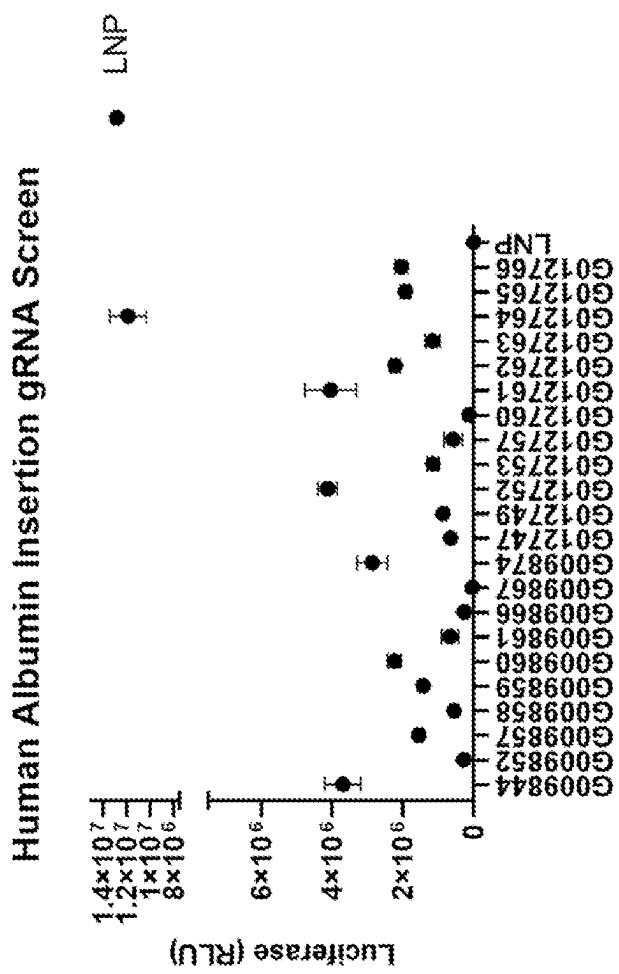


FIG. 14C

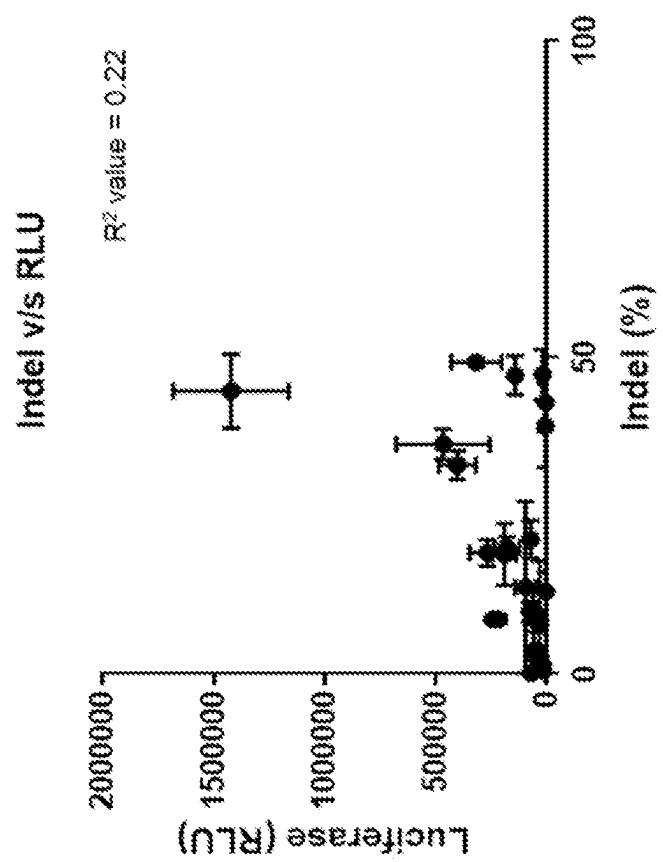
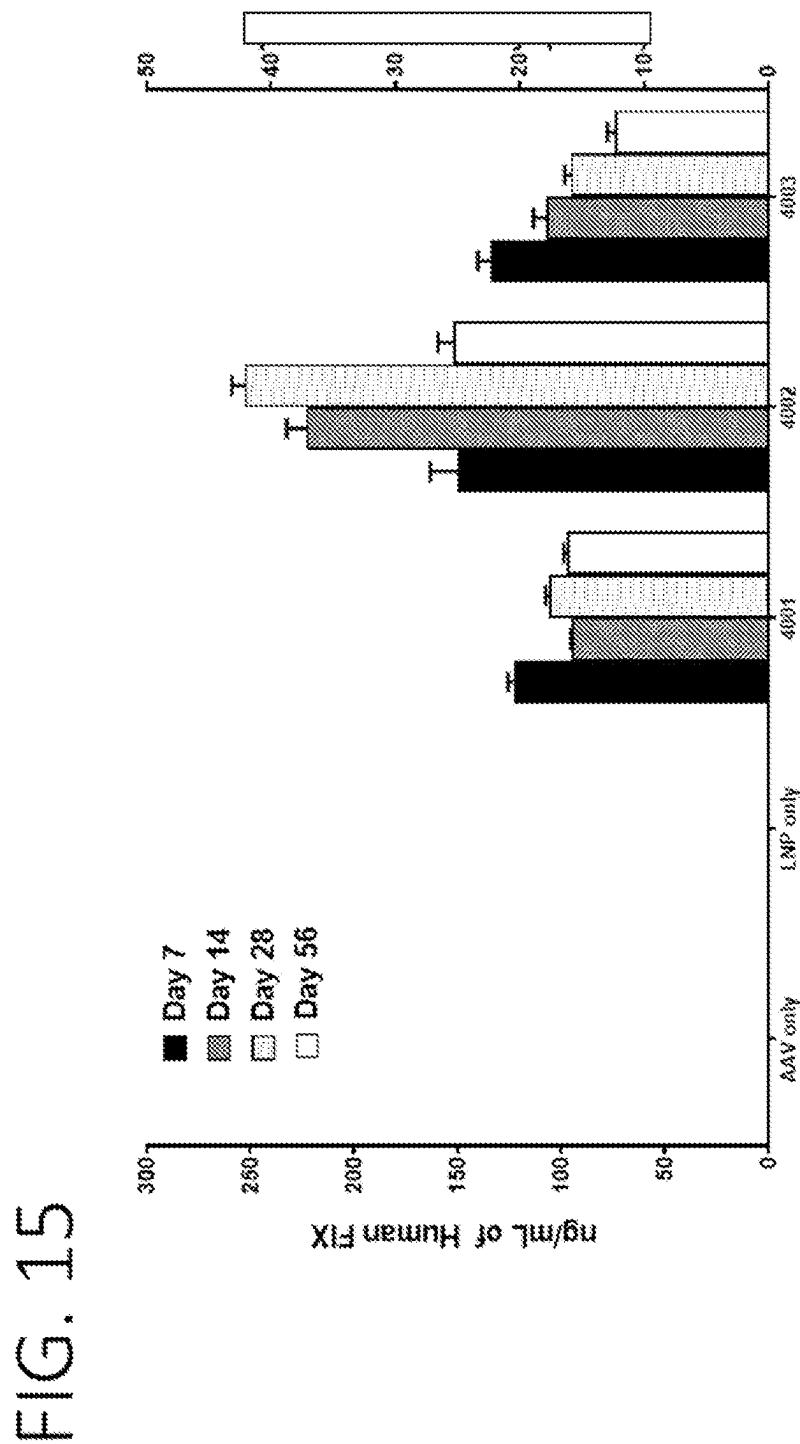


FIG. 14D



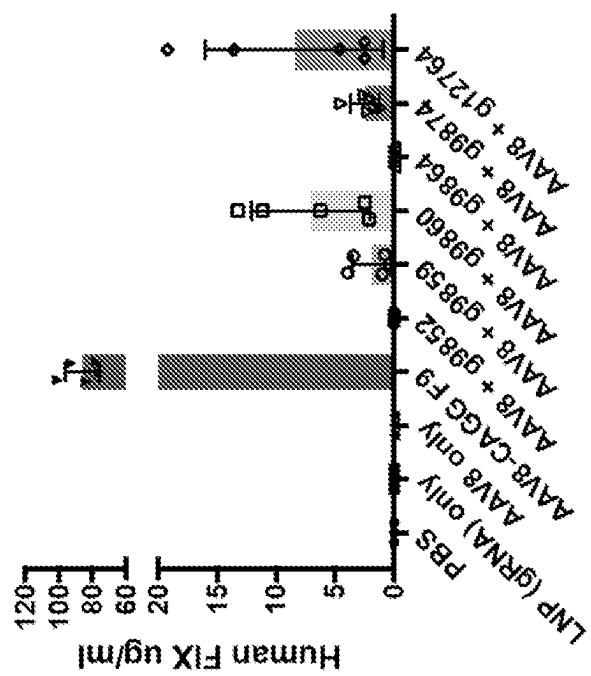


FIG. 16A

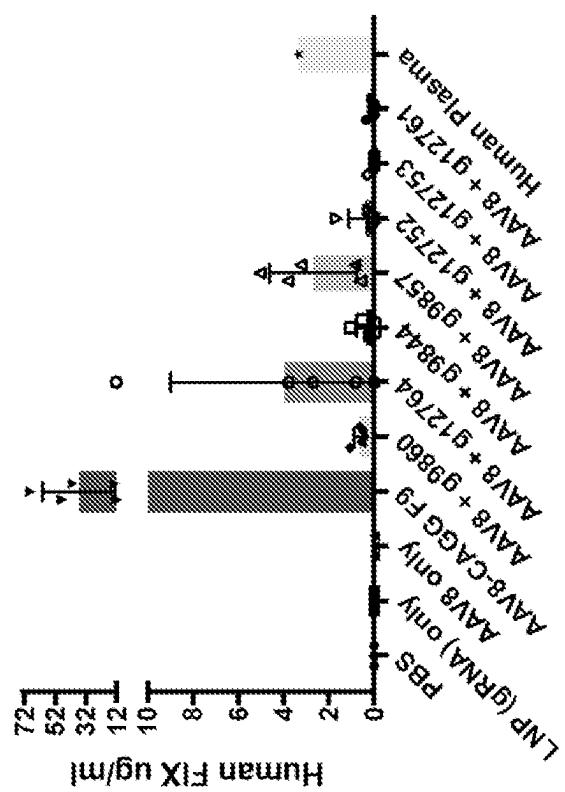


FIG. 16B

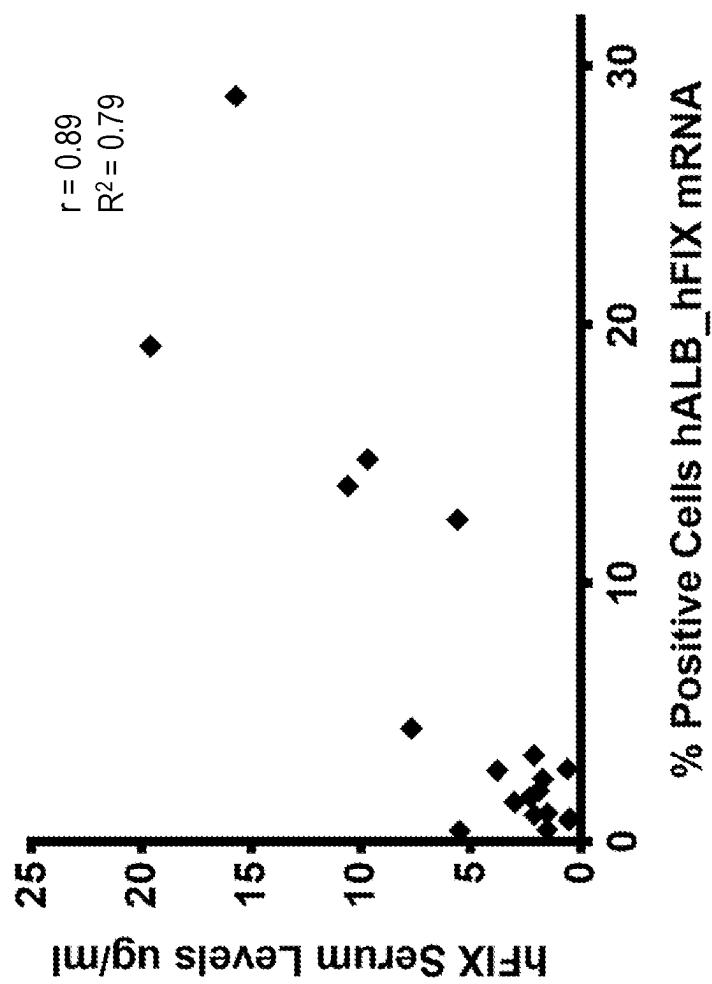


FIG. 17

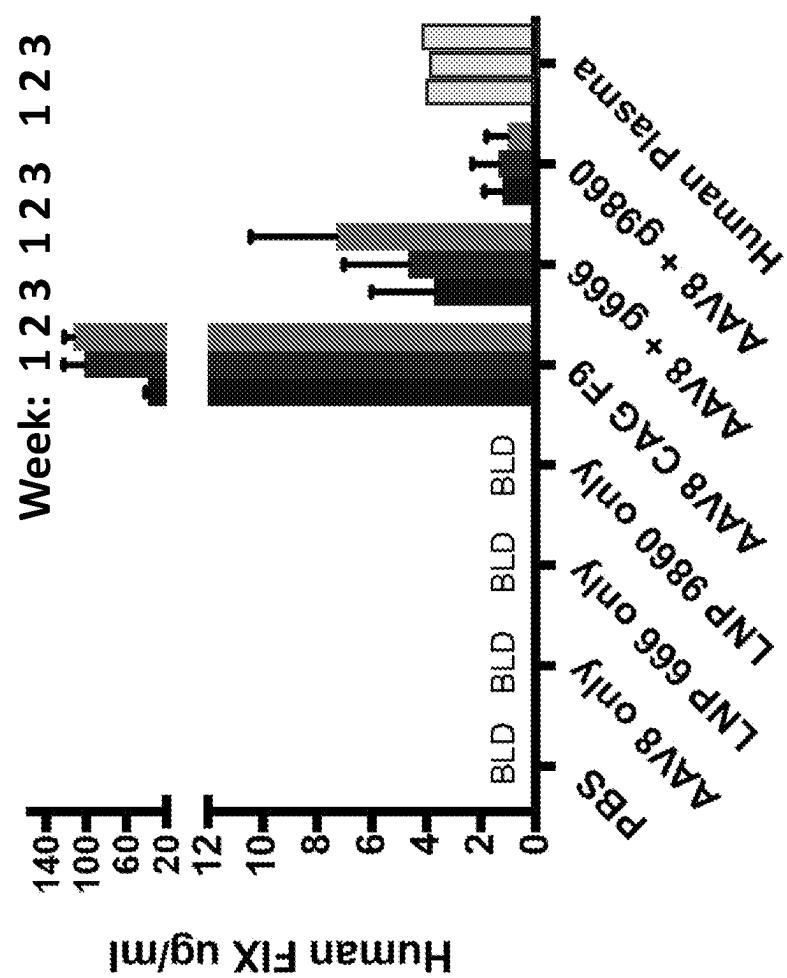


FIG. 18

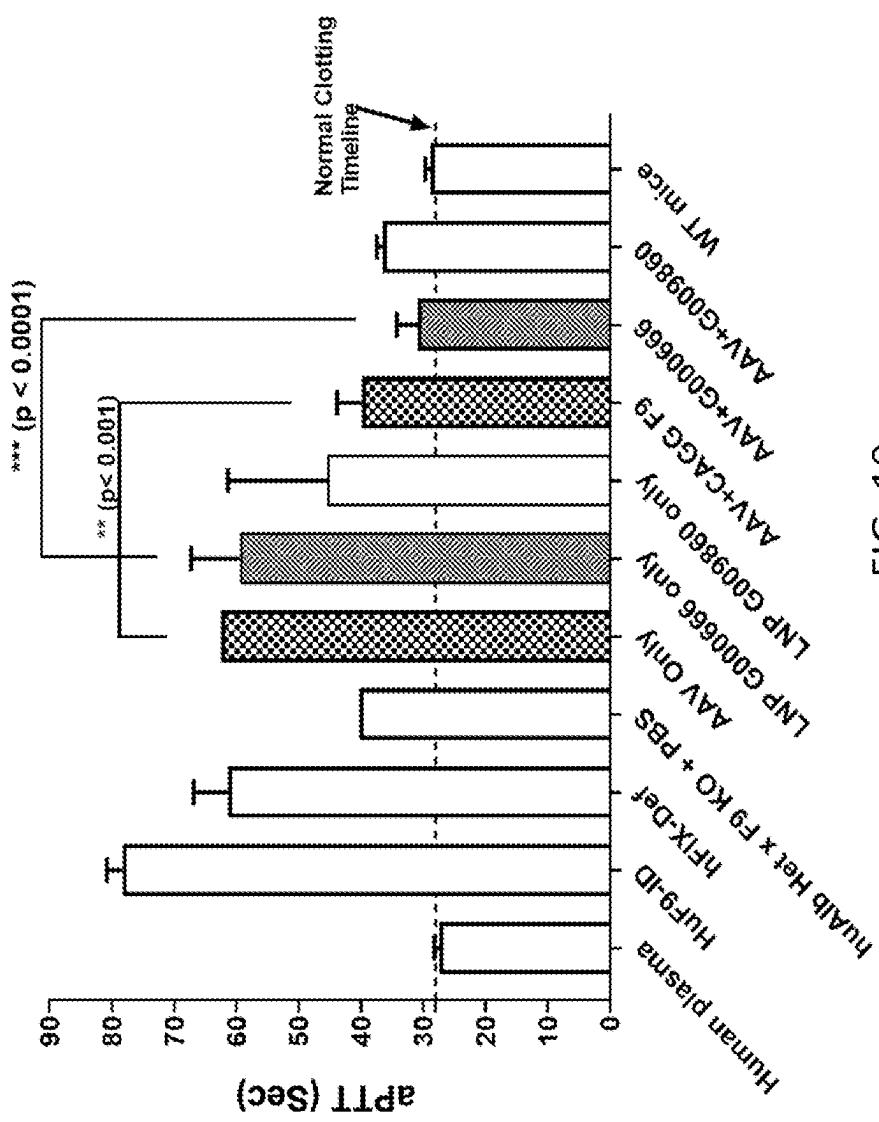


FIG. 19

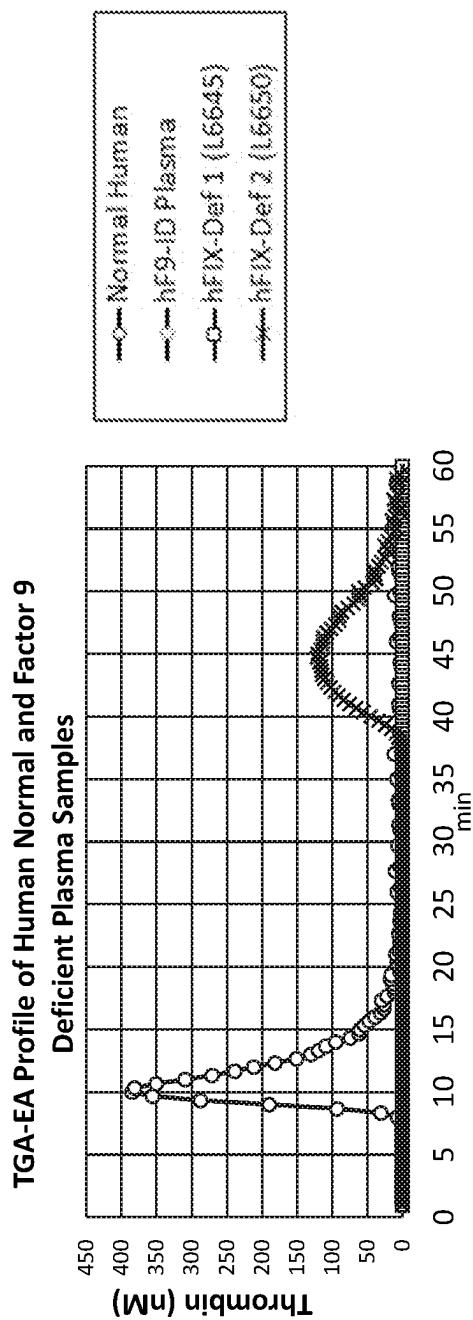


FIG. 20A
TGA-EA Profile of Mice Plasma from hF9 Insertion in huAlb Het X F9 KO Mice Study

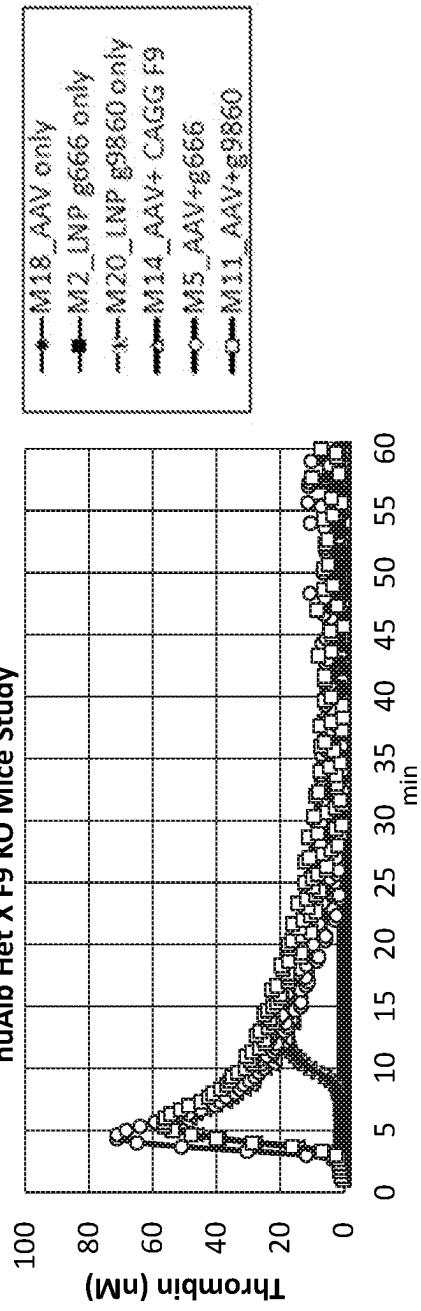


FIG. 20B

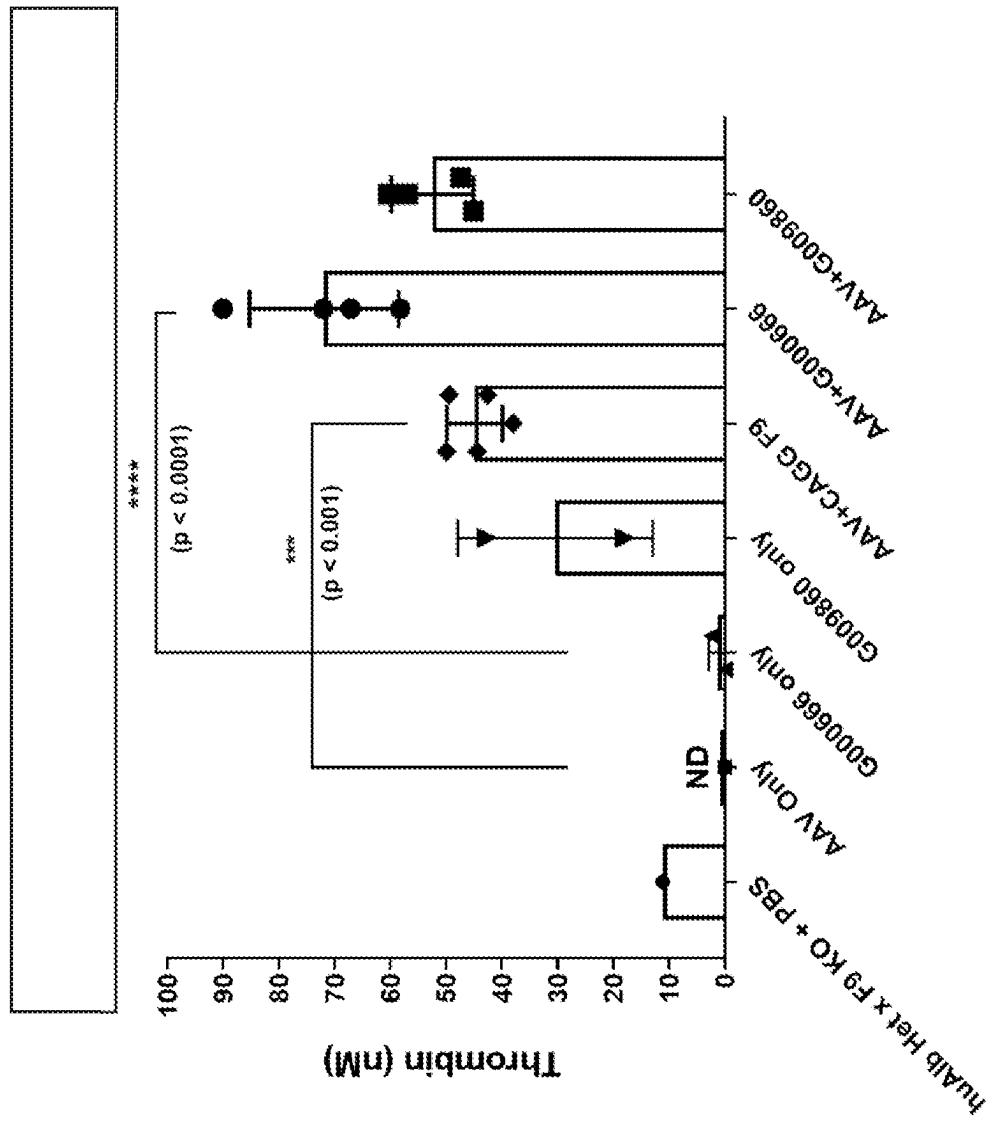


FIG. 21

COMPOSITIONS AND METHODS FOR EXPRESSING FACTOR IX

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Continuation of U.S. Non-Provisional application Ser. No. 16/657,961, filed Oct. 18, 2019, which claims the benefit of priority from U.S. Provisional Application No. 62/747,509, filed on Oct. 18, 2018, U.S. Provisional Application No. 62/829,009, filed on Apr. 3, 2019, U.S. Provisional Application No. 62/829,621, filed on Apr. 4, 2019 and U.S. Provisional Application No. 62/840,352, filed on Apr. 29, 2019, each of which is hereby incorporated by reference in its entirety.

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS AN XML FILE

[0002] The Sequence Listing written in file 625637SEQLIST.xml is 484,218 bytes, was created on May 1, 2025, and is hereby incorporated by reference in its entirety.

BACKGROUND AND SUMMARY

[0003] Bleeding disorders are caused by inadequate blood clotting. This deficiency may be caused by congenital coagulation disorders, acquired coagulation disorders, or hemorrhagic conditions induced by trauma. Bleeding is one of the most serious and significant manifestations of disease, and may occur from a local site or be generalized. Localized bleeding may be associated with lesions and may be further complicated by a defective haemostatic mechanism. Congenital or acquired deficiencies of any of the coagulation factors may be associated with a hemorrhagic tendency. Classic examples of bleeding disorders include hemophilia, such as hemophilia A, which results from a deficiency in factor VIII, or hemophilia B (Christmas Disease), which results from a deficiency in factor IX. Hemophilia occurs in all racial and ethnic groups, and affects many people in the United States and worldwide.

[0004] Traditional therapy for bleeding disorders includes parenteral replacement of deficient clotting factors, such as factor VII, factor VIII or factor IX. For example, current treatments for Hemophilia B rely on chronic, repeated intravenous infusions of purified recombinant Factor IX. However, those treatments suffer from a number of drawbacks including the need for repeated intravenous infusions, being associated with inhibitor formation, and generally being more prophylactic rather than curative. See, e.g., Petrini 2001, *Hemophilia* 7:99; Fischer et al. 2002, *Blood* 99 (7):2337.

[0005] Gene therapy, which involves introducing a copy of a missing or defective gene into a patient, provide one possible method of introducing Factor IX to patients for a longer duration. However, there exists a need for additional compositions and methods that offer improved, long term expression of Factor IX.

[0006] The present disclosure provides compositions and methods useful for expressing Factor IX in a host cell or a population of host cells (in vitro or in vivo), and for treating hemophilia (e.g., hemophilia B). Provided herein are guide RNAs for use in targeted insertion of a sequence encoding Factor IX into a human genomic locus, e.g., a safe harbor site, such as an albumin safe harbor site. Also provided are

donor constructs (e.g., bidirectional constructs), comprising a sequence encoding Factor IX, for use in targeted insertion into a safe harbor site, such as intron 1 of the albumin safe harbor site. In some embodiments, the guide RNA disclosed herein can be used in combination with an RNA-guided DNA binding agent (e.g., Cas nuclease) and a donor construct (e.g., bidirectional construct) comprising a Factor IX transgene. In some embodiments, the donor construct (e.g., bidirectional construct) can be used with a gene editing system (e.g., CRISPR/Cas system; zinc finger nuclelease (ZFN) system; transcription activator-like effector nuclelease (TALEN) system). In some embodiments, the guide RNA disclosed herein can be used in combination with an RNA-guided DNA binding agent (e.g., Cas nuclease) and a donor construct (e.g., bidirectional construct) that comprises a Factor IX transgene. The following embodiments are provided.

[0007] In some aspects, provided herein is a method of introducing a Factor IX nucleic acid to a cell or a population of cells, comprising administering: i) a nucleic acid construct comprising a Factor IX protein coding sequence; ii) an RNA-guided DNA binding agent; and iii) a guide RNA (gRNA) comprising a sequence. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID Nos: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNA comprises a sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNA comprises a sequence that is a sequence selected from the group consisting of SEQ ID NOS: 34, 40, 45, 51, 60, 61, 63, 64, 65, 66, 72, 77, 83, 92, 93, 95, 96, and 97. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNA comprises a sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNA comprises a sequence that is selected from the group consisting of SEQ ID NOS: 34-97. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 98-119. In some embodiments, the guide RNA comprises a sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 98-119. In some embodiments, the guide RNA comprises a sequence that is a sequence selected from the group consisting of SEQ ID NOS: 120-163.

[0008] In some aspects, provided herein is a method of expressing Factor IX in a cell or population of cells, comprising administering: i) a nucleic acid construct comprising a Factor IX protein coding sequence; ii) an RNA-guided DNA binding agent; and iii) a guide RNA (gRNA) comprising a sequence. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID Nos: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNA comprises a sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID

NOs: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNA comprises a sequence that is selected from the group consisting of SEQ ID NOs: 34, 40, 45, 51, 60, 61, 63, 64, 65, 66, 72, 77, 83, 92, 93, 95, 96, and 97. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOs: 2-33. In some embodiments, the guide RNA comprises a sequence that is least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 2-33. In some embodiments, the guide RNA comprises a sequence selected from the group consisting of SEQ ID NOs: 34-97. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOs: 98-119. In some embodiments, the guide RNA comprises a sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 98-119. In some embodiments, the guide RNA comprises a sequence that is selected from the group consisting of SEQ ID NOs: 120-163.

[0009] In some aspects, provided herein is a method of introducing or expressing Factor IX in a cell or population of cells, comprising administering: i) a nucleic acid construct comprising a Factor IX protein coding sequence; ii) an RNA-guided DNA binding agent; and iii) a guide RNA (gRNA) comprising a sequence wherein the administration is *in vitro*.

[0010] In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID Nos: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNA comprises at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNA comprises a sequence selected from the group consisting of SEQ ID NOs: 34, 40, 45, 51, 60, 61, 63, 64, 65, 66, 72, 77, 83, 92, 93, 95, 96, and 97. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOs: 2-33. In some embodiments, the guide RNA comprises at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 2-33. In some embodiments, the guide RNA comprises a sequence selected from the group consisting of SEQ ID NOs: 34-97.

[0011] In some embodiments, the nucleic acid construct is administered in a nucleic acid vector and/or a lipid nanoparticle. In some embodiments, the RNA-guided DNA binding agent and/or gRNA is administered in a nucleic acid vector and/or lipid nanoparticle. In some embodiments, the nucleic acid vector is a viral vector. In some embodiments, the viral vector is selected from the group consisting of an adeno associate viral (AAV) vector, adenovirus vector, retrovirus vector, and lentivirus vector. In some embodiments, the AAV vector is selected from the group consisting of AAV1, AAV3, AAV4, AAV5, AAV6, AAV8, AAV-DJ, and AAV2/8.

[0012] In some embodiments, the nucleic acid construct, RNA-guided DNA binding agent, and gRNA are administered sequentially, in any order and/or in any combination. In some embodiments, wherein the nucleic acid construct, RNA-guided DNA binding agent, and gRNA, individually

or in any combination, are administered simultaneously. In some embodiments, the RNA-guided DNA binding agent, or RNA-guided DNA binding agent and gRNA in combination, is administered prior to administering the nucleic acid construct. In some embodiments, the nucleic acid construct is administered prior to administering the gRNA and/or RNA-guided DNA binding agent.

[0013] In some embodiments, the RNA-guided DNA binding agent is a Cas nuclease. In some embodiments, the Cas nuclease is a class 2 Cas nuclease. In some embodiments the Cas nuclease is Cas9. In some embodiments, the Cas nuclease is an *S. pyogenes* Cas9 nuclease. In some embodiments, the Cas nuclease is a nickase.

[0014] In some embodiments, the nucleic acid construct is a bidirectional nucleic acid construct. In some embodiments, the nucleic acid construct is single-stranded or double-stranded. In some embodiments, the nucleic acid construct is a single-stranded DNA or a double-stranded DNA. In some embodiments, the bidirectional construct does not comprise a promoter that drives the expression of the Factor IX protein. In some embodiments, the cell or population of cells expresses Factor IX with a heterologous peptide, such as an albumin signal peptide.

[0015] In some embodiments, the cell or population of cells includes a liver cell. In some embodiments, the liver cell is a hepatocyte.

[0016] In some embodiments, the nucleic acid encodes a wild-type Factor IX protein. In some embodiments, the nucleic acid encodes a mutant Factor IX protein. In some embodiments, the nucleic acid encodes a Factor IX protein having a mutation R338L.

[0017] In some aspects, provided herein is a method of introducing a Factor IX nucleic acid to a cell or population of cells, comprising administering to the cell or population of cells a bidirectional nucleic acid construct comprising a Factor IX protein coding sequence, thereby expressing Factor IX in the cell or population of cells. Provided herein is a method of expressing Factor IX in a cell or population of cells, comprising administering to the cell or population of cells a bidirectional nucleic acid construct comprising a Factor IX protein coding sequence, thereby expressing Factor IX expression in the cell or population of cells.

[0018] In some embodiments, the bidirectional nucleic acid construct comprises: a) a first segment comprising a coding sequence for Factor IX; and b) a second segment comprising a reverse complement of a coding sequence of Factor IX, wherein the construct does not comprise a promoter that drives the expression of Factor IX. In some embodiments, the bidirectional nucleic acid construct comprises: a) a first segment comprising a coding sequence for Factor IX; and b) a second segment comprising a reverse complement of a coding sequence of a second polypeptide, wherein the construct does not comprise a promoter that drives the expression of the polypeptide.

[0019] In some embodiments, the method of introducing a Factor IX nucleic acid to a cell or population of cells further comprises administering an RNA-guided DNA binding agent. In some embodiments, the method further comprises administering a gRNA. In some embodiments, the bidirectional nucleic acid construct is administered in a nucleic acid vector and/or a lipid nanoparticle. In some embodiments, the RNA-guided DNA binding agent is administered in a nucleic acid vector and/or lipid nanoparticle. In some embodiments, the gRNA is administered in a nucleic acid

vector and/or lipid nanoparticle. In some embodiments, the nucleic acid vector is a viral vector. In some embodiments, the viral vector is selected from the group consisting of an adeno associate viral (AAV) vector, adenovirus vector, retrovirus vector, and lentivirus vector. In some embodiments, the AAV vector is selected from the group consisting of AAV1, AAV3, AAV4, AAV5, AAV6, AAV8, AAV-DJ, and AAV2/8.

[0020] In some embodiments, the bidirectional nucleic acid construct, RNA-guided DNA binding agent, and gRNA are administered sequentially, in any order and/or in any combination. In some embodiments, the bidirectional nucleic acid construct, RNA-guided DNA binding agent, and gRNA, in any combination, are administered simultaneously. In some embodiments, the RNA-guided DNA binding agent, or RNA-guided DNA binding agent and gRNA in combination, is administered prior to administering the bidirectional nucleic acid construct. In some embodiments, the bidirectional nucleic acid construct is administered prior to administering the gRNA and/or RNA-guided DNA binding agent.

[0021] In some embodiments, the RNA-guided DNA binding agent is a Cas nuclease. In some embodiments, the Cas nuclease is a class 2 Cas nuclease. In some embodiments, the Cas nuclease is selected from the group consisting of *S. pyogenes* nuclease, *S. aureus* nuclease, *C. jejuni* nuclease, *S. thermophilus* nuclease, *N. meningitidis* nuclease, and variants thereof. In some embodiments, the Cas nuclease is Cas9. In some embodiments, the Cas nuclease is a nickase.

[0022] In some embodiments, the bidirectional construct does not comprise a promoter that drives the expression of the Factor IX protein. In some embodiments, the bidirectional construct is single-stranded or double-stranded. In some embodiments, the nucleic acid construct is a single-stranded DNA or a double-stranded DNA. In some embodiments, the gRNA comprises at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 2-33 or a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOs: 2-33.

[0023] In some aspects, provided herein is a composition for use in expressing Factor IX in a cell, wherein the composition comprises: i) a nucleic acid construct comprising a Factor IX protein coding sequence; ii) an RNA-guided DNA binding agent; and iii) a guide RNA (gRNA) comprising a guide sequence selected from the group consisting of SEQ ID NOs: 2-33 or a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOs: 2-33. Provided herein is a composition for use in expressing Factor IX in a cell or population of cells, wherein the composition comprises a bidirectional nucleic acid construct comprising a Factor IX protein coding sequence. In some embodiments, a host cell is made by the method of any preceding embodiment.

[0024] In some embodiments, the host cell is a liver cell. In some embodiments, the host cell is a non-dividing cell type. In some embodiments, the host cell expresses the Factor IX polypeptide encoded by the bidirectional construct. In some embodiments, the host cell is a hepatocyte.

[0025] In some embodiments of the method, construct, or host cell of any above method, the gRNA comprises SEQ ID NO: 401.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] FIG. 1 shows construct formats as represented in AAV genomes. SA=slice acceptor; pA=polyA signal sequence; HA=homology arm; LHA=left homology arm; RHA=right homology arm

[0027] FIG. 2 shows vectors without homology arms are not effective in an immortalized liver cell line (Hepa1-6). An scAAV derived from plasmid P00204 comprising 200 bp homology arms resulted in expression of hFIX in the dividing cells. Use of the AAV vectors derived from P00123 (scAAV lacking homology arms) and P00147 (ssAAV bidirectional construct lacking homology arms) did not result in detectable expression of hFIX.

[0028] FIGS. 3A and 3B show results from in vivo testing of insertion templates with and without homology arms using vectors derived from P00123, P00147, or P00204. FIG. 3A shows liver editing levels as measured by indel formation of ~60% were detected in each group of animals treated with LNPs comprising CRISPR/Cas9 components. FIG. 3B shows animals receiving the ssAAV vectors without homology arms (derived from P00147) in combination with LNP treatment resulted in the highest level of hFIX expression in serum.

[0029] FIGS. 4A and 4B show results from in vivo testing of ssAAV insertion templates with and without homology arms. FIG. 4A compares targeted insertion with vectors derived from plasmids P00350, P00356, P00362 (having asymmetrical homology arms as shown), and P00147 (bidirectional construct as shown in FIG. 4B). FIG. 4B compares insertion into a second site targeted with vectors derived from plasmids P00353, P00354 (having symmetrical homology arms as shown), and P00147.

[0030] FIGS. 5A-5D show results of targeted insertion of bidirectional constructs across 20 target sites in primary mouse hepatocytes. FIG. 5A shows the schematics of each of the vectors tested. FIG. 5B shows editing as measured by indel formation for each of the treatment groups across each combination tested. FIG. 5C and FIG. 5D show that significant levels of editing (as indel formation at a specific target site) did not necessarily result in more efficient insertion or expression of the transgenes. hSA=human F9 splice acceptor; mSA=mouse albumin splice acceptor; HiBit=tag for luciferase based detection; pA=polyA signal sequence; Nluc=nanoluciferase reporter; GFP=green fluorescent reporter.

[0031] FIG. 6 shows results from in vivo screening of targeted insertion with bidirectional constructs across 10 target sites using with ssAAV derived from P00147. As shown, significant levels of indel formation do not necessarily result in high levels of transgene expression.

[0032] FIGS. 7A-7D show results from in vivo screening of bidirectional constructs across 20 target sites using ssAAV derived from P00147. FIG. 7A shows varied levels of editing as measured by indel formation were detected for each of the treatment groups across each LNP/vector combination tested. FIG. 7B provides corresponding targeted insertion data. The results show poor correlation between indel formation and insertion or expression of the bidirectional constructs (FIG. 7B and FIG. 7D), and a positive correlation between in vitro and in vivo results (FIG. 7C).

[0033] FIGS. 8A and 8B show insertion of the bidirectional construct at the cellular level using in situ hybridization method using probes that can detect the junctions between the hFIX transgene and the mouse albumin exon 1

sequence (FIG. 8A). Circulating hFIX levels correlated with the number of cells that were positive for the hybrid transcript (FIG. 8B).

[0034] FIG. 9 shows the effect on targeted insertion of varying the timing between delivery of the ssAAV comprising the bidirectional hFIX construct and LNP.

[0035] FIG. 10 shows the effect on targeted insertion of varying the number of LNP doses (e.g., 1, 2, or 3) following delivery of the bidirectional hFIX construct.

[0036] FIG. 11A shows the durability of hFIX expression in vivo. FIG. 11B demonstrates expression from intron 1 of albumin was sustained.

[0037] FIG. 12A and FIG. 12B show that varying AAV or LNP dose can modulate the amount of expression of hFIX from intron 1 of the albumin gene in vivo.

[0038] FIGS. 13A-13C show results from screening bidirectional constructs across target sites in primary cynomolgus hepatocytes. FIG. 13A shows varied levels of editing as measured by indel formation detected for each of the samples. FIG. 13B and FIG. 13C show that significant levels of indel formation was not predictive for insertion or expression of the bidirectional constructs into intron 1 of albumin.

[0039] FIGS. 14A-14C show results from screening bidirectional constructs across target sites in primary human hepatocytes. FIG. 14A shows editing as measured by indel formation detected for each of the samples. FIG. 14B, FIGS. 14C, and 14D show that significant levels of indel formation was not predictive for insertion or expression of the bidirectional constructs into intron 1 of the albumin gene.

[0040] FIG. 15 shows the results of in vivo studies where non-human primates were dosed with LNPs along with a bi-directional hFIX insertion template (derived from P00147). Systemic hFIX levels were achieved only in animals treated with both LNPs and AAV, with no hFIX detectable using AAV or LNPs alone.

[0041] FIG. 16A and FIG. 16B show human Factor IX expression levels in the plasma samples at week 6 post-injection.

[0042] FIG. 17 shows week 7 serum levels and % positive cells across the multiple lobes for each animal.

[0043] FIG. 18 shows human Factor IX expression levels in the plasma samples in each group at weeks 1, 2, and 4 post-injection.

[0044] FIG. 19 shows insertion of the hF9 transgene and clotting function in the APTT assay.

[0045] FIG. 20A and FIG. 20B show insertion of the hF9 transgene and thrombin generation in TGA-EA analysis.

[0046] FIG. 21 shows insertion of the hF9 transgene and thrombin generation.

DETAILED DESCRIPTION

[0047] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying drawings. While the invention is described in conjunction with the illustrated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the invention as defined by the appended embodiments.

[0048] Before describing the present teachings in detail, it is to be understood that the disclosure is not limited to specific compositions or process steps, as such may vary. It should be noted that, as used in this specification and the

appended embodiments, the singular form "a" "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a conjugate" includes a plurality of conjugates and reference to "a cell" includes a plurality or population of cells and the like. As used herein, the term "include" and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

[0049] Numeric ranges are inclusive of the numbers defining the range. Measured and measureable values are understood to be approximate, taking into account significant digits and the error associated with the measurement. Also, the use of "comprise", "comprises", "comprising", "contain", "contains", "containing", "include", "includes", and "including" are not intended to be limiting. It is to be understood that both the foregoing general description and detailed description are exemplary and explanatory only and are not restrictive of the teachings.

[0050] Unless specifically noted in the specification, embodiments in the specification that recite "comprising" various components are also contemplated as "consisting of" or "consisting essentially of" the recited components; embodiments in the specification that recite "consisting of" various components are also contemplated as "comprising" or "consisting essentially of" the recited components; and embodiments in the specification that recite "consisting essentially of" various components are also contemplated as "consisting of" or "comprising" the recited components (this interchangeability does not apply to the use of these terms in the claims). The term "or" is used in an inclusive sense, i.e., equivalent to "and/or," unless the context clearly indicates otherwise. The term "about", when used before a list, modifies each member of the list. The term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined.

[0051] The term "about", when used before a list, modifies each member of the list. The term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined.

[0052] The section headings used herein are for organizational purposes only and are not to be construed as limiting the desired subject matter in any way. In the event that any material incorporated by reference contradicts any term defined in this specification or any other express content of this specification, this specification controls.

I. Definitions

[0053] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[0054] "Polynucleotide" and "nucleic acid" are used herein to refer to a multimeric compound comprising nucleosides or nucleoside analogs which have nitrogenous heterocyclic bases or base analogs linked together along a backbone, including conventional RNA, DNA, mixed RNA-DNA, and polymers that are analogs thereof. A nucleic acid "backbone" can be made up of a variety of linkages, including one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds ("peptide nucleic acids" or PNA; PCT No. WO 95/32305), phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar

moieties of a nucleic acid can be ribose, deoxyribose, or similar compounds with substitutions, e.g., 2' methoxy or 2' halide substitutions. Nitrogenous bases can be conventional bases (A, G, C, T, U), analogs thereof (e.g., modified uridines such as 5-methoxyuridine, pseudouridine, or N1-methylpseudouridine, or others); inosine; derivatives of purines or pyrimidines (e.g., N⁴-methyl deoxyguanosine, deaza- or aza-purines, deaza- or aza-pyrimidines, pyrimidine bases with substituent groups at the 5 or 6 position (e.g., 5-methylcytosine), purine bases with a substituent at the 2, 6, or 8 positions, 2-amino-6-methylaminopurine, O⁶-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-dimethylhydrazine-pyrimidines, and O⁴-alkyl-pyrimidines; U.S. Pat. No. 5,378,825 and PCT No. WO 93/13121). For general discussion see *The Biochemistry of the Nucleic Acids* 5-36, Adams et al., ed., 11th ed., 1992). Nucleic acids can include one or more “abasic” residues where the backbone includes no nitrogenous base for position(s) of the polymer (U.S. Pat. No. 5,585,481). A nucleic acid can comprise only conventional RNA or DNA sugars, bases and linkages, or can include both conventional components and substitutions (e.g., conventional bases with 2' methoxy linkages, or polymers containing both conventional bases and one or more base analogs). Nucleic acid includes “locked nucleic acid” (LNA), an analogue containing one or more LNA nucleotide monomers with a bicyclic furanose unit locked in an RNA mimicking sugar conformation, which enhance hybridization affinity toward complementary RNA and DNA sequences (Vester and Wengel, 2004, *Biochemistry* 43(42): 13233-41). RNA and DNA have different sugar moieties and can differ by the presence of uracil or analogs thereof in RNA and thymine or analogs thereof in DNA.

[0055] “Guide RNA”, “gRNA”, and simply “guide” are used herein interchangeably to refer to either a guide that comprises a guide sequence, e.g., crRNA (also known as CRISPR RNA), or the combination of a crRNA and a trRNA (also known as tracrRNA). The crRNA and trRNA may be associated as a single RNA molecule (single guide RNA, sgRNA) or, for example, in two separate RNA molecules (dual guide RNA, dgRNA). “Guide RNA” or “gRNA” refers to each type. The trRNA may be a naturally-occurring sequence, or a trRNA sequence with modifications or variations compared to naturally-occurring sequences. Guide RNAs, such as sgRNAs or dgRNAs, can include modified RNAs as described herein.

[0056] As used herein, a “guide sequence” refers to a sequence within a guide RNA that is complementary to a target sequence and functions to direct a guide RNA to a target sequence for binding or modification (e.g., cleavage) by an RNA-guided DNA binding agent. A “guide sequence” may also be referred to as a “targeting sequence,” or a “spacer sequence.” A guide sequence can be 20 base pairs in length, e.g., in the case of *Streptococcus pyogenes* (i.e., Spy Cas9) and related Cas9 homologs/orthologs. Shorter or longer sequences can also be used as guides, e.g., 15-, 16-, 17-, 18-, 19-, 21-, 22-, 23-, 24-, or 25-nucleotides in length. For example, in some embodiments, the guide sequence comprises at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs:2-33. In some embodiments, the target sequence is in a gene or on a chromosome, for example, and is complementary to the guide sequence. In some embodiments, the degree of complementarity or identity between a guide sequence and its corresponding target sequence may be about 75%, 80%,

85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. For example, in some embodiments, the guide sequence comprises a sequence with about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 2-33. In some embodiments, the guide sequence and the target region may be 100% complementary or identical. In other embodiments, the guide sequence and the target region may contain at least one mismatch. For example, the guide sequence and the target sequence may contain 1, 2, 3, or 4 mismatches, where the total length of the target sequence is at least 15, 16, 17, 18, 19, 20 or more base pairs. In some embodiments, the guide sequence and the target region may contain 1-4 mismatches where the guide sequence comprises at least 15, 16, 17, 18, 19, 20 or more nucleotides. In some embodiments, the guide sequence and the target region may contain 1, 2, 3, or 4 mismatches where the guide sequence comprises 20 nucleotides.

[0057] Target sequences for RNA-guided DNA binding agents include both the positive and negative strands of genomic DNA (i.e., the sequence given and the sequence's reverse complement), as a nucleic acid substrate for an RNA-guided DNA binding agent is a double stranded nucleic acid. Accordingly, where a guide sequence is said to be “complementary to a target sequence”, it is to be understood that the guide sequence may direct a guide RNA to bind to the reverse complement of a target sequence. Thus, in some embodiments, where the guide sequence binds the reverse complement of a target sequence, the guide sequence is identical to certain nucleotides of the target sequence (e.g., the target sequence not including the PAM) except for the substitution of U for T in the guide sequence.

[0058] As used herein, an “RNA-guided DNA-binding agent” means a polypeptide or complex of polypeptides having RNA and DNA binding activity, or a DNA-binding subunit of such a complex, wherein the DNA binding activity is sequence-specific and depends on the sequence of the RNA. The term RNA-guided DNA binding-agent also includes nucleic acids encoding such polypeptides. Exemplary RNA-guided DNA-binding agents include Cas cleavases/nickases.

[0059] Exemplary RNA-guided DNA-binding agents may include inactivated forms thereof (“dCas DNA-binding agents”), e.g. if those agents are modified to permit DNA cleavage, e.g. via fusion with a FokI cleavase domain. “Cas nuclease”, as used herein, encompasses Cas cleavases and Cas nickases. Cas cleavases and Cas nickases include a Csm or Cmr complex of a type III CRISPR system, the Cas10, Csm1, or Cmr2 subunit thereof, a Cascade complex of a type I CRISPR system, the Cas3 subunit thereof, and Class 2 Cas nucleases. As used herein, a “Class 2 Cas nuclease” is a single-chain polypeptide with RNA-guided DNA binding activity. Class 2 Cas nucleases include Class 2 Cas cleavases/nickases (e.g., H840A, D10A, or N863A variants), which further have RNA-guided DNA cleavases or nickase activity, and Class 2 dCas DNA-binding agents, in which cleavase/nickase activity is inactivated”), if those agents are modified to permit DNA cleavage. Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, C2c3, HF Cas9 (e.g., N497A, R661A, Q695A, Q926A variants), HypaCas9 (e.g., N692A, M694A, Q695A, H698A variants), eSPCas9(1.0) (e.g., K810A, K1003A, R1060A variants), and eSPCas9(1.1) (e.g., K848A, K1003A, R1060A variants)

proteins and modifications thereof. Cpf1 protein, Zetsche et al., *Cell*, 163: 1-13 (2015), also contains a RuvC-like nuclease domain. Cpf1 sequences of Zetsche are incorporated by reference in their entirety. See, e.g., Zetsche, Tables Si and S3. See, e.g., Makarova et al., *Nat Rev Microbiol*, 13(11): 722-36 (2015); Shmakov et al., *Molecular Cell*, 60:385-397 (2015). As used herein, delivery of an RNA-guided DNA-binding agent (e.g. a Cas nuclease, a Cas9 nuclease, or an *S. pyogenes* Cas9 nuclease) includes delivery of the polypeptide or mRNA.

[0060] As used herein, “ribonucleoprotein” (RNP) or “RNP complex” refers to a guide RNA together with an RNA-guided DNA binding agent, such as a Cas nuclease, e.g., a Cas cleavage, Cas nickase, or dCas DNA binding agent (e.g., Cas9). In some embodiments, the guide RNA guides the RNA-guided DNA binding agent such as Cas9 to a target sequence, and the guide RNA hybridizes with and the agent binds to the target sequence; in cases where the agent is a cleavage or nickase, binding can be followed by cleaving or nicking.

[0061] As used herein, a first sequence is considered to “comprise a sequence with at least X % identity to” a second sequence if an alignment of the first sequence to the second sequence shows that X % or more of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence AAGA comprises a sequence with 100% identity to the sequence AAG because an alignment would give 100% identity in that there are matches to all three positions of the second sequence. The differences between RNA and DNA (generally the exchange of uridine for thymidine or vice versa) and the presence of nucleoside analogs such as modified uridines do not contribute to differences in identity or complementarity among polynucleotides as long as the relevant nucleotides (such as thymidine, uridine, or modified uridine) have the same complement (e.g., adenine for all of thymidine, uridine, or modified uridine; another example is cytosine and 5-methylcytosine, both of which have guanosine or modified guanosine as a complement). Thus, for example, the sequence 5'-AXG where X is any modified uridine, such as pseudouridine, N1-methyl pseudouridine, or 5-methoxyuridine, is considered 100% identical to AUG in that both are perfectly complementary to the same sequence (5'-CAU). Exemplary alignment algorithms are the Smith-Waterman and Needleman-Wunsch algorithms, which are well-known in the art. One skilled in the art will understand what choice of algorithm and parameter settings are appropriate for a given pair of sequences to be aligned; for sequences of generally similar length and expected identity >50% for amino acids or >75% for nucleotides, the Needleman-Wunsch algorithm with default settings of the Needleman-Wunsch algorithm interface provided by the EBI at the www.ebi.ac.uk web server is generally appropriate.

[0062] As used herein, a first sequence is considered to be “X % complementary to” a second sequence if X % of the bases of the first sequence base pairs with the second sequence. For example, a first sequence 5'AAGA3' is 100% complementary to a second sequence 3'TTCT5', and the second sequence is 100% complementary to the first sequence. In some embodiments, a first sequence 5'AAGA3' is 100% complementary to a second sequence 3'TTCTGTGA5', whereas the second sequence is 50% complementary to the first sequence.

[0063] As used herein, “mRNA” is used herein to refer to a polynucleotide that is entirely or predominantly RNA or modified RNA and comprises an open reading frame that can be translated into a polypeptide (i.e., can serve as a substrate for translation by a ribosome and amino-acylated tRNAs). mRNA can comprise a phosphate-sugar backbone including ribose residues or analogs thereof, e.g., 2'-methoxy ribose residues. In some embodiments, the sugars of an mRNA phosphate-sugar backbone consist essentially of ribose residues, 2'-methoxy ribose residues, or a combination thereof.

[0064] Guide sequences useful in the guide RNA compositions and methods described herein are shown in Table 1 throughout the application.

[0065] As used herein, “indels” refer to insertion/deletion mutations consisting of a number of nucleotides that are either inserted or deleted at the site of double-stranded breaks (DSBs) in a target nucleic acid.

[0066] As used herein, “Factor IX” is used interchangeably with “FIX” or “F9”, and is also known as Christmas Factor. The human wild-type Factor IX protein sequence is available at NCBI NP_000124; gene sequence is available at NCBI NM_000133. Examples of the Factor IX protein sequence are described herein (e.g. SEQ ID NO: 700, SEQ ID NO: 701, and/or SEQ ID NO: 702). As used herein, Factor IX also encompasses a variant of Factor IX, e.g., a variant that possesses increased coagulation activity as compared to wild type Factor IX. A hyperactive variant of Factor IX may comprise a substitution of R338. An example of such a variant Factor IX comprises the mutation R338L relative to SEQ ID NO: 701. The terms hyperactive and hyperfunctional are being used interchangeably herein. Further examples of variant Factor IX comprise an amino acid at residue 338 chosen from alanine, leucine, valine, isoleucine, phenylalanine, tryptophan, methionine, serine, and threonine. Further Factor IX variants comprise an amino acid at residue 338 chosen from leucine, cysteine, aspartic acid, glutamic acid, histidine, lysine, asparagine, glutamine, or tyrosine. As used herein, Factor IX also encompasses a variant that is 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, having at least 60%, 70%, 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild-type Factor IX. As used herein, Factor IX also encompasses a variant that is 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, having at least 60%, 70%, 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to SEQ ID NO: 701 or SEQ ID NO: 702. As used herein, Factor IX also encompasses a fragment that possesses at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type Factor IX. In some embodiments, a Factor IX variant may be a hyperactive Factor IX variant. In certain instances, the Factor IX variant possesses between about 80% and about 100%, 120%, 140%, 160%, 180%, or 200% of the activity as compared to the wild-type Factor IX. The specific activity of the Factor IX variant can be used to calculate its functionally normalized activity, for example as described in Example 13. The specific activities of Factor IX variants, e.g. R338L, are known in the literature and can be calculated using known methods. A hyperfunctional Factor IX variant may have about 1.2, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, or 15 times the specific activity of a corresponding wild type Factor IX protein. In one embodiment, the hyperfunctional Factor IX may have

about 8-12 times the specific activity of a corresponding wild type Factor IX protein. In another embodiment, the hyperfunctional Factor IX may have 1.2-5 times the specific activity of a corresponding wild type Factor IX protein. Exemplary sequences are known in the art, and include sequences in U.S. Pat. Nos. 4,770,999, 4,994,371, 5,521,070, 6,046,380, 6,531,298, and 8,383,388, for example.

[0067] As used herein, a “target sequence” refers to a sequence of nucleic acid in a target gene that has complementarity to the guide sequence of the gRNA. The interaction of the target sequence and the guide sequence directs an RNA-guided DNA binding agent to bind, and potentially nick or cleave (depending on the activity of the agent), within the target sequence.

[0068] As used herein, “hemophilia” refers to a disorder caused by a missing or defective Factor IX gene or polypeptide. The disorder includes conditions that are inherited and/or acquired (e.g., caused by a spontaneous mutation in the gene), and includes hemophilia B. In some embodiments, the defective Factor IX gene or polypeptide results in reduced Factor IX level in the plasma and/or a reduced coagulation activity of Factor IX. As used herein, hemophilia includes mild, moderate, and severe hemophilia. For example, individuals with less than about 1% active factor are classified as having severe haemophilia, those with about 1-5% active factor have moderate haemophilia, and those with mild haemophilia have between about 5-40% of normal levels of active clotting factor.

[0069] As used herein, “normal” or “healthy” individuals include those having between 50 and 160% of normal pooled plasma level of Factor IX activity and antigen levels. Based on its purification from human plasma, the concentration of Factor IX in the normal adult (normal pooled plasma level of Factor IX) is about 300-400 µg/ml of plasma. In some embodiments, the level of Factor IX, e.g., circulating Factor IX, can be measured by a coagulation and/or an immunologic assay, e.g., an sandwich immunoassay, ELISA (see, e.g., Example 13), MSD (see, e.g., Example 14). Factor IX procoagulant activity is determined by the ability of the patient’s plasma to correct the clotting time of Factor IX-deficient plasma.

[0070] As used herein, “treatment” refers to any administration or application of a therapeutic for disease or disorder in a subject, and includes inhibiting the disease, arresting its development, relieving one or more symptoms of the disease, curing the disease, or preventing reoccurrence of one or more symptoms of the disease. For example, treatment of hemophilia may comprise alleviating symptoms of hemophilia.

[0071] As used herein, a “bidirectional nucleic acid construct” (interchangeably referred to herein as a “bidirectional construct”) comprises at least two nucleic acid segments, wherein one segment (the first segment) comprises a coding sequence that encodes a polypeptide of interest (the coding sequence may be referred to herein as “transgene” or a first transgene), while the other segment (the second segment) comprises a sequence wherein the complement of the sequence encodes a polypeptide of interest, or a second transgene. That is, the at least two segments can encode identical or different polypeptides. When the two segments encode the identical polypeptide, the coding sequence of the first segment need not be identical to the complement of the sequence of the second segment. In some embodiments, the sequence of the second segment is a reverse complement of

the coding sequence of the first segment. A bidirectional construct can be single-stranded or double-stranded. The bidirectional construct disclosed herein encompasses a construct that is capable of expressing any polypeptide of interest.

[0072] In some embodiments, a bidirectional nucleic acid construct comprises a first segment that comprises a coding sequence that encodes a first polypeptide (a first transgene), and a second segment that comprises a sequence wherein the complement of the sequence encodes a second polypeptide (a second transgene). In some embodiments, the first and the second polypeptides are at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical. In some embodiments, the first and the second polypeptides comprise an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical, e.g. across 50, 100, 200, 500, 1000 or more amino acid residues.

[0073] As used herein, a “reverse complement” refers to a sequence that is a complement sequence of a reference sequence, wherein the complement sequence is written in the reverse orientation. For example, for a hypothetical sequence 5'CTGGACCGA3' (SEQ ID NO: 500), the “perfect” complement sequence is 3'GACCTGGCT5' (SEQ ID NO: 501), and the “perfect” reverse complement is written 5'TCGGTCCAG3' (SEQ ID NO: 502). A reverse complement sequence need not be “perfect” and may still encode the same polypeptide or a similar polypeptide as the reference sequence. Due to codon usage redundancy, a reverse complement can diverge from a reference sequence that encodes the same polypeptide. As used herein, “reverse complement” also includes sequences that are, e.g., 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the reverse complement sequence of a reference sequence.

[0074] As used herein, “polypeptide” refers to a wild-type or variant protein (e.g., mutant, fragment, fusion, or combinations thereof). A variant polypeptide may possess at least or about 5%, 10%, 15%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% functional activity of the wild-type polypeptide. In some embodiments, the variant is at least 70%, 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of the wild-type polypeptide. In some embodiments, a variant polypeptide may be a hyperactive variant. In certain instances, the variant possesses between about 80% and about 120%, 140%, 160%, 180%, 200%, 300%, 400%, 500%, or more of a functional activity of the wild-type polypeptide.

[0075] As used herein, a “heterologous gene” refers to a gene that has been introduced as an exogenous source to a site within a host cell genome (e.g., at a genomic locus such as a safe harbor locus including an albumin intron 1 site). That is, the introduced gene is heterologous with respect to its insertion site. A polypeptide expressed from such heterologous gene is referred to as a “heterologous polypeptide.” The heterologous gene can be naturally-occurring or engineered, and can be wild type or a variant. The heterologous gene may include nucleotide sequences other than the sequence that encodes the heterologous polypeptide (e.g., an internal ribosomal entry site). The heterologous gene can be a gene that occurs naturally in the host genome, as a wild type or a variant (e.g., mutant). For example, although the

host cell contains the gene of interest (as a wild type or as a variant), the same gene or variant thereof can be introduced as an exogenous source for, e.g., expression at a locus that is highly expressed. The heterologous gene can also be a gene that is not naturally occurring in the host genome, or that expresses a heterologous polypeptide that does not naturally occur in the host genome. “Heterologous gene”, “exogenous gene”, and “transgene” are used interchangeably. In some embodiments, the heterologous gene or transgene includes an exogenous nucleic acid sequence, e.g. a nucleic acid sequence is not endogenous to the recipient cell. In some embodiments, the heterologous gene or transgene includes an exogenous nucleic acid sequence, e.g. a nucleic acid sequence that does not naturally occur in the recipient cell. For example, a heterologous gene may be heterologous with respect to its insertion site and with respect to its recipient cell.

[0076] A “safe harbor” locus is a locus within the genome wherein a gene may be inserted without significant deleterious effects on the host cell, e.g. hepatocyte, e.g., without causing apoptosis, necrosis, and/or senescence, or without causing more than 5%, 10%, 15%, 20%, 25%, 30%, or 40% apoptosis, necrosis, and/or senescence as compared to a control cell. See, e.g., Hsin et al., “Hepatocyte death in liver inflammation, fibrosis, and tumorigenesis,” 2017. In some embodiments, a safe harbor locus allows overexpression of an exogenous gene without significant deleterious effects on the host cell, e.g. hepatocyte, e.g., without causing apoptosis, necrosis, and/or senescence, or without causing more than 5%, 10%, 15%, 20%, 25%, 30%, or 40% apoptosis, necrosis, and/or senescence as compared to a control cell. In some embodiments, a desirable safe harbor locus may be one in which expression of the inserted gene sequence is not perturbed by read-through expression from neighboring genes. The safe harbor may be within an albumin gene, such as a human albumin gene. The safe harbor may be within an albumin intron 1 region, e.g., human albumin intron 1. The safe harbor may be a human safe harbor, e.g., for a liver tissue or hepatocyte host cell. In some embodiments, a safe harbor allows overexpression of an exogenous gene without significant deleterious effects on the host cell or cell population, such as hepatocytes or liver cells, e.g. without causing apoptosis, necrosis, and/or senescence, or without causing more than 5%, 10%, 15%, 20%, 25%, 30%, or 40% apoptosis, necrosis, and/or senescence as compared to a control cell or cell population.

II. Compositions

A. Compositions Comprising Guide RNA (gRNAs)

[0077] Provided herein are guide RNA compositions and methods useful for inserting and expressing a Factor IX gene within a genomic locus, e.g., a safe harbor site of a host cell or a population of host cells. In particular, as exemplified herein, targeting and inserting an exogenous gene at the albumin locus (e.g., at intron 1) allows the use of albumin’s endogenous promoter to drive robust expression of the exogenous gene. The present disclosure is based, in part, on the identification of guide RNAs that specifically target sites within intron 1 of the albumin gene, and which provide efficient insertion and expression of the Factor IX gene. As shown in the Examples and further described herein, the ability of identified gRNAs to mediate high levels of editing as measured through indel forming activity, unexpectedly does not necessarily correlate with use of the same gRNAs

to mediate efficient insertion of transgenes as measured through, e.g., expression of the transgene. That is, certain gRNAs that are able to achieve a high level of indel formation are not necessarily able to mediate efficient insertion, and conversely, some gRNAs shown to achieve low levels of indel formation may mediate efficient insertion and expression of a transgene.

[0078] In some embodiments, provided herein are compositions and methods useful for inserting and expressing a Factor IX gene within a region of an albumin locus (e.g., intron 1) of a host cell. In some embodiments, disclosed herein are compositions useful for introducing or inserting a heterologous Factor IX nucleic acid within an albumin locus of a host cell, e.g., using a guide RNA disclosed herein with an RNA-guided DNA binding agent, and a construct (e.g., donor construct or template) comprising a heterologous Factor IX nucleic acid (“Factor IX transgene”). In some embodiments, disclosed herein are compositions useful for expressing a heterologous Factor IX from an albumin locus of a host cell, e.g., using a guide RNA disclosed herein with an RNA-guided DNA binding agent and a construct (e.g., donor) comprising a heterologous Factor IX nucleic acid. In some embodiments, disclosed herein are compositions useful for expressing a heterologous Factor IX from an albumin locus of a host cell, e.g., using a guide RNA disclosed herein with an RNA-guided DNA binding agent and a bidirectional construct comprising a heterologous Factor IX nucleic acid. In some embodiments, disclosed herein are compositions useful for inducing a break (e.g., double-stranded break (DSB) or single-stranded break (nick)) within the serum albumin gene of a host cell, e.g., using a guide RNA disclosed herein with an RNA-guided DNA binding agent (e.g., a CRISPR/Cas system). The compositions may be used in vitro or in vivo for, e.g., treating hemophilia.

[0079] In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that binds, or is capable of binding, within an intron of an albumin locus. In some embodiments, the guide RNAs disclosed herein bind within a region of intron 1 of the human albumin gene (SEQ ID NO: 1). It will be appreciated that not every base of the guide sequence must bind within the recited regions. For example, in some embodiments, 15, 16, 17, 18, 19, 20, or more bases of the guide RNA sequence bind with the recited regions. For example, in some embodiments, 15, 16, 17, 18, 19, 20, or more contiguous bases of the guide RNA sequence bind with the recited regions.

[0080] In some embodiments, the guide RNAs disclosed herein mediate a target-specific cutting by an RNA-guided DNA binding agent (e.g., Cas nuclease) at a site within human albumin intron 1 (SEQ ID NO: 1). It will be appreciated that, in some embodiments, the guide RNAs comprise guide sequences that bind to, or are capable of binding to, said regions.

[0081] In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOs: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOs: 2-5, 10-17, 21-27, and 29-33.

[0082] In some embodiments, the guide RNAs disclosed herein comprise a guide sequence having at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected

from the group consisting of a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID Nos: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NO: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 34, 40, 45, 51, 60, 61, 63, 64, 65, 66, 72, 77, 83, 92, 93, 95, 96, and 97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is selected from the group consisting of SEQ ID NOS: 34-97.

[0083] In some embodiments, the guide RNAs disclosed herein comprise a guide sequence having at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is selected from the group consisting of SEQ ID NOS: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97.

[0084] In some embodiments, the guide RNAs disclosed herein mediate a target-specific cutting resulting in a double-stranded break (DSB). In some embodiments, the guide RNAs disclosed herein mediate a target-specific cutting resulting in a single-stranded break (nick).

[0085] In some embodiments, the guide RNAs disclosed herein bind to a region upstream of a propospacer adjacent motif (PAM). As would be understood by those of skill in the art, the PAM sequence occurs on the strand opposite to the strand that contains the target sequence. That is, the PAM sequence is on the complement strand of the target strand (the strand that contains the target sequence to which the guide RNA binds). In some embodiments, the PAM is

selected from the group consisting of NGG, NNGRRT, NNGRR(N), NNAGAAW, NNNNG(A/C)TT, and NNNN-RYAC.

[0086] In some embodiments, the guide RNA sequences provided herein are complementary to a sequence adjacent to a PAM sequence.

[0087] In some embodiments, the guide RNA sequence comprises a sequence that is complementary to a sequence within a genomic region selected from the tables herein according to coordinates in human reference genome hg38. In some embodiments, the guide RNA sequence comprises a sequence that is complementary to a sequence that comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive nucleotides from within a genomic region selected from the tables herein. In some embodiments, the guide RNA sequence comprises a sequence that is complementary to a sequence that comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive nucleotides spanning a genomic region selected from the tables herein.

[0088] The guide RNAs disclosed herein mediate a target-specific cutting resulting in a double-stranded break (DSB). The guide RNAs disclosed herein mediate a target-specific cutting resulting in a single-stranded break (SSB or nick).

[0089] In some embodiments, the guide RNAs disclosed herein mediate target-specific cutting by an RNA-guided DNA binding agent (e.g., a Cas nuclease, as disclosed herein), resulting in insertion of a heterologous Factor IX nucleic acid within intron 1 of an albumin gene. In some embodiments, the guide RNA and/or cutting results in a rate of between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% insertion of a heterologous Factor IX gene. In some embodiments, the guide RNA and/or cutting results in a rate of at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% insertion of a heterologous Factor IX nucleic acid. Insertion rates can be measured in vitro or in vivo. For example, in some embodiments, rate of insertion can be determined by detecting and measuring the inserted Factor IX nucleic acid within a population of cells, and calculating a percentage of the population that contains the inserted Factor IX nucleic acid. Methods of measuring insertion rates are known and available in the art. In some embodiments, the guide RNA allows between 5 and 10%, 10 and 15%, 15 and 20%, 20 and 25%, 25 and 30%, 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, 95 and 99% or more increased expression of a heterologous Factor IX gene. Increased expression of a heterologous Factor IX gene can be measured in vitro or in vivo. For example, in some embodiments, increased expression can be determined by detecting and measuring the Factor IX polypeptide level and comparing the level against the Factor IX polypeptide level before, e.g., treating the cells or administration to a subject. In some embodiments, the guide RNA allows between 5 and 10%, 10 and 15%, 15 and 20%, 20 and 25%, 25 and 30%, 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, 95 and 99% or more increased activity that results from expression of a heterologous Factor IX gene. For example, increased

activity can be determined by detecting and measuring the coagulation activity and comparing the activity against the the coagulation activity before, e.g., treating the cells or administration to a subject. In some embodiments, increased activity can be determined using by assessing clotting function in an aPTT assay and/or thrombin generation in an TGA-EA assay. Such methods are available and known in the art (e.g. Simioni et al, NEJM 2009).

[0090] Each of the guide sequences shown in Table 1 at SEQ ID NOS:2-33 may further comprise additional nucleotides to form a crRNA and/or guide RNA, e.g., with the following exemplary nucleotide sequence following the guide sequence at its 3' end: GUUUUAGAGCUAGUUAAAUAAGGCUAGUCCGUUAUC (SEQ ID NO: 400) in 5' to 3' orientation. Genomic coordinates are according to human reference genome hg38. In the case of a sgRNA, the above guide sequences may further comprise additional nucleotides to form a sgRNA, e.g., with the following exemplary nucleotide sequence following the 3' end of the guide sequence:

(SEQ ID NO: 401)
GUUUUAGAGCUAGAAAUAAGCAAGUUAAAUAAGGCUAGUCCGUUAUC
AACUUGAAAAAGUGGCACCGAGUCGGUCUUUU
or

(SEQ ID NO: 402)
GUUUUAGAGCUAGAAAUAAGCAAGUUAAAUAAGGCUAGUCCGUUAUC
AACUUGAAAAAGUGGCACCGAGUCGGUGC in
5' to 3' orientation.

[0091] Each of the guide sequences in Table 1 at SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33 may further comprise additional nucleotides to form a crRNA, e.g., with the following exemplary nucleotide sequence following the guide sequence at its 3' end: GUUUUAGAGCUAGCUAUGCU-GUUUG (SEQ ID NO: 400) in 5' to 3' orientation. In the case of a sgRNA, the above guide sequences may further comprise additional nucleotides to form a sgRNA, e.g., with the following exemplary nucleotide sequence following the 3' end of the guide sequence:

(SEQ ID NO: 401)
GUUUUAGAGCUAGAAAUAAGCAAGUUAAAUAAGGCUAGUCCGUUAUC
AACUUGAAAAAGUGGCACCGAGUCGGUCUUUU
or

(SEQ ID NO: 402)
GUUUUAGAGCUAGAAAUAAGCAAGUUAAAUAAGGCUAGUCCGUUAUC
AACUUGAAAAAGUGGCACCGAGUCGGUGC in
5' to 3' orientation.

TABLE 1

TABLE 1-continued

		Human guide RNA sequences and chromosomal coordinates	
Guide ID	Guide Sequence	Human Genomic Coordinates (hg38)	SEQ ID NO:
G009857	AUUUAUGAGA UCAACAGCAC	chr4: 73404761- 73404781	5
G009858	GAUCAACAGC ACAGGUUUG	chr4: 73404753- 73404773	6
G009859	UUAAAUAAG CAUAGUGCAA	chr4: 73404727- 73404747	7
G009860	UAAAGCAUAG UGCAAUGGAU	chr4: 73404722- 73404742	8
G009861	UAGUGCAAUG GAUAGGUCUU	chr4: 73404715- 73404735	9
G009866	UACAAAACU UUAUUUUACU	chr4: 73404452- 73404472	10
G009867	AAAGUUGAAC AAUAGAAAAA	chr4: 73404418- 73404438	11
G009868	AAUGCAUAAU CUAAGUCAAA	chr4: 73405013- 73405033	12
G009874	UAAUAAAUU CAAACAUCCU	chr4: 73404561- 73404581	13
G012747	GCAUCUUAAA AGAAUUAUUU	chr4: 73404478- 73404498	14
G012748	UUUGGCAUUU AUUUCAAAAA	chr4: 73404496- 73404516	15
G012749	UGUAUUUUGUG AAGUCUUACA	chr4: 73404529- 73404549	16
G012750	UCCUAGGUAA AAAAAAAAAA	chr4: 73404577- 73404597	17
G012751	UAAAAUUUCUU UUGCGCACUA	chr4: 73404620- 73404640	18
G012752	UGACUGAAC UUCACAGAAU	chr4: 73404664- 73404684	19
G012753	GACUGAAACU UCACAGAAUA	chr4: 73404665- 73404685	20
G012754	UUCAUUUAG UCUGUCUUUC	chr4: 73404803- 73404823	21
G012755	AUUACUAAAG UUUGAAUUA	chr4: 73404859- 73404879	22
G012756	AAUUUUUAAA AUAGUAUUCU	chr4: 73404897- 73404917	23
G012757	UGAAUUAUUC UUCUGUUAAA	chr4: 73404924- 73404944	24
G012758	AUCAUCCUGA GUUUUUCUGU	chr4: 73404965- 73404985	25
G012759	UUACUAAAAC UUUAAAUAAC	chr4: 73404453- 73404473	26
G012760	ACCUUUUUUU UUUUUUACCU	chr4: 73404581- 73404601	27

TABLE 1-continued

Human guide RNA sequences and chromosomal coordinates		Human Genomic Coordinates (hg38)	SEQ ID NO.
Guide ID	Guide Sequence		
G012761	AGUGCAAUAGG AUAGGUUU	chr4: 73404714- 73404734	28
G012762	UGAUUCCUAC AGAAAAACUC	chr4: 73404973- 73404993	29
G012763	UGGGCAAGGG AAGAAAAAAA	chr4: 73405094- 73405114	30
G012764	CCUCACUCUU GUCUGGGCAA	chr4: 73405107- 73405127	31
G012765	ACCUCACUCU UGUCUGGGCA	chr4: 73405108- 73405128	32
G012766	UGAGCAACCU CACCUUGUC	chr4: 73405114- 73405134	33

[0092] The guide RNA may further comprise a trRNA. In each composition and method embodiment described herein, the crRNA and trRNA may be associated as a single RNA (sgRNA) or may be on separate RNAs (dgRNA). In the context of sgRNAs, the crRNA and trRNA components may be covalently linked, e.g., via a phosphodiester bond or other covalent bond. In some embodiments, the sgRNA comprises one or more linkages between nucleotides that is not a phosphodiester linkage.

[0093] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise two RNA molecules as a “dual guide RNA” or “dgRNA”. The dgRNA comprises a first RNA molecule comprising a crRNA comprising, e.g., a guide sequence shown in Table 1, and a second RNA molecule comprising a trRNA. The first and second RNA molecules may not be covalently linked, but may form a RNA duplex via the base pairing between portions of the crRNA and the trRNA.

[0094] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise a single RNA molecule as a “single guide RNA” or “sgRNA”. The sgRNA may comprise a crRNA (or a portion thereof) comprising a guide sequence shown in Table 1 covalently linked to a trRNA. The sgRNA may comprise 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the crRNA and the trRNA are covalently linked via a linker. In some embodiments, the sgRNA forms a stem-loop structure via the base pairing between portions of the crRNA and the trRNA. In some embodiments, the crRNA and the trRNA are covalently linked via one or more bonds that are not a phosphodiester bond.

[0095] In some embodiments, the trRNA may comprise all or a portion of a trRNA sequence derived from a naturally-occurring CRISPR/Cas system. In some embodiments, the trRNA comprises a truncated or modified wild type trRNA. The length of the trRNA depends on the CRISPR/Cas system used. In some embodiments, the trRNA comprises or consists of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or more than 100 nucleotides. In some embodiments, the trRNA may com-

prise certain secondary structures, such as, for example, one or more hairpin or stem-loop structures, or one or more bulge structures.

[0096] In some embodiments, the target sequence or region within intron 1 of a human albumin locus (SEQ ID NO: 1) may be complementary to the guide sequence of the guide RNA. In some embodiments, the degree of complementarity or identity between a guide sequence of a guide RNA and its corresponding target sequence may be at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the target sequence and the guide sequence of the gRNA may be 100% complementary or identical. In other embodiments, the target sequence and the guide sequence of the gRNA may contain at least one mismatch. For example, the target sequence and the guide sequence of the gRNA may contain 1, 2, 3, 4, or 5 mismatches, where the total length of the guide sequence is about 20, or 20. In some embodiments, the target sequence and the guide sequence of the gRNA may contain 1-4 mismatches where the guide sequence is about 20, or 20 nucleotides.

[0097] In some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding an RNA-guided DNA binding agent, such as a Cas nuclease as described herein. In some embodiments, an mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, is provided, used, or administered.

B. Modified gRNAs and mRNAs

[0098] In some embodiments, the gRNA is chemically modified. A gRNA comprising one or more modified nucleosides or nucleotides is called a “modified” gRNA or “chemically modified” gRNA, to describe the presence of one or more non-naturally and/or naturally occurring components or configurations that are used instead of or in addition to the canonical A, G, C, and U residues. In some embodiments, a modified gRNA is synthesized with a non-canonical nucleoside or nucleotide, is here called “modified.” Modified nucleosides and nucleotides can include one or more of: (i) alteration, e.g., replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage (an exemplary backbone modification); (ii) alteration, e.g., replacement, of a constituent of the ribose sugar, e.g., of the 2' hydroxyl on the ribose sugar (an exemplary sugar modification); (iii) wholesale replacement of the phosphate moiety with “dephospho” linkers (an exemplary backbone modification); (iv) modification or replacement of a naturally occurring nucleobase, including with a non-canonical nucleobase (an exemplary base modification); (v) replacement or modification of the ribose-phosphate backbone (an exemplary backbone modification); (vi) modification of the 3' end or 5' end of the oligonucleotide, e.g., removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, cap or linker (such 3' or 5' cap modifications may comprise a sugar and/or backbone modification); and (vii) modification or replacement of the sugar (an exemplary sugar modification).

[0099] Chemical modifications such as those listed above can be combined to provide modified gRNAs and/or mRNAs comprising nucleosides and nucleotides (collectively “residues”) that can have two, three, four, or more modifications. For example, a modified residue can have a modified sugar and a modified nucleobase. In some embodi-

ments, every base of a gRNA is modified, e.g., all bases have a modified phosphate group, such as a phosphorothioate group. In certain embodiments, all, or substantially all, of the phosphate groups of an gRNA molecule are replaced with phosphorothioate groups. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 5' end of the RNA. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 3' end of the RNA. Certain gRNAs comprise at least one modified residue at or near the 5' end and 3' end of the RNA.

[0100] In some embodiments, the gRNA comprises one, two, three or more modified residues. In some embodiments, at least 5% (e.g., at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the positions in a modified gRNA are modified nucleosides or nucleotides.

[0101] Unmodified nucleic acids can be prone to degradation by, e.g., intracellular nucleases or those found in serum. For example, nucleases can hydrolyze nucleic acid phosphodiester bonds. Accordingly, in one aspect the gRNAs described herein can contain one or more modified nucleosides or nucleotides, e.g., to introduce stability toward intracellular or serum-based nucleases. In some embodiments, the modified gRNA molecules described herein can exhibit a reduced innate immune response when introduced into a population of cells, both *in vivo* and *ex vivo*. The term “innate immune response” includes a cellular response to exogenous nucleic acids, including single stranded nucleic acids, which involves the induction of cytokine expression and release, particularly the interferons, and cell death.

[0102] In some embodiments of a backbone modification, the phosphate group of a modified residue can be modified by replacing one or more of the oxygens with a different substituent. Further, the modified residue, e.g., modified residue present in a modified nucleic acid, can include the wholesale replacement of an unmodified phosphate moiety with a modified phosphate group as described herein. In some embodiments, the backbone modification of the phosphate backbone can include alterations that result in either an uncharged linker or a charged linker with unsymmetrical charge distribution.

[0103] Examples of modified phosphate groups include, phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. The phosphorous atom in an unmodified phosphate group is achiral. However, replacement of one of the non-bridging oxygens with one of the above atoms or groups of atoms can render the phosphorous atom chiral. The stereogenic phosphorous atom can possess either the “R” configuration (herein Rp) or the “S” configuration (herein Sp). The backbone can also be modified by replacement of a bridging oxygen, (i.e., the oxygen that links the phosphate to the nucleoside), with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates) and carbon (bridged methylenephosphonates). The replacement can occur at either linking oxygen or at both of the linking oxygens.

[0104] The phosphate group can be replaced by non-phosphorus containing connectors in certain backbone modifications. In some embodiments, the charged phosphate group can be replaced by a neutral moiety. Examples of

moieties which can replace the phosphate group can include, without limitation, e.g., methyl phosphonate, hydroxylamino, siloxane, carbonate, carboxymethyl, carbamate, amide, thioether, ethylene oxide linker, sulfonate, sulfonamide, thioformacetal, formacetal, oxime, methyleneimino, methylenemethylimino, methylenehydrazo, methylenedimethylhydrazo and methyleneoxymethylimino.

[0105] Scaffolds that can mimic nucleic acids can also be constructed wherein the phosphate linker and ribose sugar are replaced by nuclease resistant nucleoside or nucleotide surrogates. Such modifications may comprise backbone and sugar modifications. In some embodiments, the nucleobases can be tethered by a surrogate backbone. Examples can include, without limitation, the morpholino, cyclobutyl, pyrrolidine and peptide nucleic acid (PNA) nucleoside surrogates.

[0106] The modified nucleosides and modified nucleotides can include one or more modifications to the sugar group, i.e. at sugar modification. For example, the 2' hydroxyl group (OH) can be modified, e.g. replaced with a number of different “oxy” or “deoxy” substituents. In some embodiments, modifications to the 2' hydroxyl group can enhance the stability of the nucleic acid since the hydroxyl can no longer be deprotonated to form a 2'-alkoxide ion.

[0107] Examples of 2' hydroxyl group modifications can include alkoxy or aryloxy (OR, wherein “R” can be, e.g., alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or a sugar); polyethyleneglycols (PEG), O(CH₂CH₂O)_nCH₂CH₂OR wherein R can be, e.g., H or optionally substituted alkyl, and n can be an integer from 0 to 20 (e.g., from 0 to 4, from 0 to 8, from 0 to 10, from 0 to 16, from 1 to 4, from 1 to 8, from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to 8, from 2 to 10, from 2 to 16, from 2 to 20, from 4 to 8, from 4 to 10, from 4 to 16, and from 4 to 20). In some embodiments, the 2' hydroxyl group modification can be 2'-O-Me. In some embodiments, the 2' hydroxyl group modification can be a 2'-fluoro modification, which replaces the 2' hydroxyl group with a fluoride. In some embodiments, the 2' hydroxyl group modification can be a 2'-H, which replaces the 2' hydroxyl group with a hydrogen. In some embodiments, the 2' hydroxyl group modification can include “locked” nucleic acids (LNA) in which the 2' hydroxyl can be connected, e.g., by a C₁₋₆ alkylene or C₁₋₆ heteroalkylene bridge, to the 4' carbon of the same ribose sugar, where exemplary bridges can include methylene, propylene, ether, or amino bridges; O-amino (wherein amino can be, e.g., NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylarnino, or diheteroarylarnino, ethylenediamine, or polyamino) and aminoalkoxy, O(CH₂)_n-amino, (wherein amino can be, e.g., NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylarnino, or diheteroarylarnino, ethylenediamine, or polyamino). In some embodiments, the 2' hydroxyl group modification can include “unlocked” nucleic acids (UNA) in which the ribose ring lacks the C2'-C3' bond. In some embodiments, the 2' hydroxyl group modification can include the methoxyethyl group (MOE), (OCH₂CH₂OCH₃, e.g., a PEG derivative).

[0108] “Deoxy” 2' modifications can include hydrogen (i.e. deoxyribose sugars, e.g., at the overhang portions of partially dsRNA); halo (e.g., bromo, chloro, fluoro, or iodo); amino (wherein amino can be, e.g., NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylarnino, diheteroarylarnino, or amino acid); NH(CH₂CH₂NH)_nCH₂CH₂— amino (wherein amino can

be, e.g., as described herein), —NHC(O)R (wherein R can be, e.g., alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), cyano; mercapto; alkyl-thio-alkyl; thioalkoxy; and alkyl, cycloalkyl, aryl, alkenyl and alkynyl, which may be optionally substituted with e.g., an amino as described herein.

[0109] The sugar modification can comprise a sugar group which may also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a modified nucleic acid can include nucleotides containing e.g., arabinose, as the sugar. The modified nucleic acids can also include abasic sugars. These abasic sugars can also be further modified at one or more of the constituent sugar atoms. The modified nucleic acids can also include one or more sugars that are in the L form, e.g. L-nucleosides.

[0110] The modified nucleosides and modified nucleotides described herein, which can be incorporated into a modified nucleic acid, can include a modified base, also called a nucleobase. Examples of nucleobases include, but are not limited to, adenine (A), guanine (G), cytosine (C), and uracil (U). These nucleobases can be modified or wholly replaced to provide modified residues that can be incorporated into modified nucleic acids. The nucleobase of the nucleotide can be independently selected from a purine, a pyrimidine, a purine analog, or pyrimidine analog. In some embodiments, the nucleobase can include, for example, naturally-occurring and synthetic derivatives of a base.

[0111] In embodiments employing a dual guide RNA, each of the crRNA and the tracr RNA can contain modifi-

cations. Such modifications may be at one or both ends of the crRNA and/or tracr RNA. In embodiments comprising an sgRNA, one or more residues at one or both ends of the sgRNA may be chemically modified, and/or internal nucleosides may be modified, and/or the entire sgRNA may be chemically modified. Certain embodiments comprise a 5' end modification. Certain embodiments comprise a 3' end modification.

[0112] In some embodiments, the guide RNAs disclosed herein comprise one of the modification patterns disclosed in WO2018/107028 A1, filed Dec. 8, 2017, titled “Chemically Modified Guide RNAs,” the contents of which are hereby incorporated by reference in their entirety. In some embodiments, the guide RNAs disclosed herein comprise one of the structures/modification patterns disclosed in US20170114334, the contents of which are hereby incorporated by reference in their entirety. In some embodiments, the guide RNAs disclosed herein comprise one of the structures/modification patterns disclosed in WO2017/136794, WO2017004279, US2018187186, US2019048338, the contents of which are hereby incorporated by reference in their entirety.

[0113] In some embodiments, the sgRNA of the present disclosure comprises the modification patterns shown below in Table 2. “Full Sequence” in Table 2 refers to an sgRNA sequence for each of the guides listed in Table 1. “Full Sequence Modified” shows a modification pattern for each sgRNA.

TABLE 2

sgRNA and modification patterns to sgRNA of human albumin guide sequences					
Guide ID	Full Sequence	SEQ ID NO:	Full Sequence Modified	SEQ ID NO:	
G009844	GAGCAACCUCACUCUUGUCUGUUUU AGAGCUAGAAAAGCAAGUUAAA AAGGCUGGUCCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	34	mG*mA*mG*CAACCUCACUCUUGUCUGU UUUAGAmGmCmUmAmGmAmAmUm AmGmCAAGUAAAUAAGGCUAGUCC GUUAUCAmAmCmUmUmGmAmAmAm AmGmUmGmGmCmAmCmCmGmAmGmUm CmGmGmUmGmCmU*mU*mU*mU	66	
G009851	AUGCAUUUGUUUCAAAAUAUGUUUU AGAGCUAGAAAAGCAAGUUAAA AAGGCUGGUCCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	35	mA*mU*mG*CAUUUGUUUCAAAAUAUG UUUAGAmGmCmUmAmGmAmAmUm AmGmCAAGUAAAUAAGGCUAGUCC UUUAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	67	
G009852	UGCAUUUGUUUCAAAAUAUGUUUU AGAGCUAGAAAAGCAAGUUAAA AAGGCUGGUCCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	36	mU*mG*mC*AUUUGUUUCAAAAUAUGU UUUAGAmGmCmUmAmGmAmAmAmUmAm GmCAAGUAAAUAAGGCUAGUCCGUUA UCAmAmCmUmUmGmAmAmAmAmGmUm GmGmCmAmCmCmGmAmGmUmCmGmUm GmCmU*mU*mU*mU	68	
G009857	AUUUAUGAGAUCAACAGCACGUUUU AGAGCUAGAAAAGCAAGUUAAA AAGGCUGGUCCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	37	mA*mU*mU*UAUGAGAUCAACAGCACGU UUUAGAmGmCmUmAmGmAmAmAmUmAm GmCAAGUAAAUAAGGCUAGUCCGUUA UCAmAmCmUmUmGmAmAmAmAmAmGm UmGmGmCmAmCmCmGmAmGmUmCmGmUm GmGmCmU*mU*mU*mU	69	

TABLE 2 -continued

		sgRNA and modification patterns to sqRNA of human albumin guide sequences			
Guide ID	Full Sequence	SEQ ID NO:	Full Sequence	Modified	SEQ ID NO:
G009858	GAUCAACAGCACAGGUUUUGGUUU AGAGCUAGAAAAGCAAGGUAAAA AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	38	mG*mA*mU*CAACAGCACAGGUUUUGGU UUUAGAmGmCmUmAmGmAmAmUmAm GmCAAGGUAAAAGGUAGUCGUUA UCAmAmCmUmUmGmAmAmAmAmGm UmGmGmCmAmCmCmGmAmGmUmCmGm GmUmGmCmU*mU*mU*mU		70
G009859	UUAAAAGCAUAGUGCAAGGUUU AGAGCUAGAAAAGCAAGGUAAAA AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	39	mU*mU*mA*AAUAAAGCAUAGUGCAAGGU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGGUAAAAGGUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU		71
G009860	UAAAGCAUAGUGCAAUGGAUGUUUU AGAGCUAGAAAAGCAAGGUAAAA AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	40	mU*mA*mA*AGCAUAGUGCAAUGGAUGUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGGUAAAAGGUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU		72
G009861	UAGUGCAAUGGUAGGUUAGGUUU AGAGCUAGAAAAGCAAGGUAAAA AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	41	mU*mA*mG*UGCAAUGGUAGGUUAGGU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGGUAAAAGGUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU		73
G009866	UACUAAAACUUUACUUUACUGUUUU AGAGCUAGAAAAGCAAGGUAAAA AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	42	mU*mA*mC*UAAAACUUUACUUUACUGUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGGUAAAAGGUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU		74
G009867	AAAGGUAGAACAAUAGAAAAAGUUUU AGAGCUAGAAAAGCAAGGUAAAA AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	43	mA*mA*mA*GUUGAACAAUAGAAAAAGUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGGUAAAAGGUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU		75
G009868	AAUGCAUAAUCUAAGUCAAAGGUUU AGAGCUAGAAAAGCAAGGUAAAA AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	44	mA*mA*mU*GCAUAAUCUAAGUCAAAGUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGGUAAAAGGUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU		76
G009874	UAAAUAUUAACAAACAUCCUGUUUU AGAGCUAGAAAAGCAAGGUAAAA AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	45	mU*mA*mA*UAAAUAUCAACAUCCUGUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGGUAAAAGGUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU		77
G012747	GCAUCUUAAAAGAAUUAUUGUUUU AGAGCUAGAAAAGCAAGGUAAAA AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	46	mG*mC*mA*UCUUAAAAGAAUUAUUGUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGGUAAAAGGUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU		78
G012748	UUUGGCAUUUACUUUACUUAAAAGUUUU AGAGCUAGAAAAGCAAGGUAAAA AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	47	mU*mU*mU*GGCAUUUACUUAAAAGUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGGUAAAAGGUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmGmUmGmCm U*mU*mU*mU		79

TABLE 2 -continued

		sgRNA and modification patterns to sqRNA of human albumin guide sequences			
Guide ID	Full Sequence	SEQ ID NO:	Full Sequence	Modified	SEQ ID NO:
G012749	UGUAUUUGUGAAGUCUUACAGUUUU AGAGCUAGAAAUGCAGUAAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	48	mU*mG*mU*AUUUGUGAAGUCUUACAGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		80
G012750	UCCUAGGUAAAAAAAAAGUUUU AGAGCUAGAAAUGCAGUAAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	49	mU*mC*mC*UAGGUAAAAAAAAAGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		81
G012751	UAUUUUUUUUUGCGCACUAGUUUU AGAGCUAGAAAUGCAGUAAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	50	mU*mA*mA*UUUUUUUUUGCGCACUAGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		82
G012752	UGACUGAAACUUCACAGAAUGUUUU AGAGCUAGAAAUGCAGUAAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	51	mU*mG*mA*CUGAACUUUCACAGAAUGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		83
G012753	GACUGAAACUUCACAGAAUAGUUUU AGAGCUAGAAAUGCAGUAAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	52	mG*mA*mC*UGAACUUUCACAGAAUAGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		84
G012754	UUCAUUUUAGCUGUCUUUCUGUUUU AGAGCUAGAAAUGCAGUAAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	53	mU*mU*mC*AUUUUAGUCUGCUUCUGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		85
G012755	AUUAUCUAAGUUUGAAUUAUGUUUU AGAGCUAGAAAUGCAGUAAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	54	mA*mU*mU*AUCUAAGUUUGAAUUAUGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		86
G012756	AAUUUUUUAAAUAGUAAUCUGUUUU AGAGCUAGAAAUGCAGUAAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	55	mA*mA*mU*UUUUUUAAAAGUAUUUCUGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		87
G012757	UGAAUUUUUUUCUUCUGUUUAAGUUUU AGAGCUAGAAAUGCAGUAAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	56	mU*mG*mA*AUUAUUUUUCUGUUUAAGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		88
G012758	AUCAUCCUGAGUUUUUCUGUGUUUU AGAGCUAGAAAUGCAGUAAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	57	mA*mU*mC*AUCCUGAGUUUUUCUGUGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		89

TABLE 2 -continued

Guide ID	Full Sequence	sgRNA and modification patterns to sqRNA of human albumin guide sequences			SEQ ID NO:
		SEQ ID NO:	Full Sequence	Modified	
G012759	UUACUAAAACUUUAAAAACGUUUU AGAGCUAGAAAUAAGCAAGGUAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	58	mU*mU*mA*CUAAAACUUUAAAAACGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		90
G012760	ACCUUUUUUUUUUUUUUACCUGUUUU AGAGCUAGAAAUAAGCAAGGUAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	59	mA*mC*mC*UUUUUUUUUUUUACCUGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		91
G012761	AGUGCAAUGGAUAGGUUUUUGUUUU AGAGCUAGAAAUAAGCAAGGUAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	60	mA*mG*mU*GCAAUGGAUAGGUUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		92
G012762	UGAUUCCUACAGAAAAACUCGUUUU AGAGCUAGAAAUAAGCAAGGUAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	61	mU*mG*mA*UUCCUACAGAAAAACUCGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		93
G012763	UGGGCAAGGGAGAGAAAAAGUUUU AGAGCUAGAAAUAAGCAAGGUAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	62	mU*mG*mG*GCAAGGGAGAGAAAAAGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		94
G012764	CCUCACUCUUGUCUGGGCAAGUUUU AGAGCUAGAAAUAAGCAAGGUAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	63	mC*mC*mU*CACUCUUGUCUGGGCAAGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		95
G012765	ACCUCACUCUUGUCUGGGCAGUUUU AGAGCUAGAAAUAAGCAAGGUAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	64	mA*mC*mC*UACUCUUGUCUGGGCAGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		96
G012766	UGAGCAACCUCACUCUUGUCGUUUU AGAGCUAGAAAUAAGCAAGGUAAAAU AAGGCUAGUCGUUAUCAACUUGAA AAAGUGGCACCGAGUCGGUGCUUUU	65	mU*mG*mA*GCAACCUCACUCUUGUCGUUU UAGAmGmCmUmAmGmAmAmUmAmGmC AAAGUUAAAUAAGGCUAGUCGUUAUCAm AmCmUmUmGmAmAmAmAmAmGmUmGmGm CmAmCmCmGmAmGmUmCmGmUmGmCm U*mU*mU*mU		97

[0114] In some embodiments, the modified sgRNA comprises the following sequence: mN*mN*mN*NNNNNNNNNNNNNNNNNNGUUUU AGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAUAAGGCUAGUCGUUAUCAm mAmCmUmUmGmAmAmAmAm GmUmGmGmC mAmCmCmGmAmGmUmCmGmGm UmGmCmU*mU*mU*mU (SEQ ID NO: 300), where "N" may be any natural or non-natural nucleotide, and wherein the totality of N's comprise an albumin intron 1 guide

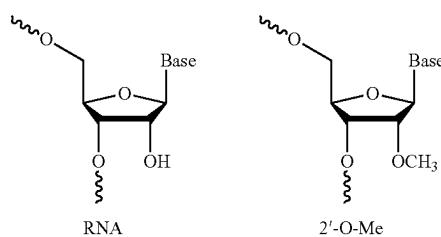
sequence as described in Table 1. For example, encompassed herein is SEQ ID NO: 300, where the N's are replaced with any of the guide sequences disclosed herein in Table 1 (SEQ ID Nos: 2-33).

[0115] For example, encompassed herein is SEQ ID NO: 300, where the N's are replaced with any of the guide sequences disclosed herein in Table 1 (SEQ ID NOs: 2-5, 10-17, 21-27, and 29-33).

[0116] Any of the modifications described below may be present in the gRNAs and mRNAs described herein.

[0117] The terms “mA,” “mC,” “mU,” or “mG” may be used to denote a nucleotide that has been modified with 2'-O-Me.

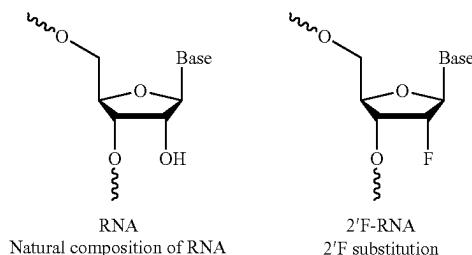
[0118] Modification of 2'-O-methyl can be depicted as follows:



[0119] Another chemical modification that has been shown to influence nucleotide sugar rings is halogen substitution. For example, 2'-fluoro (2'-F) substitution on nucleotide sugar rings can increase oligonucleotide binding affinity and nuclease stability.

[0120] In this application, the terms “fA,” “fC,” “fU,” or “fG” may be used to denote a nucleotide that has been substituted with 2'-F.

[0121] Substitution of 2'-F can be depicted as follows:

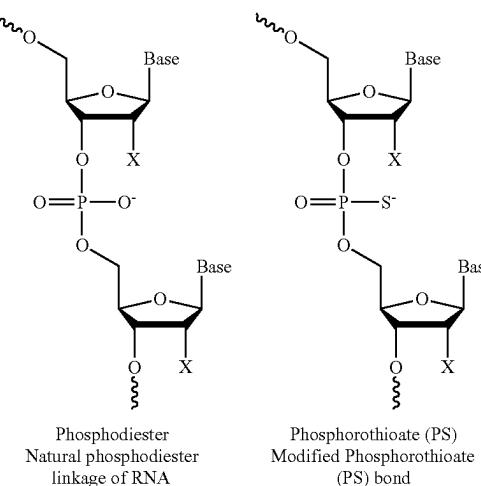


[0122] Phosphorothioate (PS) linkage or bond refers to a bond where a sulfur is substituted for one nonbridging phosphate oxygen in a phosphodiester linkage, for example in the bonds between nucleotides bases. When phosphorothioates are used to generate oligonucleotides, the modified oligonucleotides may also be referred to as S-oligos.

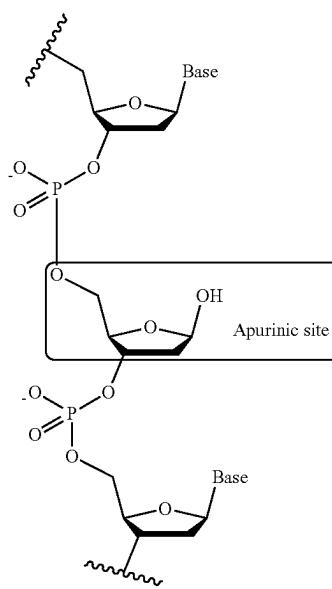
[0123] A “*” may be used to depict a PS modification. In this application, the terms A*, C*, U*, or G* may be used to denote a nucleotide that is linked to the next (e.g., 3') nucleotide with a PS bond.

[0124] In this application, the terms “mA*,” “mC*,” “mU*,” or “mG*” may be used to denote a nucleotide that has been substituted with 2'-O-Me and that is linked to the next (e.g., 3') nucleotide with a PS bond.

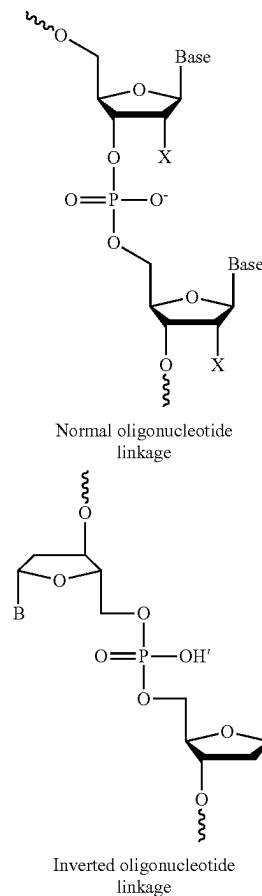
[0125] The diagram below shows the substitution of S— into a nonbridging phosphate oxygen, generating a PS bond in lieu of a phosphodiester bond:



[0126] A basic nucleotides refer to those which lack nitrogenous bases. The figure below depicts an oligonucleotide with an abasic (also known as apurinic) site that lacks a base:



[0127] Inverted bases refer to those with linkages that are inverted from the normal 5' to 3' linkage (i.e., either a 5' to 5' linkage or a 3' to 3' linkage). For example:



[0128] An abasic nucleotide can be attached with an inverted linkage. For example, an abasic nucleotide may be attached to the terminal 5' nucleotide via a 5' to 5' linkage, or an abasic nucleotide may be attached to the terminal 3' nucleotide via a 3' to 3' linkage. An inverted abasic nucleotide at either the terminal 5' or 3' nucleotide may also be called an inverted abasic end cap.

[0129] In some embodiments, one or more of the first three, four, or five nucleotides at the 5' terminus, and one or more of the last three, four, or five nucleotides at the 3' terminus are modified. In some embodiments, the modification is a 2'-O-Me, 2'-F, inverted abasic nucleotide, PS bond, or other nucleotide modification well known in the art to increase stability and/or performance.

[0130] In some embodiments, the first four nucleotides at the 5' terminus, and the last four nucleotides at the 3' terminus are linked with phosphorothioate (PS) bonds.

[0131] In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise a 2'-O-methyl (2'-O-Me) modified nucleotide. In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise a 2'-fluoro (2'-F) modified nucleotide. In some embodiments, the first three nucleotides at the 5'

terminus, and the last three nucleotides at the 3' terminus comprise an inverted abasic nucleotide.

[0132] In some embodiments, the guide RNA comprises a modified sgRNA. In some embodiments, the sgRNA comprises the modification pattern shown in SEQ ID No: 300, where N is any natural or non-natural nucleotide, and where the totality of the N's comprise a guide sequence that directs a nuclease to a target sequence in human albumin intron 1, e.g., as shown in Table 1.

[0133] In some embodiments, the guide RNA comprises a sgRNA shown in any one of SEQ ID No: 34-97. In some embodiments, the guide RNA comprises a sgRNA comprising any one of the guide sequences of SEQ ID No: 2-33 and the nucleotides of SEQ ID No: 300 wherein the nucleotides of SEQ ID No: 300 are on the 3' end of the guide sequence, and wherein the sgRNA may be modified, e.g., as shown in SEQ ID NO: 300.

[0134] In some embodiments, the guide RNA comprises a sgRNA shown in any one of SEQ ID NOs: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97. In some embodiments, the guide RNA comprises a sgRNA comprising any one of the guide sequences of SEQ ID NOs: 2-5, 10-17, 21-27, and 29-33 and the nucleotides of SEQ ID No: 300 wherein the nucleotides of SEQ ID NO: 300 are on the 3' end of the guide sequence, and wherein the sgRNA may be modified, e.g., as shown in SEQ ID NO: 300.

[0135] As noted above, in some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding an RNA-guided DNA binding agent, such as a Cas nuclease as described herein. In some embodiments, an mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, is provided, used, or administered. As described below, the mRNA comprising a Cas nuclease may comprise a Cas9 nuclease, such as an *S. pyogenes* Cas9 nuclease having cleavage, nickase, and/or site-specific DNA binding activity. In some embodiments, the ORF encoding an RNA-guided DNA nuclease is a “modified RNA-guided DNA binding agent ORF” or simply a “modified ORF,” which is used as shorthand to indicate that the ORF is modified.

[0136] Cas9 ORFs, including modified Cas9 ORFs, are provided herein and are known in the art. As one example, the Cas9 ORF can be codon optimized, such that coding sequence includes one or more alternative codons for one or more amino acids. An “alternative codon” as used herein refers to variations in codon usage for a given amino acid, and may or may not be a preferred or optimized codon (codon optimized) for a given expression system. Preferred codon usage, or codons that are well-tolerated in a given system of expression, is known in the art. The Cas9 coding sequences, Cas9 mRNAs, and Cas9 protein sequences of WO2013/176772, WO2014/065596, WO2016/106121, and WO2019/067910 are hereby incorporated by reference. In particular, the ORFs and Cas9 amino acid sequences of the table at paragraph [0449] WO2019/067910, and the Cas9 mRNAs and ORFs of paragraphs [0214]-[0234] of WO2019/067910 are hereby incorporated by reference.

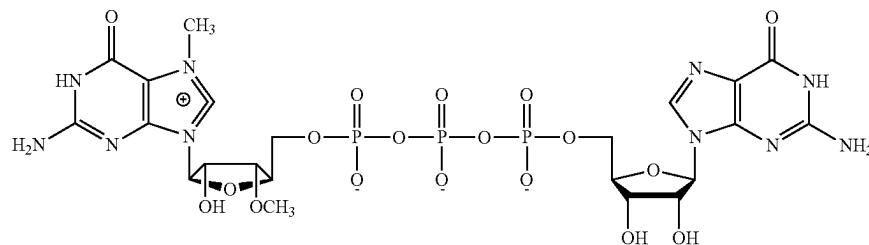
[0137] In some embodiments, the modified ORF may comprise a modified uridine at least at one, a plurality of, or all uridine positions. In some embodiments, the modified uridine is a uridine modified at the 5 position, e.g., with a halogen, methyl, or ethyl. In some embodiments, the modified uridine is a pseudouridine modified at the 1 position, e.g., with a halogen, methyl, or ethyl. The modified uridine can be, for example, pseudouridine, N1-methyl-pseudouridine, 5-methoxyuridine, 5-iodouridine, or a combination thereof. In some embodiments, the modified uridine is

5-methoxyuridine. In some embodiments, the modified uridine is 5-iodouridine. In some embodiments, the modified uridine is pseudouridine. In some embodiments, the modified uridine is N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and 5-methoxyuridine. In some embodiments, the modified uridine is a combination of N1-methyl pseudouridine and 5-methoxyuridine. In some embodiments, the modified uridine is a combination of 5-iodouridine and N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and 5-iodouridine. In some embodiments, the modified uridine is a combination of 5-iodouridine and 5-methoxyuridine.

[0138] In some embodiments, an mRNA disclosed herein comprises a 5' cap, such as a Cap0, Cap1, or Cap2. A 5' cap is generally a 7-methylguanine ribonucleotide (which may be further modified, as discussed below e.g. with respect to ARCA) linked through a 5'-triphosphate to the 5' position of the first nucleotide of the 5'-to-3' chain of the mRNA, i.e., the first cap-proximal nucleotide. In Cap0, the riboses of the first and second cap-proximal nucleotides of the mRNA both

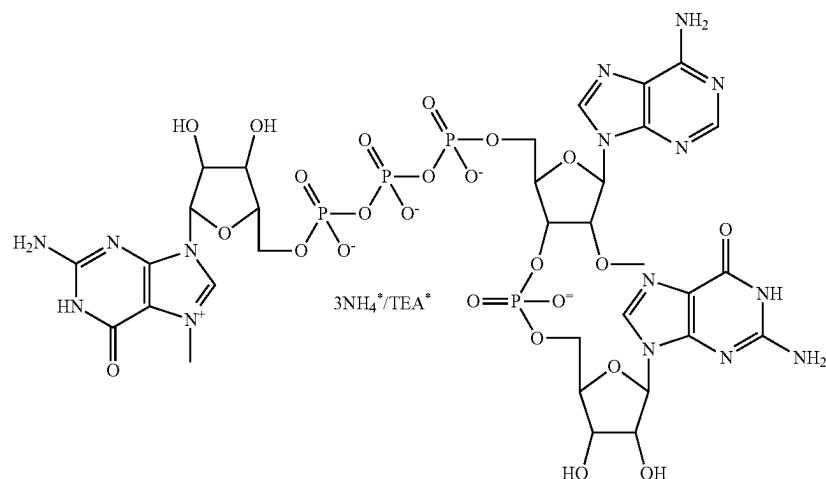
mammalian mRNAs such as human mRNAs, comprise Cap1 or Cap2. Cap0 and other cap structures differing from Cap1 and Cap2 may be immunogenic in mammals, such as humans, due to recognition as "non-self" by components of the innate immune system such as IFIT-1 and IFIT-5, which can result in elevated cytokine levels including type I interferon. Components of the innate immune system such as IFIT-1 and IFIT-5 may also compete with eIF4E for binding of an mRNA with a cap other than Cap1 or Cap2, potentially inhibiting translation of the mRNA.

[0139] A cap can be included co-transcriptionally. For example, ARCA (anti-reverse cap analog; Thermo Fisher Scientific Cat. No. AM8045) is a cap analog comprising a 7-methylguanine 3'-methoxy-5'-triphosphate linked to the 5' position of a guanine ribonucleotide which can be incorporated in vitro into a transcript at initiation. ARCA results in a Cap0 cap in which the 2' position of the first cap-proximal nucleotide is hydroxyl. See, e.g., Stepinski et al., (2001) "Synthesis and properties of mRNAs containing the novel 'anti-reverse' cap analogs 7-methyl(3'-O-methyl)GpppG and 7-methyl(3'deoxy)GpppG," *RNA* 7: 1486-1495. The ARCA structure is shown below.



comprise a 2'-hydroxyl. In Cap1, the riboses of the first and second transcribed nucleotides of the mRNA comprise a 2'-methoxy and a 2'-hydroxyl, respectively. In Cap2, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-methoxy. See, e.g., Katibah et al. (2014) *Proc Natl Acad Sci USA* 111(33):12025-30; Abbas et al. (2017) *Proc Natl Acad Sci USA* 114(11):E2106-E2115. Most endogenous higher eukaryotic mRNAs, including

[0140] CleanCap™ AG (m7G(5')ppp(5')(2'OMeA)pG; TriLink Biotechnologies Cat. No. N-7113) or CleanCap™ GG (m7G(5')ppp(5')(2'OMeG)pG; TriLink Biotechnologies Cat. No. N-7133) can be used to provide a Cap1 structure co-transcriptionally. 3'-O-methylated versions of CleanCap™ AG and CleanCap™ GG are also available from TriLink Biotechnologies as Cat. Nos. N-7413 and N-7433, respectively. The CleanCap™ AG structure is shown below.



[0141] Alternatively, a cap can be added to an RNA post-transcriptionally. For example, Vaccinia capping enzyme is commercially available (New England Biolabs Cat. No. M2080S) and has RNA triphosphatase and guanyltransferase activities, provided by its D1 subunit, and guanine methyltransferase, provided by its D12 subunit. As such, it can add a 7-methylguanine to an RNA, so as to give Cap0, in the presence of S-adenosyl methionine and GTP. See, e.g., Guo, P. and Moss, B. (1990) *Proc. Natl. Acad. Sci. USA* 87, 4023-4027; Mao, X. and Shuman, S. (1994) *J. Biol. Chem.* 269, 24472-24479.

[0142] In some embodiments, the mRNA further comprises a poly-adenylated (poly-A) tail. In some embodiments, the poly-A tail comprises at least 20, 30, 40, 50, 60, 70, 80, 90, or 100 adenines, optionally up to 300 adenines. In some embodiments, the poly-A tail comprises 95, 96, 97, 98, 99, or 100 adenine nucleotides.

C. Donor Constructs

[0143] The compositions and methods described herein include the use of a nucleic acid construct that comprises a sequence encoding a heterologous Factor IX gene to be inserted into a cut site created by a guide RNA of the present disclosure and an RNA-guided DNA binding agent. As used herein, such a construct is sometimes referred to as a “donor construct/template”. In some embodiments, the construct is a DNA construct. Methods of designing and making various functional/structural modifications to donor constructs are known in the art. In some embodiments, the construct may comprise any one or more of a polyadenylation tail sequence, a polyadenylation signal sequence, splice acceptor site, or selectable marker. In some embodiments, the polyadenylation tail sequence is encoded, e.g., as a “poly-A” stretch, at the 3' end of the coding sequence. Methods of designing a suitable polyadenylation tail sequence and/or polyadenylation signal sequence are well known in the art. For example, the polyadenylation signal sequence AAUAAA (SEQ ID NO: 800) is commonly used in mammalian systems, although variants such as UAUAAA (SEQ ID NO: 801) or AU/GUAAA (SEQ ID NO: 802) have been identified. See, e.g., NJ Proudfoot, *Genes & Dev.* 25(17): 1770-82, 2011.

[0144] In some embodiments, the donor construct comprises a sequence encoding Factor IX, wherein the Factor IX sequence is wild type Factor IX, e.g., SEQ ID NO: 700. In some embodiments, the donor construct comprises a sequence encoding Factor IX, wherein the Factor IX sequence is wild type Factor IX, e.g., SEQ ID NO: 701. In some embodiments, the sequence encodes a variant of Factor IX. For example, the variant can possess increased coagulation activity than wild type Factor IX. For example, the variant Factor IX can comprise one or mutations, such as an amino acid substitution in position R338 (e.g., R338L), relative to SEQ ID NO: 701. In some embodiments, the sequence encodes a Factor IX variant that is 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, SEQ ID NO: 701, or SEQ ID NO: 702, having at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type Factor IX. In some embodiments, the sequence encodes a fragment of Factor IX, wherein the fragment possesses at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type Factor IX.

[0145] In some embodiments, the donor construct comprises a sequence encoding a Factor IX variant, wherein the Factor IX variant activates coagulation in the absence of its cofactor, Factor VIII. Such Factor IX variants can further maintain the activity of wild type Factor IX. Such Factor IX variants can be used to treat hemophilia, such as hemophilia B. For example, such a Factor IX variant can comprise an amino acid substitution at position L6, V181, K265, 1383, E185, or a combination thereof relative to wild type Factor IX (e.g., relative to SEQ ID NO: 701). For example, such a Factor IX variant can comprise an L6F mutation, a V181I mutation, a K265A mutation, an 1383V mutation, an E185D mutation, or a combination thereof relative to wild type Factor IX (e.g., relative to SEQ ID NO: 701).

[0146] In one example, the Factor IX protein can comprise amino acid substitutions at positions L6 and V181. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6 and K265. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6 and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6 and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181 and K265. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181 and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181 and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions K265 and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions K265 and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions 1383 and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, and K265. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, K265, and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, K265, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, 1383, and E186. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181, K265, and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181, K265, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181, K265, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181, 1383, and E186. In another example, the Factor IX protein can comprise amino acid substitutions at positions K265, 1383, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, K265, and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, K265, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, 1383, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, K265, 1383, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181, K265, 1383, and E185.

[0147] In a specific example, the Factor IX protein can comprise amino acid substitutions at positions V181, K265, and 1383. In another specific example, the Factor IX protein

can comprise amino acid substitutions at positions V181, K265, 1383, and E185. In another specific example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, K265, and 1383.

[0148] In one example, the Factor IX protein can comprise an L6F mutation and a V181I mutation. In another example, the Factor IX protein can comprise an L6F mutation and a K265A mutation. In another example, the Factor IX protein can comprise an L6F mutation and an I383V mutation. In another example, the Factor IX protein can comprise an L6F mutation and an E185D mutation. In another example, the Factor IX protein can comprise a V181I mutation and a K265A mutation. In another example, the Factor IX protein can comprise a V181I mutation and an I383V mutation. In another example, the Factor IX protein can comprise a V181I mutation and an E185D mutation. In another example, the Factor IX protein can comprise a K265A mutation and an E185D mutation. In another example, the Factor IX protein can comprise an I383V mutation and an E185D mutation. In another example, the Factor IX protein can comprise an L6F mutation, a V181I mutation, and a K265A mutation. In another example, the Factor IX protein can comprise an L6F mutation, a V181I mutation, and an I383V mutation. In another example, the Factor IX protein can comprise an L6F mutation, a K265A mutation, and an E185D mutation. In another example, the Factor IX protein can comprise an L6F mutation, an I383V mutation, and an E186D mutation. In another example, the Factor IX protein can comprise a V181I mutation, a K265A mutation, and an I383V mutation. In another example, the Factor IX protein can comprise a V181I mutation, a K265A mutation, and an E185D mutation. In another example, the Factor IX protein can comprise a V181I mutation, an I383V mutation, and an E186D mutation. In another example, the Factor IX protein can comprise a K265A mutation, an I383V mutation, and an E185D mutation. In another example, the Factor IX protein can comprise an L6F mutation, a V181I mutation, a K265A mutation, and an I383V mutation. In another example, the Factor IX protein can comprise an L6F mutation, a K265A mutation, an I383V mutation, and an E185D mutation. In some embodiments, the Factor IX variant is at least 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, having at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type Factor IX. In certain embodiments, the Factor IX variant is at least 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, having at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type

Factor IX and comprises a V181I mutation, a K265A mutation, an I383V mutation, and/or an E185D mutation. In another specific example, the Factor IX protein can comprise an L6F mutation, a V181I mutation, a K265A mutation, and an I383V mutation. In some embodiments, the Factor IX variant is at least 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, having at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type Factor IX and comprises an L6F mutation, a V181I mutation, a K265A mutation, and/or an I383V mutation.

[0150] The length of the construct can vary, depending on the size of the gene to be inserted, and can be, for example, from 200 base pairs (bp) to about 5000 bp, such as about 200 bp to about 2000 bp, such as about 500 bp to about 1500 bp. In some embodiments, the length of the DNA donor template is about 200 bp, or is about 500 bp, or is about 800 bp, or is about 1000 base pairs, or is about 1500 base pairs. In other embodiments, the length of the donor template is at least 200 bp, or is at least 500 bp, or is at least 800 bp, or is at least 1000 bp, or is at least 1500 bp. In other embodiments, the length of the donor template is at least 200 bp, or is at least 500 bp, or is at least 800 bp, or is at least 1000 bp, or is at least 1500 bp, or at least 2000, or at least 2500, or at least 3000, or at least 3500, or at least 4000, or at least 4500, or at least 5000.

[0151] The construct can be DNA or RNA, single-stranded, double-stranded or partially single- and partially double-stranded and can be introduced into a host cell in linear or circular (e.g., minicircle) form. See, e.g., U.S. Patent Publication Nos. 2010/0047805, 2011/0281361, 2011/0207221. If introduced in linear form, the ends of the donor sequence can be protected (e.g., from exonucleolytic degradation) by methods known to those of skill in the art. For example, one or more dideoxynucleotide residues are added to the 3' terminus of a linear molecule and/or self-complementary oligonucleotides are ligated to one or both ends. See, for example, Chang et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:4959-4963; Nehls et al. (1996) *Science* 272: 886-889. Additional methods for protecting exogenous polynucleotides from degradation include, but are not limited to, addition of terminal amino group(s) and the use of modified internucleotide linkages such as, for example, phosphorothioates, phosphoramidates, and O-methyl ribose or deoxyribose residues. A construct can be introduced into a cell as part of a vector molecule having additional sequences such as, for example, replication origins, promoters and genes encoding antibiotic resistance. A construct may omit viral elements. Moreover, donor constructs can be introduced as naked nucleic acid, as nucleic acid complexed with an agent such as a liposome or poloxamer, or can be delivered by viruses (e.g., adenovirus, AAV, herpesvirus, retrovirus, lentivirus).

[0152] In some embodiments, the construct may be inserted so that its expression is driven by the endogenous promoter at the insertion site (e.g., the endogenous albumin promoter when the donor is integrated into the host cell's albumin locus). In such cases, the transgene may lack control elements (e.g., promoter and/or enhancer) that drive its expression (e.g., a promoterless construct). Nonetheless, it will be apparent that in other cases the construct may comprise a promoter and/or enhancer, for example a constitutive promoter or an inducible or tissue specific (e.g., liver- or platelet-specific) promoter that drives expression of

[0149] In a specific example, the Factor IX protein can comprise a V181I mutation, an K265A mutation, and an I383V mutation. In another specific example, the Factor IX protein can comprise a V181I mutation, a K265A mutation, an I383V mutation, and an E185D mutation. In some embodiments, the Factor IX variant is at least 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, having at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type Factor IX. In certain embodiments, the Factor IX variant is at least 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, having at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type

the functional protein upon integration. The construct may comprise a sequence encoding a heterologous Factor IX protein downstream of and operably linked to a signal sequence encoding a signal peptide. In some embodiments, the nucleic acid construct works in homology-independent insertion of a nucleic acid that encodes a Factor IX protein. In some embodiments, the nucleic acid construct works in non-dividing cells, e.g., cells in which NHEJ, not HR, is the primary mechanism by which double-stranded DNA breaks are repaired. The nucleic acid may be a homology-independent donor construct.

[0153] Some donor constructs comprising a heterologous Factor IX nucleic acid (Factor IX transgene) are capable of insertion into a cut site in a target DNA sequence for a gene editing system (e.g., capable of insertion into a safe harbor gene, such as an albumin locus) by non-homologous end joining. In some cases, such constructs do not comprise homology arms. For example, such constructs can be inserted into a blunt end double-strand break following cleavage with a gene editing system (e.g., CRISPR/Cas system) as disclosed herein. In a specific example, the construct can be delivered via AAV and can be capable of insertion by non-homologous end joining (e.g., the construct can be one that does not comprise homology arms).

[0154] In a specific example, the construct can be inserted via homology-independent targeted integration. For example, the heterologous Factor IX nucleic acid in the construct can be flanked on each side by a target site for a gene editing system (e.g., the same target site as in the target DNA sequence for targeted insertion (e.g., in a safe harbor gene), and the same gene editing system being used to cleave the target DNA sequence for targeted insertion). The gene editing system can then cleave the target sites flanking the heterologous Factor IX nucleic acid. In a specific example, the construct is delivered AAV-mediated delivery, and cleavage of the target sites flanking the heterologous Factor IX nucleic acid can remove the inverted terminal repeats (ITRs) of the AAV. In some methods, the target DNA sequence for targeted insertion (e.g., target DNA sequence in a safe harbor locus, e.g., a gRNA target sequence including the flanking protospacer adjacent motif) is no longer present if the heterologous Factor IX nucleic acid is inserted into the cut site or target DNA sequence in the correct orientation but it is reformed if the heterologous Factor IX nucleic acid is inserted into the cut site or target DNA sequence in the opposite orientation. This can help ensure that the heterologous Factor IX nucleic acid is inserted in the correct orientation for expression.

[0155] Also described herein are bidirectional nucleic acid constructs that allow enhanced insertion and expression of a Factor IX gene. Briefly, various bidirectional constructs disclosed herein comprise at least two nucleic acid segments, wherein one segment (the first segment) comprises a coding sequence that encodes Factor IX (sometimes interchangeably referred to herein as “transgene”), while the other segment (the second segment) comprises a sequence wherein the complement of the sequence encodes Factor IX.

[0156] In one embodiment, a bidirectional construct comprise at least two nucleic acid segments in *cis*, wherein one segment (the first segment) comprises a coding sequence (sometimes interchangeably referred to herein as “transgene”), while the other segment (the second segment) comprises a sequence wherein the complement of the sequence encodes a transgene. The first transgene and the second

transgene may be the same or different. The bidirectional constructs may comprise at least two nucleic acid segments in *cis*, wherein one segment (the first segment) comprises a coding sequence that encodes a heterologous gene in one orientation, while the other segment (the second segment) comprises a sequence wherein its complement encodes the heterologous gene in the other orientation. That is, the first segment is a complement of the second segment (not necessarily a perfect complement); the complement of the second segment is the reverse complement of the first segment (not necessarily a perfect reverse complement though both encode the same heterologous protein). A bidirectional construct may comprise a first coding sequence that encodes a heterologous gene linked to a splice acceptor and a second coding sequence wherein the complement encodes a heterologous gene in the other orientation, also linked to a splice acceptor.

[0157] When used in combination with a gene editing system (e.g., CRISPR/Cas system; zinc finger nuclease (ZFN) system; transcription activator-like effector nuclease (TALEN) system) as described herein, the bidirectionality of the nucleic acid constructs allows the construct to be inserted in either direction (is not limited to insertion in one direction) within a target insertion site, allowing the expression of Factor IX from either a) a coding sequence of one segment (e.g., the left segment encoding “Human F9” of FIG. 1 upper left ssAAV construct), or b) a complement of the other segment (e.g., the complement of the right segment encoding “Human F9” indicated upside down in the upper left ssAAV construct FIG. 1), thereby enhancing insertion and expression efficiency, as exemplified herein. Various known gene editing systems can be used in the practice of the present disclosure, including, e.g., CRISPR/Cas system; zinc finger nuclease (ZFN) system; transcription activator-like effector nuclease (TALEN) system.

[0158] The bidirectional constructs disclosed herein can be modified to include any suitable structural feature as needed for any particular use and/or that confers one or more desired function. In some embodiments, the bidirectional nucleic acid construct disclosed herein does not comprise a homology arm. In some embodiments, the bidirectional nucleic acid construct disclosed herein is a homology-independent donor construct. In some embodiments, owing in part to the bidirectional function of the nucleic acid construct, the bidirectional construct can be inserted into a genomic locus in either direction as described herein to allow for efficient insertion and/or expression of a polypeptide of interest (e.g., Factor IX).

[0159] In some embodiments, the bidirectional nucleic acid construct does not comprise a promoter that drives the expression of Factor IX. For example, the expression of Factor IX is driven by a promoter of the host cell (e.g., the endogenous albumin promoter when the transgene is integrated into a host cell's albumin locus).

[0160] In some embodiments, the bidirectional nucleic acid construct comprises a first segment comprising a coding sequence for Factor IX and a second segment comprising a reverse complement of a coding sequence of Factor IX. Thus, the coding sequence in the first segment is capable of expressing Factor IX, while the complement of the reverse complement in the second segment is also capable of expressing Factor IX. As used herein, “coding sequence” when referring to the second segment comprising a reverse complement sequence refers to the complementary (coding)

strand of the second segment (i.e., the complement coding sequence of the reverse complement sequence in the second segment).

[0161] In some embodiments, the coding sequence that encodes Factor IX in the first segment is less than 100% complementary to the reverse complement of a coding sequence that also encodes Factor IX. That is, in some embodiments, the first segment comprises a coding sequence (1) for Factor IX, and the second segment is a reverse complement of a coding sequence (2) for Factor IX, wherein the coding sequence (1) is not identical to the coding sequence (2). For example, coding sequence (1) and/or coding sequence (2) that encodes for Factor IX can be codon optimized, such that coding sequence (1) and the reverse complement of coding sequence (2) possess less than 100% complementarity. In some embodiments, the coding sequence of the second segment encodes Factor IX using one or more alternative codons for one or more amino acids of the same (i.e., same amino acid sequence) Factor IX encoded by the coding sequence in the first segment. An “alternative codon” as used herein refers to variations in codon usage for a given amino acid, and may or may not be a preferred or optimized codon (codon optimized) for a given expression system. Preferred codon usage, or codons that are well-tolerated in a given system of expression is known in the art.

[0162] In some embodiments, the second segment comprises a reverse complement sequence that adopts different codon usage from that of the coding sequence of the first segment in order to reduce hairpin formation. Such a reverse complement forms base pairs with fewer than all nucleotides of the coding sequence in the first segment, yet it optionally encodes the same polypeptide. In such cases, the coding sequence, e.g. for Polypeptide A, of the first segment many be homologous to, but not identical to, the coding sequence, e.g. for Polypeptide A of the second half of the bidirectional construct. In some embodiments, the second segment comprises a reverse complement sequence that is not substantially complementary (e.g., not more than 70% complementary) to the coding sequence in the first segment. In some embodiments, the second segment comprises a reverse complement sequence that is highly complementary (e.g., at least 90% complementary) to the coding sequence in the first segment. In some embodiments, the second segment comprises a reverse complement sequence having at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, or about 99% complementarity to the coding sequence in the first segment.

[0163] In some embodiments, the second segment comprises a reverse complement sequence having 100% complementarity to the coding sequence in the first segment. That is, the sequence in the second segment is a perfect reverse complement of the coding sequence in the first segment. By way of example, the first segment comprises a hypothetical sequence 5' CTGGACCGA 3' (SEQ ID NO: 500) and the second segment comprises the reverse complement of SEQ ID NO: 1—i.e., 5' TCGGTCCAG 3' (SEQ ID NO: 502).

[0164] In some embodiments, the bidirectional nucleic acid construct comprises a first segment comprising a coding sequence for Factor IX (a first polypeptide) and a second segment comprising a reverse complement of a coding sequence of a (second) polypeptide. In some embodiments,

the first and second segments each comprise a coding sequence that encodes the same polypeptide (e.g., Factor IX), as described above. In some embodiments, the first and second segments each comprise a coding sequence that encodes different polypeptides. For example, the first polypeptide is Factor IX and the second polypeptide is Polypeptide B. As a further example, the first polypeptide is Factor IX and the second polypeptide is a variant (e.g., a fragment, mutant, fusion) of Factor IX (e.g., having R338L mutation described herein). A coding sequence that encodes a polypeptide may optionally comprise one or more additional sequences, such as sequences encoding amino- or carboxy-terminal amino acid sequences such as a signal sequence, label sequence (e.g. HiBit), or heterologous functional sequence (e.g. nuclear localization sequence (NLS) or self-cleaving peptide) linked to the polypeptide. A coding sequence that encodes a polypeptide may optionally comprise sequences encoding one or more amino-terminal signal peptide sequences. Each of these additional sequences can be the same or different in the first segment and second segment of the construct.

[0165] In some embodiments, the bidirectional nucleic acid construct is linear. For example, the first and second segments are joined in a linear manner through a linker sequence. In some embodiments, the 5' end of the second segment that comprises a reverse complement sequence is linked to the 3' end of the first segment. In some embodiments, the 5' end of the first segment is linked to the 3' end of the second segment that comprises a reverse complement sequence. In some embodiments, the linker sequence is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 500, 1000, 1500, 2000 or more nucleotides in length. As would be appreciate by those of skill in the art, other structural elements in addition to, or instead of a linker sequence, can be inserted between the first and second segments.

[0166] The bidirectional constructs disclosed herein can be modified to include any suitable structural feature as needed for any particular use and/or that confers one or more desired function. In some embodiments, the bidirectional nucleic acid construct disclosed herein does not comprise a homology arm. In some embodiments, owing in part to the bidirectional function of the nucleic acid construct, the bidirectional construct can be inserted into a genomic locus in either direction (orientation) as described herein to allow for efficient insertion and/or expression of a polypeptide of interest (e.g., a heterologous Factor IX).

[0167] In some embodiments, one or both of the first and second segment comprises a polyadenylation tail sequence. Methods of designing a suitable polyadenylation tail sequence are well known in the art.

[0168] In some embodiments, one or both of the first and second segment comprises a polyadenylation tail sequence and/or a polyadenylation signal sequence downstream of an open reading frame. In some embodiments, the polyadenylation tail sequence is encoded, e.g., as a “poly-A” stretch, at the 3' end of the first and/or second segment. In some embodiments, a polyadenylation tail sequence is provided co-transcriptionally as a result of a polyadenylation signal sequence that is encoded at or near the 3' end of the first and/or second segment. In some embodiments, a poly-A tail comprises at least 20, 30, 40, 50, 60, 70, 80, 90, or 100 adenines, optionally up to 300 adenines. In some embodi-

ments, the poly-A tail comprises 95, 96, 97, 98, 99, or 100 adenine nucleotides. Methods of designing a suitable polyadenylation tail sequence and/or polyadenylation signal sequence are well known in the art. Suitable splice acceptor sequences are disclosed and exemplified herein, including mouse albumin and human FIX splice acceptor sites.

[0169] In some embodiments, the polyadenylation signal sequence AAUAAA (SEQ ID NO: 800) is commonly used in mammalian systems, although variants such as UAUAAA (SEQ ID NO: 801) or AU/GUAAA (SEQ ID NO: 802) have been identified. See, e.g., NJ Proudfoot, *Genes & Dev.* 25(17):1770-82, 2011. In some embodiments, a polyA tail sequence is included.

[0170] In some embodiments, the constructs disclosed herein can be DNA or RNA, single-stranded, double-stranded, or partially single- and partially double-stranded. For example, the constructs can be single- or double-stranded DNA. In some embodiments, the nucleic acid can be modified (e.g., using nucleoside analogs), as described herein.

[0171] In some embodiments, the constructs disclosed herein comprise a splice acceptor site on either or both ends of the construct, e.g., 5' of an open reading frame in the first and/or second segments, or 5' of one or both transgene sequences. In some embodiments, the splice acceptor site comprises NAG. In further embodiments, the splice acceptor site consists of NAG. In some embodiments, the splice acceptor is an albumin splice acceptor, e.g., an albumin splice acceptor used in the splicing together of exons 1 and 2 of albumin. In some embodiments, the splice acceptor is derived from the human albumin gene. In some embodiments, the splice acceptor is derived from the mouse albumin gene. In some embodiments, the splice acceptor is a F9 (or "FIX") splice acceptor, e.g., the F9 splice acceptor used in the splicing together of exons 1 and 2 of F9. In some embodiments, the splice acceptor is derived from the human F9 gene. In some embodiments, the splice acceptor is derived from the mouse F9 gene. Additional suitable splice acceptor sites useful in eukaryotes, including artificial splice acceptors are known and can be derived from the art. See, e.g., Shapiro, et al., 1987, *Nucleic Acids Res.*, 15, 7155-7174, Burset, et al., 2001, *Nucleic Acids Res.*, 29, 255-259.

[0172] In some embodiments, the bidirectional constructs disclosed herein can be modified on either or both ends to include one or more suitable structural features as needed, and/or to confer one or more functional benefit. For example, structural modifications can vary depending on the method(s) used to deliver the constructs disclosed herein to a host cell—e.g., use of viral vector delivery or packaging into lipid nanoparticles for delivery. Such modifications include, without limitation, e.g., terminal structures such as inverted terminal repeats (ITR), hairpin, loops, and other structures such as toroid. In some embodiments, the constructs disclosed herein comprise one, two, or three ITRs. In some embodiments, the constructs disclosed herein comprise no more than two ITRs. Various methods of structural modifications are known in the art.

[0173] In some embodiments, one or both ends of the construct can be protected (e.g., from exonucleolytic degradation) by methods known in the art. For example, one or more dideoxynucleotide residues are added to the 3' terminus of a linear molecule and/or self-complementary oligonucleotides are ligated to one or both ends. See, for example, Chang et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:4959-

4963; Nehls et al. (1996) *Science* 272:886-889. Additional methods for protecting the constructs from degradation include, but are not limited to, addition of terminal amino group(s) and the use of modified internucleotide linkages such as, for example, phosphorothioates, phosphoramidates, and O-methyl ribose or deoxyribose residues.

[0174] In some embodiments, the constructs disclosed herein can be introduced into a cell as part of a vector having additional sequences such as, for example, replication origins, promoters and genes encoding antibiotic resistance. In some embodiments, the constructs can be introduced as naked nucleic acid, as nucleic acid complexed with an agent such as a liposome, polymer, or poloxamer, or can be delivered by viral vectors (e.g., adenovirus, AAV, herpesvirus, retrovirus, lentivirus).

[0175] In some embodiments, although not required for expression, the constructs disclosed herein may also include transcriptional or translational regulatory sequences, for example, promoters, enhancers, insulators, internal ribosome entry sites, sequences encoding peptides, and/or polyadenylation signals.

[0176] In some embodiments, the constructs comprising a coding sequence for Factor IX may include one or more of the following modifications: codon optimization (e.g., to human codons) and/or addition of one or more glycosylation sites. See, e.g., McIntosh et al. (2013) *Blood* (17):3335-44.

D. Gene Editing System

[0177] Various known gene editing systems can be used for targeted insertion of the Factor IX gene in the practice of the present disclosure, including, e.g., CRISPR/Cas system; zinc finger nuclease (ZFN) system; transcription activator-like effector nuclease (TALEN) system. Generally, the gene editing systems involve the use of engineered cleavage systems to induce a double strand break (DSB) or a nick (e.g., a single strand break, or SSB) in a target DNA sequence. Cleavage or nicking can occur through the use of specific nucleases such as engineered ZFN, TALENs, or using the CRISPR/Cas system with an engineered guide RNA to guide specific cleavage or nicking of a target DNA sequence. Further, targeted nucleases are being developed based on the Argonaute system (e.g., from *T. thermophilus*, known as 'TtAgo', see Swarts et al (2014) *Nature* 507 (7491): 258-261), which also may have the potential for uses in genome editing and gene therapy.

[0178] It will be appreciated that for methods that use the guide RNAs disclosed herein, the methods include the use of the CRISPR/Cas system (and any of the donor construct disclosed herein that comprises a sequence encoding Factor IX). It will also be appreciated that the present disclosure contemplates methods of targeted insertion and expression of Factor IX using the bidirectional constructs disclosed herein, which can be performed with or without the guide RNAs disclosed herein (e.g., using a ZFN system to cause a break in a target DNA sequence, creating a site for insertion of the bidirectional construct).

[0179] In some embodiments, a CRISPR/Cas system (e.g., a guide RNA and RNA-guided DNA binding agent) can be used to create a site of insertion at a desired locus within a host genome, at which site a donor construct (e.g., bidirectional construct) comprising a sequence encoding Factor IX disclosed herein can be inserted to express Factor IX. The Factor IX may be heterologous with respect to its insertion site or locus, for example a safe harbor locus from which

Factor IX is not normally expressed, as described herein. Alternatively, in some embodiments, Factor IX may be non-heterologous with respect to its insertion site, for example, insertion of a wild type Factor IX into the endogenous locus to correct a defective Factor IX gene. The safe harbor may be within an albumin gene, such as a human albumin gene. The safe harbor may be within an albumin intron 1 region, e.g., human albumin intron 1. The safe harbor may be a human safe harbor, e.g., for a liver tissue or hepatocyte host cell. In some embodiments, a guide RNA described herein can be used according to the present methods with an RNA-guided DNA binding agent (e.g., Cas nuclease) to create a site of insertion, at which site a donor construct (e.g., bidirectional construct) comprising a sequence encoding Factor IX can be inserted to express Factor IX. The guide RNAs useful for targeted insertion of Factor IX into intron 1 of the human albumin locus are exemplified and described herein (see, e.g., Table 1).

[0180] Methods of using various RNA-guided DNA-binding agents, e.g., a nuclease, such as a Cas nuclease, e.g., Cas9, are also well known in the art. While the use of a bidirectional nucleic acid with a CRISPR/Cas system is exemplified herein, it will be appreciated that suitable variations to the system can also be used. It will be appreciated that, depending on the context, the RNA-guided DNA-binding agent can be provided as a nucleic acid (e.g., DNA or mRNA) or as a protein. In some embodiments, the present method can be practiced in a host cell that already comprises and/or expresses an RNA-guided DNA-binding agent.

[0181] In some embodiments, the RNA-guided DNA-binding agent, such as a Cas9 nuclease, has cleavage activity, which can also be referred to as double-strand endonuclease activity. In some embodiments, the RNA-guided DNA-binding agent, such as a Cas9 nuclease, has nuclease activity, which can also be referred to as single-strand endonuclease activity. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nuclease. Examples of Cas nucleases include those of the type II CRISPR systems of *S. pyogenes*, *S. aureus*, and other prokaryotes (see, e.g., the list in the next paragraph), and variant or mutant (e.g., engineered, non-naturally occurring, naturally occurring, or other variant) versions thereof. See, e.g., US2016/0312198 A1; US 2016/0312199 A1.

[0182] Non-limiting exemplary species that the Cas nuclease can be derived from include *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus* sp., *Staphylococcus aureus*, *Listeria innocua*, *Lactobacillus gasseri*, *Francisella novicida*, *Wolinella succinogenes*, *Sutterella wadsworthensis*, *Gammaproteobacterium*, *Neisseria meningitidis*, *Campylobacter jejuni*, *Pasteurella multocida*, *Fibrobacter succinogenes*, *Rhodospirillum rubrum*, *Nocardiopsis dassonvillei*, *Streptomyces pristinaespiralis*, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Streptosporangium roseum*, *Ali-cyclobacillus acidocaldarius*, *Bacillus pseudomycoides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Lactobacillus buchneri*, *Treponema denticola*, *Microscilla marina*, *Burkholderiales bacterium*, *Polaromonas naphthalenivorans*, *Polaromonas* sp., *Crocosphaera watsonii*, *Cyanobacteria* sp., *Microcystis aeruginosa*, *Synechococcus* sp., *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicelulosiruptor beccii*, *Candidatus Desulforudis*, *Clostridium botulinum*, *Clostridium difficile*, *Finegoldia*

magna, *Natranaerobius thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter* sp., *Nitrosococcus halophilus*, *Nitrosococcus watsoni*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc* sp., *Arthrobacteria maxima*, *Arthrobacteria platensis*, *Arthrobacteria* sp., *Lynghya* sp., *Microcoleus chthonoplastes*, *Oscillatoria* sp., *Petrotoga mobilis*, *Thermosiphon africanus*, *Streptococcus pasteurianus*, *Neisseria cinerea*, *Campylobacter lari*, *Parvibaculum lavamentivorans*, *Corynebacterium diphtheriae*, *Acidaminococcus* sp., *Lachnospiraceae* bacterium ND2006, and *Acaryochloris marina*.

[0183] In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus thermophilus*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Neisseria meningitidis*. In some embodiments, the Cas nuclease is the Cas9 nuclease is from *Staphylococcus aureus*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella novicida*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Acidaminococcus* sp. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Lachnospiraceae bacterium* ND2006. In further embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella tularensis*, *Lachnospiraceae bacterium*, *Butyrivibrio proteo-clasticus*, *Perigrinibacteria bacterium*, *Parcubacteria bacterium*, *Smithella*, *Acidaminococcus*, *Candidatus Methanoplasma termitum*, *Eubacterium eligens*, *Moraxella bovoculi*, *Lepotspira inadai*, *Porphyromonas crevioricanis*, *Prevotella disiens*, or *Porphyromonas macacae*. In certain embodiments, the Cas nuclease is a Cpf1 nuclease from an *Acidaminococcus* or *Lachnospiraceae*.

[0184] In some embodiments, the gRNA together with an RNA-guided DNA-binding agent is called a ribonucleoprotein complex (RNP). In some embodiments, the RNA-guided DNA-binding agent is a Cas nuclease. In some embodiments, the gRNA together with a Cas nuclease is called a Cas RNP. In some embodiments, the RNP comprises Type-I, Type-II, or Type-III components. In some embodiments, the Cas nuclease is the Cas9 protein from the Type-II CRISPR/Cas system. In some embodiment, the gRNA together with Cas9 is called a Cas9 RNP.

[0185] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 protein comprises more than one RuvC domain and/or more than one HNH domain. In some embodiments, the Cas9 protein is a wild type Cas9. In each of the composition, use, and method embodiments, the Cas induces a double strand break in target DNA.

[0186] In some embodiments, chimeric Cas nucleases are used, where one domain or region of the protein is replaced by a portion of a different protein. In some embodiments, a Cas nuclease domain may be replaced with a domain from a different nuclease such as Fok1. In some embodiments, a Cas nuclease may be a modified nuclease.

[0187] In other embodiments, the Cas nuclease may be from a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a component of the Cascade complex of a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a Cas3 protein. In some

embodiments, the Cas nuclease may be from a Type-III CRISPR/Cas system. In some embodiments, the Cas nuclease may have an RNA cleavage activity.

[0188] In some embodiments, the RNA-guided DNA-binding agent has single-strand nickase activity, i.e., can cut one DNA strand to produce a single-strand break, also known as a “nick.” In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. A nickase is an enzyme that creates a nick in dsDNA, i.e., cuts one strand but not the other of the DNA double helix. In some embodiments, a Cas nickase is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which an endonucleolytic active site is inactivated, e.g., by one or more alterations (e.g., point mutations) in a catalytic domain. See, e.g., U.S. Pat. No. 8,889,356 for discussion of Cas nickases and exemplary catalytic domain alterations. In some embodiments, a Cas nickase such as a Cas9 nickase has an inactivated RuvC or HNH domain.

[0189] In some embodiments, the RNA-guided DNA-binding agent is modified to contain only one functional nuclelease domain. For example, the agent protein may be modified such that one of the nuclelease domains is mutated or fully or partially deleted to reduce its nucleic acid cleavage activity. In some embodiments, a nickase is used having a RuvC domain with reduced activity. In some embodiments, a nickase is used having an inactive RuvC domain. In some embodiments, a nickase is used having an HNH domain with reduced activity. In some embodiments, a nickase is used having an inactive HNH domain.

[0190] In some embodiments, a conserved amino acid within a Cas protein nuclease domain is substituted to reduce or alter nuclease activity. In some embodiments, a Cas nuclease may comprise an amino acid substitution in the RuvC or RuvC-like nuclease domain. Exemplary amino acid substitutions in the RuvC or RuvC-like nuclease domain include D10A (based on the *S. pyogenes* Cas9 protein). See, e.g., Zetsche et al. (2015) *Cell* Oct 22:163(3): 759-771. In some embodiments, the Cas nuclease may comprise an amino acid substitution in the HNH or HNH-like nuclease domain. Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain include E762A, H840A, N863A, H983A, and D986A (based on the *S. pyogenes* Cas9 protein). See, e.g., Zetsche et al. (2015). Further exemplary amino acid substitutions include D917A, E1006A, and D1255A (based on the *Francisella novicida* U112 Cpf1 (FnCpf1) sequence (UniProtKB-AOQ7Q2 (CPF1_FRATN)).

[0191] In some embodiments, a nickase is provided in combination with a pair of guide RNAs that are complementary to the sense and antisense strands of the target sequence, respectively. In this embodiment, the guide RNAs direct the nickase to a target sequence and introduce a DSB by generating a nick on opposite strands of the target sequence (i.e., double nicking). In some embodiments, a nickase is used together with two separate guide RNAs targeting opposite strands of DNA to produce a double nick in the target DNA. In some embodiments, a nickase is used together with two separate guide RNAs that are selected to be in close proximity to produce a double nick in the target DNA.

[0192] In some embodiments, the RNA-guided DNA-binding agent comprises one or more heterologous functional domains (e.g., is or comprises a fusion polypeptide).

[0193] In some embodiments, the heterologous functional domain may facilitate transport of the RNA-guided DNA-binding agent into the nucleus of a cell. For example, the heterologous functional domain may be a nuclear localization signal (NLS). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-10 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-5 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with one NLS. Where one NLS is used, the NLS may be linked at the N-terminus or the C-terminus of the RNA-guided DNA-binding agent sequence. It may also be inserted within the RNA-guided DNA-binding agent sequence. In other embodiments, the RNA-guided DNA-binding agent may be fused with more than one NLS. In some embodiments, the RNA-guided DNA-binding agent may be fused with 2, 3, 4, or 5 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs. In certain circumstances, the two NLSs may be the same (e.g., two SV40 NLSs) or different. In some embodiments, the RNA-guided DNA-binding agent is fused to two SV40 NLS sequences linked at the carboxy terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs, one linked at the N-terminus and one at the C-terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with 3 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with no NLS. In some embodiments, the NLS may be a monopartite sequence, such as, e.g., the SV40 NLS, PKKRKV (SEQ ID NO: 600) or PKKKRRV (SEQ ID NO: 601). In some embodiments, the NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATK-KAGQAKKKK (SEQ ID NO: 602). In a specific embodiment, a single PKKKRKV (SEQ ID NO: 600) NLS may be linked at the C-terminus of the RNA-guided DNA-binding agent. One or more linkers are optionally included at the fusion site.

III. Delivery Methods

[0194] The guide RNA, RNA-guided DNA binding agents (e.g., Cas nuclease), and nucleic acid constructs (e.g., bidirectional construct) disclosed herein can be delivered to a host cell or population of host cells or a subject, in vivo or ex vivo, using various known and suitable methods available in the art. The guide RNA, RNA-guided DNA binding agents, and nucleic acid constructs can be delivered individually or together in any combination, using the same or different delivery methods as appropriate.

[0195] Conventional viral and non-viral based gene delivery methods can be used to introduce the guide RNA disclosed herein as well as the RNA-guided DNA binding agent and donor construct in cells (e.g., mammalian cells) and target tissues. As further provided herein, non-viral vector delivery systems nucleic acids such as non-viral vectors, plasmid vectors, and, e.g. naked nucleic acid, and nucleic acid complexed with a delivery vehicle such as a liposome, lipid nanoparticle (LNP), or polyplex. Viral vector delivery systems include DNA and RNA viruses.

[0196] Methods and compositions for non-viral delivery of nucleic acids include electroporation, lipofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, LNPs, polycation or lipid:nucleic acid conjugates, naked nucleic acid (e.g., naked DNA/RNA), artificial viruses, and agent-enhanced uptake of DNA. Sonoporation

using, e.g., the Sonitron 2000 system (Rich-Mar) can also be used for delivery of nucleic acids.

[0197] Additional exemplary nucleic acid delivery systems include those provided by AmaxaBiosystems (Cologne, Germany), Maxcyte, Inc. (Rockville, Md.), BTX Molecular Delivery Systems (Holliston, Ma.) and Copernicus Therapeutics Inc., (see for example U.S. Pat. No. 6,008,336). Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386; 4,946,787; and 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known in the art, and as described herein.

[0198] Various delivery systems (e.g., vectors, liposomes, LNPs) containing the guide RNAs, RNA-guided DNA binding agent, and donor construct, singly or in combination, can also be administered to an organism for delivery to cells *in vivo* or administered to a cell or cell culture *ex vivo*. Administration is by any of the routes normally used for introducing a molecule into ultimate contact with blood, fluid, or cells including, but not limited to, injection, infusion, topical application and electroporation. Suitable methods of administering such nucleic acids are available and well known to those of skill in the art.

[0199] In certain embodiments, the present disclosure provides DNA or RNA vectors encoding any of the compositions disclosed herein—e.g., a guide RNA comprising any one or more of the guide sequences described herein; or a construct (e.g., bidirectional construct) comprising a sequence encoding Factor IX. In some embodiments, the vector also comprises a sequence encoding an RNA-guided DNA binding agent. In certain embodiments, the invention comprises DNA or RNA vectors encoding any one or more of the compositions described herein, or in any combination. In some embodiments, the vectors further comprise, e.g., promoters, enhancers, and regulatory sequences. In some embodiments, the vector that comprises a bidirectional construct comprising a sequence that encodes Factor IX does not comprise a promoter that drives Factor IX expression. For example, the expression of the Factor IX polypeptide is driven by a promoter of the host cell (e.g., the endogenous albumin promoter when the transgene is integrated into a host cell's albumin locus). In some embodiments, the bidirectional nucleic acid construct includes a first segment and a second segment, each having a splice acceptor upstream of a transgene. In certain embodiments, the splice acceptor is compatible with the splice donor sequence of the host cell's safe harbor site, e.g. the splice donor of intron 1 of a human albumin gene. In some embodiments, the vector that comprises a guide RNA comprising any one or more of the guide sequences described herein also comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, or a crRNA and trRNA, as disclosed herein.

[0200] In some embodiments, the vector comprises a nucleotide sequence encoding a guide RNA described herein. In some embodiments, the vector comprises one copy of the guide RNA. In other embodiments, the vector comprises more than one copy of the guide RNA. In embodiments with more than one guide RNA, the guide RNAs may be non-identical such that they target different target sequences, or may be identical in that they target the same target sequence. In some embodiments where the vectors comprise more than one guide RNA, each guide

RNA may have other different properties, such as activity or stability within a complex with an RNA-guided DNA nuclease, such as a Cas RNP complex. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to at least one transcriptional or translational control sequence, such as a promoter, a 3' UTR, or a 5' UTR. In one embodiment, the promoter may be a tRNA promoter, e.g., tRNA^{Lys3}, or a tRNA chimera. See Mefford et al., *RNA*. 2015 21:1683-9; Scherer et al., *Nucleic Acids Res.* 2007 35: 2620-2628. In some embodiments, the promoter may be recognized by RNA polymerase III (Pol III). Non-limiting examples of Pol III promoters include U6 and H1 promoters. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human U6 promoter. In other embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human H1 promoter. In embodiments with more than one guide RNA, the promoters used to drive expression may be the same or different. In some embodiments, the nucleotide encoding the crRNA of the guide RNA and the nucleotide encoding the trRNA of the guide RNA may be provided on the same vector. In some embodiments, the nucleotide encoding the crRNA and the nucleotide encoding the trRNA may be driven by the same promoter. In some embodiments, the crRNA and trRNA may be transcribed into a single transcript. For example, the crRNA and trRNA may be processed from the single transcript to form a double-molecule guide RNA. Alternatively, the crRNA and trRNA may be transcribed into a single-molecule guide RNA (sgRNA). In other embodiments, the crRNA and the trRNA may be driven by their corresponding promoters on the same vector. In yet other embodiments, the crRNA and the trRNA may be encoded by different vectors.

[0201] In some embodiments, the nucleotide sequence encoding the guide RNA may be located on the same vector comprising the nucleotide sequence encoding an RNA-guided DNA binding agent such as a Cas protein. In some embodiments, expression of the guide RNA and of the RNA-guided DNA binding agent such as a Cas protein may be driven by their own corresponding promoters. In some embodiments, expression of the guide RNA may be driven by the same promoter that drives expression of the RNA-guided DNA binding agent such as a Cas protein. In some embodiments, the guide RNA and the RNA-guided DNA binding agent such as a Cas protein transcript may be contained within a single transcript. For example, the guide RNA may be within an untranslated region (UTR) of the RNA-guided DNA binding agent such as a Cas protein transcript. In some embodiments, the guide RNA may be within the 5' UTR of the transcript. In other embodiments, the guide RNA may be within the 3' UTR of the transcript. In some embodiments, the intracellular half-life of the transcript may be reduced by containing the guide RNA within its 3' UTR and thereby shortening the length of its 3' UTR. In additional embodiments, the guide RNA may be within an intron of the transcript. In some embodiments, suitable splice sites may be added at the intron within which the guide RNA is located such that the guide RNA is properly spliced out of the transcript. In some embodiments, expression of the RNA-guided DNA binding agent such as a Cas protein and the guide RNA from the same vector in close temporal proximity may facilitate more efficient formation of the CRISPR RNP complex.

[0202] In some embodiments, the nucleotide sequence encoding the guide RNA and/or RNA-guided DNA binding agent may be located on the same vector comprising the construct that comprises a Factor IX gene. In some embodiments, proximity of the construct comprising the Factor IX gene and the guide RNA (and/or the RNA-guided DNA binding agent) on the same vector may facilitate more efficient insertion of the construct into a site of insertion created by the guide RNA/RNA-guided DNA binding agent.

[0203] In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a sgRNA and an mRNA encoding an RNA-guided DNA binding agent, which can be a Cas protein, such as Cas9 or Cpf1. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, and an mRNA encoding an RNA-guided DNA binding agent, which can be a Cas protein, such as, Cas9 or Cpf1. In one embodiment, the Cas9 is from *Streptococcus pyogenes* (i.e., Spy Cas9). In some embodiments, the nucleotide sequence encoding the crRNA, trRNA, or crRNA and trRNA (which may be a sgRNA) comprises or consists of a guide sequence flanked by all or a portion of a repeat sequence from a naturally-occurring CRISPR/Cas system. The nucleic acid comprising or consisting of the crRNA, trRNA, or crRNA and trRNA may further comprise a vector sequence wherein the vector sequence comprises or consists of nucleic acids that are not naturally found together with the crRNA, trRNA, or crRNA and trRNA.

[0204] In some embodiments, the crRNA and the trRNA are encoded by non-contiguous nucleic acids within one vector. In other embodiments, the crRNA and the trRNA may be encoded by a contiguous nucleic acid. In some embodiments, the crRNA and the trRNA are encoded by opposite strands of a single nucleic acid. In other embodiments, the crRNA and the trRNA are encoded by the same strand of a single nucleic acid.

[0205] In some embodiments, the vector comprises a donor construct (e.g., the bidirectional nucleic acid construct) comprising a sequence that encodes Factor IX, as disclosed herein. In some embodiments, in addition to the donor construct (e.g., bidirectional nucleic acid construct) disclosed herein, the vector may further comprise nucleic acids that encode the guide RNAs described herein and/or nucleic acid encoding an RNA-guided DNA-binding agent (e.g., a Cas nuclease such as Cas9). In some embodiments, a nucleic acid encoding an RNA-guided DNA-binding agent are each or both on a separate vector from a vector that comprises the donor construct (e.g., bidirectional construct) disclosed herein. In any of the embodiments, the vector may include other sequences that include, but are not limited to, promoters, enhancers, regulatory sequences, as described herein. In some embodiments, the promoter does not drive the expression of Factor IX of the donor construct (e.g., bidirectional construct). In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, or a crRNA and trRNA. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a sgRNA and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas nuclease (e.g., Cas9). In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas nuclease, such as, Cas9. In some embodiments, the Cas9 is from *Streptococcus pyo-*

genes (i.e., Spy Cas9). In some embodiments, the nucleotide sequence encoding the crRNA, trRNA, or crRNA and trRNA (which may be a sgRNA) comprises or consists of a guide sequence flanked by all or a portion of a repeat sequence from a naturally-occurring CRISPR/Cas system. The nucleic acid comprising or consisting of the crRNA, trRNA, or crRNA and trRNA may further comprise a vector sequence wherein the vector sequence comprises or consists of nucleic acids that are not naturally found together with the crRNA, trRNA, or crRNA and trRNA.

[0206] In some embodiments, the vector may be circular. In other embodiments, the vector may be linear. In some embodiments, the vector may be enclosed in a lipid nanoparticle, liposome, non-lipid nanoparticle, or viral capsid. Non-limiting exemplary vectors include plasmids, phage-mids, cosmids, artificial chromosomes, minichromosomes, transposons, viral vectors, and expression vectors.

[0207] In some embodiments, the vector may be a viral vector. In some embodiments, the viral vector may be genetically modified from its wild type counterpart. For example, the viral vector may comprise an insertion, deletion, or substitution of one or more nucleotides to facilitate cloning or such that one or more properties of the vector is changed. Such properties may include packaging capacity, transduction efficiency, immunogenicity, genome integration, replication, transcription, and translation. In some embodiments, a portion of the viral genome may be deleted such that the virus is capable of packaging exogenous sequences having a larger size. In some embodiments, the viral vector may have an enhanced transduction efficiency. In some embodiments, the immune response induced by the virus in a host may be reduced. In some embodiments, viral genes (such as, e.g., integrase) that promote integration of the viral sequence into a host genome may be mutated such that the virus becomes non-integrating. In some embodiments, the viral vector may be replication defective. In some embodiments, the viral vector may comprise exogenous transcriptional or translational control sequences to drive expression of coding sequences on the vector. In some embodiments, the virus may be helper-dependent. For example, the virus may need one or more helper virus to supply viral components (such as, e.g., viral proteins) required to amplify and package the vectors into viral particles. In such a case, one or more helper components, including one or more vectors encoding the viral components, may be introduced into a host cell or population of host cells along with the vector system described herein. In other embodiments, the virus may be helper-free. For example, the virus may be capable of amplifying and packaging the vectors without a helper virus. In some embodiments, the vector system described herein may also encode the viral components required for virus amplification and packaging.

[0208] Non-limiting exemplary viral vectors include adeno-associated virus (AAV) vector, lentivirus vectors, adenovirus vectors, helper dependent adenoviral vectors (HDAd), herpes simplex virus (HSV-1) vectors, bacteriophage T4, baculovirus vectors, and retrovirus vectors. In some embodiments, the viral vector may be an AAV vector. In other embodiments, the viral vector may a lentivirus vector.

[0209] In some embodiments, "AAV" refers all serotypes, subtypes, and naturally-occurring AAV as well as recombinant AAV. "AAV" may be used to refer to the virus itself or

a derivative thereof. The term "AAV" includes AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV6.2, AAV7, AA VRh.64R1, AA Vhu.37, AA VRh.8, AA VRh.32.33, AAV8, AAV9, AAV-DJ, AAV2/8, AA VRh10, AA VLK03, AV10, AAV11, AAV12, rh10, and hybrids thereof, avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, nonprimate AAV, and ovine AAV. The genomic sequences of various serotypes of AAV, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. A "AAV vector" as used herein refers to an AAV vector comprising a heterologous sequence not of AAV origin (i.e., a nucleic acid sequence heterologous to AAV), typically comprising a sequence encoding a heterologous polypeptide of interest. The construct may comprise an AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV6.2, AAV7, AA VRh.64R1, AA Vhu.37, AA VRh.8, AA VRh.32.33, AAV8, AAV9, AAV-DJ, AAV2/8, AA VRh10, AA VLK03, AV10, AAV11, AAV12, rh10, and hybrids thereof, avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, nonprimate AAV, and ovine AAV capsid sequence. In general, the heterologous nucleic acid sequence (the transgene) is flanked by at least one, and generally by two, AAV inverted terminal repeat sequences (ITRs). An AAV vector may either be single-stranded (ssAAV) or self-complementary (scAAV).

[0210] In some embodiments, the lentivirus may be non-integrating. In some embodiments, the viral vector may be an adenovirus vector. In some embodiments, the adenovirus may be a high-cloning capacity or "gutless" adenovirus, where all coding viral regions apart from the 5' and 3' inverted terminal repeats (ITRs) and the packaging signal ('I') are deleted from the virus to increase its packaging capacity. In yet other embodiments, the viral vector may be an HSV-1 vector. In some embodiments, the HSV-1-based vector is helper dependent, and in other embodiments it is helper independent. For example, an amplicon vector that retains only the packaging sequence requires a helper virus with structural components for packaging, while a 30kb-deleted HSV-1 vector that removes non-essential viral functions does not require helper virus. In additional embodiments, the viral vector may be bacteriophage T4. In some embodiments, the bacteriophage T4 may be able to package any linear or circular DNA or RNA molecules when the head of the virus is emptied. In further embodiments, the viral vector may be a baculovirus vector. In yet further embodiments, the viral vector may be a retrovirus vector. In embodiments using AAV or lentiviral vectors, which have smaller cloning capacity, it may be necessary to use more than one vector to deliver all the components of a vector system as disclosed herein. For example, one AAV vector may contain sequences encoding an RNA-guided DNA binding agent such as a Cas protein (e.g., Cas9), while a second AAV vector may contain one or more guide sequences.

[0211] In some embodiments, the vector system may be capable of driving expression of one or more nuclease components in a cell. In some embodiments, the bidirectional construct, optionally as part of a vector system, may comprise a promoter capable of driving expression of a coding sequence in a cell. In some embodiments, the vector does not comprise a promoter that drives expression of one or more coding sequences once it is integrated in a cell (e.g.,

uses the host cell's endogenous promoter such as when inserted at intron 1 of an albumin locus, as exemplified herein). In some embodiments, the cell may be a eukaryotic cell, such as, e.g., a yeast, plant, insect, or mammalian cell. In some embodiments, the eukaryotic cell may be a mammalian cell. In some embodiments, the eukaryotic cell may be a rodent cell. In some embodiments, the eukaryotic cell may be a human cell. Suitable promoters to drive expression in different types of cells are known in the art. In some embodiments, the promoter may be wild type. In other embodiments, the promoter may be modified for more efficient or efficacious expression. In yet other embodiments, the promoter may be truncated yet retain its function. For example, the promoter may have a normal size or a reduced size that is suitable for proper packaging of the vector into a virus.

[0212] In some embodiments, the vector may comprise a nucleotide sequence encoding an RNA-guided DNA binding agent such as a Cas protein (e.g., Cas9) described herein. In some embodiments, the nuclease encoded by the vector may be a Cas protein. In some embodiments, the vector system may comprise one copy of the nucleotide sequence encoding the nuclease. In other embodiments, the vector system may comprise more than one copy of the nucleotide sequence encoding the nuclease. In some embodiments, the nucleotide sequence encoding the nuclease may be operably linked to at least one transcriptional or translational control sequence. In some embodiments, the nucleotide sequence encoding the nuclease may be operably linked to at least one promoter.

[0213] In some embodiments, the vector may comprise any one or more of the constructs comprising a heterologous Factor IX gene described herein. In some embodiments, the Factor IX gene may be operably linked to at least one transcriptional or translational control sequence. In some embodiments, the Factor IX gene may be operably linked to at least one promoter. In some embodiments, the Factor IX gene is not linked to a promoter that drives the expression of the heterologous gene.

[0214] In some embodiments, the promoter may be constitutive, inducible, or tissue-specific. In some embodiments, the promoter may be a constitutive promoter. Non-limiting exemplary constitutive promoters include cytomegalovirus immediate early promoter (CMV), simian virus (SV40) promoter, adenovirus major late (MLP) promoter, Rous sarcoma virus (RSV) promoter, mouse mammary tumor virus (MMTV) promoter, phosphoglycerate kinase (PGK) promoter, elongation factor-alpha (EF1a) promoter, ubiquitin promoters, actin promoters, tubulin promoters, immunoglobulin promoters, a functional fragment thereof, or a combination of any of the foregoing. In some embodiments, the promoter may be a CMV promoter. In some embodiments, the promoter may be a truncated CMV promoter. In other embodiments, the promoter may be an EF1a promoter. In some embodiments, the promoter may be an inducible promoter. Non-limiting exemplary inducible promoters include those inducible by heat shock, light, chemicals, peptides, metals, steroids, antibiotics, or alcohol. In some embodiments, the inducible promoter may be one that has a low basal (non-induced) expression level, such as, e.g., the Tet-On® promoter (Clontech).

[0215] In some embodiments, the promoter may be a tissue-specific promoter, e.g., a promoter specific for expression in the liver.

[0216] In some embodiments, the compositions comprise a vector system. In some embodiments, the vector system may comprise one single vector. In other embodiments, the vector system may comprise two vectors. In additional embodiments, the vector system may comprise three vectors. When different guide RNAs are used for multiplexing, or when multiple copies of the guide RNA are used, the vector system may comprise more than three vectors.

[0217] In some embodiments, the vector system may comprise inducible promoters to start expression only after it is delivered to a target cell. Non-limiting exemplary inducible promoters include those inducible by heat shock, light, chemicals, peptides, metals, steroids, antibiotics, or alcohol. In some embodiments, the inducible promoter may be one that has a low basal (non-induced) expression level, such as, e.g., the Tet-On® promoter (Clontech).

[0218] In additional embodiments, the vector system may comprise tissue-specific promoters to start expression only after it is delivered into a specific tissue.

[0219] The vector comprising: a guide RNA, RNA-binding DNA binding agent, or donor construct comprising a sequence encoding Factor IX, individually or in any combination, may be delivered by liposome, a nanoparticle, an exosome, or a microvesicle. The vector may also be delivered by a lipid nanoparticle (LNP). One or more guide RNA, RNA-binding DNA binding agent (e.g. mRNA), or donor construct comprising a sequence encoding a heterologous protein, individually or in any combination, may be delivered by liposome, a nanoparticle, an exosome, or a microvesicle. One or more guide RNA, RNA-binding DNA binding agent (e.g. mRNA), or donor construct comprising a sequence encoding a heterologous protein, individually or in any combination, may be delivered by LNP.

[0220] Lipid nanoparticles (LNPs) are a well-known means for delivery of nucleotide and protein cargo, and may be used for delivery of any of the guide RNAs, RNA-guided DNA binding agent, and/or donor construct (e.g., bidirectional construct) disclosed herein. In some embodiments, the LNPs deliver the compositions in the form of nucleic acid (e.g., DNA or mRNA), or protein (e.g., Cas nuclease), or nucleic acid together with protein, as appropriate.

[0221] In some embodiments, provided herein is a method for delivering any of the guide RNAs described herein and/or donor construct (e.g., bidirectional construct) disclosed herein, alone or in combination, to a host cell or a population of host cells or a subject, wherein any one or more of the components is associated with an LNP. In some embodiments, the method further comprises an RNA-guided DNA binding agent (e.g., Cas9 or a sequence encoding Cas9).

[0222] In some embodiments, provided herein is a composition comprising any of the guide RNAs described herein and/or donor construct (e.g., bidirectional construct) disclosed herein, alone or in combination, with an LNP. In some embodiments, the composition further comprises an RNA-guided DNA binding agent (e.g., Cas9 or a sequence encoding Cas9).

[0223] In some embodiments, the LNPs comprise cationic lipids. In some embodiments, the LNPs comprise (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate) or another ionizable lipid.

See, e.g., lipids of PCT/US2018/053559 (filed Sep. 28, 2018), WO/2017/173054, WO2015/095340, and WO2014/136086, as well as references provided therein. In some embodiments, the LNPs comprise molar ratios of a cationic lipid amine to RNA phosphate (N:P) of about 4.5, 5.0, 5.5, 6.0, or 6.5. In some embodiments, the term cationic and ionizable in the context of LNP lipids is interchangeable, e.g., wherein ionizable lipids are cationic depending on the pH.

[0224] In some embodiments, LNPs associated with the bidirectional construct disclosed herein are for use in preparing a medicament for treating a disease or disorder. The disease or disorder may be a Factor IX deficiency such as hemophilia B.

[0225] In some embodiments, any of the guide RNAs described herein, RNA-guided DNA binding agents, and/or donor construct (e.g., bidirectional construct) disclosed herein, alone or in combination, whether naked or as part of a vector, is formulated in or administered via a lipid nanoparticle; see e.g., WO/2017/173054 the contents of which are hereby incorporated by reference in their entirety.

[0226] In some embodiments, an LNP composition is encompassed comprising: an RNA component and a lipid component, wherein the lipid component comprises an amine lipid such as a biodegradable, ionizable lipid. In some instances, the lipid component comprises biodegradable, ionizable lipid, cholesterol, DSPC, and PEG-DMG.

[0227] It will be apparent that a guide RNA disclosed herein, an RNA-guided DNA binding agent (e.g., Cas nuclease or a nucleic acid encoding a Cas nuclease), and a donor construct (e.g., bidirectional construct) comprising a sequence encoding Factor IX can be delivered using the same or different systems. For example, the guide RNA, Cas nuclease, and construct can be carried by the same vector (e.g., AAV). Alternatively, the Cas nuclease (as a protein or mRNA) and/or gRNA can be carried by a plasmid or LNP, while the donor construct can be carried by a vector such as AAV. Furthermore, the different delivery systems can be administered by the same or different routes (e.g. by infusion; by injection, such as intramuscular injection, tail vein injection, or other intravenous injection; by intraperitoneal administration and/or intramuscular injection).

[0228] The different delivery systems can be delivered in vitro or in vivo simultaneously or in any sequential order. In some embodiments, the donor construct, guide RNA, and Cas nuclease can be delivered in vitro or in vivo simultaneously, e.g., in one vector, two vectors, individual vectors, one LNP, two LNPs, individual LNPs, or a combination thereof. In some embodiments, the donor construct can be delivered in vivo or in vitro, as a vector and/or associated with a LNP, prior to (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more days) delivering the guide RNA and/or Cas nuclease, as a vector and/or associated with a LNP singly or together as a ribonucleoprotein (RNP). As a further example, the guide RNA and Cas nuclease, as a vector and/or associated with a LNP singly or together as a ribonucleoprotein (RNP), can be delivered in vivo or in vitro, prior to (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more days) delivering the construct, as a vector and/or associated with a LNP.

[0229] In some embodiments, the present disclosure also provides pharmaceutical formulations for administering any of the guide RNAs disclosed herein. In some embodiments, the pharmaceutical formulation includes an RNA-guided

DNA binding agent (e.g., Cas nuclease) and a donor construct comprising a coding sequence of a therapeutic heterologous gene, as disclosed herein. Pharmaceutical formulations suitable for delivery into a subject (e.g., human subject) are well known in the art.

IV. Methods of Use

[0230] The gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents described herein are useful for introducing a Factor IX nucleic acid to a host cell or population of host cells, *in vivo* or *in vitro*. In some embodiments, the gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents described herein are useful for expressing Factor IX in a host cell or population of host cells, or in a subject in need thereof. In some embodiments, the gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents described herein are useful for treating hemophilia (e.g., hemophilia B) in a subject in need thereof. Administration of any one or more of the gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents described herein will increase Factor IX protein levels and/or Factor IX activity levels, e.g. circulating, serum, or plasma levels. In some embodiments, the effectiveness of the treatment can be assessed by measuring serum or plasma Factor IX activity, wherein an increase in the subject's plasma level and/or activity of Factor IX indicates effectiveness of the treatment. In some embodiments, the effectiveness of the treatment can be assessed by measuring serum or plasma Factor IX protein and/or activity levels, wherein an increase in the subject's plasma level and/or activity of Factor IX indicates effectiveness of the treatment. In some embodiments, effectiveness of the treatment can be determined by assessing clotting function in an aPTT assay and/or thrombin generation in an TGA-EA assay. In some embodiments, effectiveness of the treatment can be determined by assessing the level of Factor IX, e.g., circulating Factor IX, can be measured by a coagulation and/or an immunologic assay, e.g., an sandwich immunoassay, ELISA (see, e.g., Example 13), MSD (see, e.g., Example 14).

[0231] In normal or healthy individuals, Factor IX activity and antigen levels vary between about 50 and 160% of normal pooled plasma which is about 3-5 µg/ml, based on its purification from adult human plasma Amiral et al., Clin. Chem. 30(9), 1512-16, 1984 at Table 2; see also Osterud et al., 1978. Individuals having less than 50% of normal plasma level of Factor IX activity and/or antigen levels are classified as having hemophilia. In particular, individuals with less than about 1% active factor are classified as having severe haemophilia, while those with about 1-5% active factor have moderate haemophilia. Individuals with mild haemophilia have between about 6-49% of normal levels of active clotting factor. In some embodiments, the level of circulating factor IX can be measured by a coagulation and/or an immunologic assay, which methods are well known in the art (e.g. Simioni et al, NEJM 2009, Adcock et al., Coagulation Handbook, Esoterix Laboratory Services, 2006). An immunologic method for detecting hFIX protein, and a method of functionally normalizing Factor IX activity of a hyperfunctional hFIX variant is found in Example 13.

In some embodiments, Factor IX, e.g., circulating Factor IX, can be measured by a coagulation and/or an immunologic assay, e.g., an sandwich immunoassay, ELISA (see, e.g., Example 13), MSD (see, e.g., Example 14).

[0232] Accordingly, in some embodiments, the compositions and methods disclosed herein are useful for increasing plasma levels of Factor IX or Factor IX activity levels in a subject having hemophilia to about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, or more, of normal level.

[0233] In some embodiments, the compositions and methods disclosed herein are useful for increasing Factor IX activity and/or levels, for example increasing circulating FIX protein levels to about 0.05, 0.1, 0.2, 0.5, 1, 2, 3, or 4 µg/ml. FIX protein levels may reach about 150 µg/ml, or more. In some embodiments, the compositions and methods disclosed herein are useful for increasing Factor IX protein levels to about 4 µg/ml. In some embodiments, the compositions and methods disclosed herein are useful for increasing Factor IX protein levels to about 4 µg/ml to about 5 µg/ml, about 4 µg/ml to 6 µg/ml, about 4 µg/ml to 8 µg/ml, about 4 µg/ml to about 10 µg/ml, or more. In some embodiments, the compositions and methods disclosed herein are useful for increasing Factor IX protein levels to about 0.1 µg/ml to about 10 µg/ml, about 1 µg/ml to about 10 µg/ml, about 0.1 µg/ml to about 6 µg/ml, about 1 µg/ml to about 6 µg/ml, about 2 µg/ml to about 5 µg/ml, or about 3 µg/ml to about 5 µg/ml. For example, the compositions and methods disclosed herein are useful for increasing plasma levels of Factor IX in a subject having hemophilia to about 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150 µg/ml, or more.

[0234] In some embodiments, the compositions and methods disclosed herein are useful for increasing plasma levels of Factor IX activity and/or levels in a subject having hemophilia by about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 1%, 6%, 17%, %18%, %19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, or more, as compared to the subject's plasma level and/or activity of Factor IX before administration.

[0235] In some embodiments, the compositions and methods disclosed herein are useful for increasing Factor IX protein and/or Factor IX activity in a host cell or population of host cells by about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, or more as compared to a Factor IX level and/or activity before administration to the host cell or population of host cells, e.g. a normal level. In some embodiments, the cell is a liver cell or a population of liver

cells. In some embodiments, the liver cell is hepatocyte or the population of liver cells are hepatocytes.

[0236] In some embodiments, the method comprises administering a guide RNA and an RNA-guided DNA binding agent (such as an mRNA encoding a Cas9 nuclease) in an LNP. In further embodiments, the method comprises administering an AAV nucleic acid construct encoding a Factor IX protein, such as an bidirectional FIX construct. CRISPR/Cas9 LNP, comprising guide RNA and an mRNA encoding a Cas9, can be administered intravenously. AAV FIX donor construct can be administered intravenously. Exemplary dosing of CRISPR/Cas9 LNP includes about 0.1, 0.25, 0.3, 0.5, 1, 2, 3, 4, 5, 6, 8, or 10 mpk (RNA). The units mg/kg and mpk are being used interchangeably herein. Exemplary dosing of AAV comprising a nucleic acid encoding a FIX protein includes an MOI of about 10^{11} , 10^{12} , 10^{13} , and 10^{14} vg/kg, optionally the MOI may be about 1×10^{13} to 1×10^{14} vg/kg.

[0237] In some embodiments, the method comprises expressing a therapeutically effective amount of the Factor IX protein. In some embodiments, the method comprises achieving a therapeutically effective level of circulating Factor IX coagulation activity in an individual. In particular embodiments, the method comprises achieving Factor IX activity of at least about 5% to about 50% of normal. The method may comprise achieving Factor IX activity of at least about 50% to about 150% of normal. In certain embodiments, the method comprises achieving an increase in Factor IX activity over the patient's baseline Factor IX activity of at least about 1% to about 50% of normal Factor IX activity, or at least about 5% to about 50% of normal Factor IX activity, or at least about 50% to about 150% of normal Factor IX activity.

[0238] In some embodiments, the method further comprises achieving a durable effect, e.g. at least 1 month, 2 months, 6 months, 1 year, or 2 year effect. In some embodiments, the method further comprises achieving the therapeutic effect in a durable and sustained manner, e.g. at least 1 month, 2 months, 6 months, 1 year, or 2 year effect. In some embodiments, the level of circulating Factor IX activity and/or level is stable for at least 1 month, 2 months, 6 months, 1 year, or more. In some embodiments a steady-state activity and/or level of FIX protein is achieved by at least 7 days, at least 14 days, or at least 28 days. In additional embodiments, the method comprises maintaining Factor IX activity and/or levels after a single dose for at least 1, 2, 4, or 6 months, or at least 1, 2, 3, 4, or 5 years.

[0239] In additional embodiments involving insertion into the albumin locus, the individual's circulating albumin levels are normal. The method may comprise maintaining the individual's circulating albumin levels within +5%, +10%, +15%, +20%, or +50% of normal circulating albumin levels. In certain embodiments, the individual's albumin levels are unchanged as compared to the albumin levels of untreated individuals by at least week 4, week 8, week 12, or week 20. In certain embodiments, the individual's albumin levels transiently drop then return to normal levels. In particular, the methods may comprise detecting no significant alterations in levels of plasma albumin.

[0240] In some embodiments, the invention comprises a method or use of modifying (e.g., creating a double strand break in) an albumin gene, such as a human albumin gene, comprising, administering or delivering to a host cell or population of host cells any one or more of the gRNAs,

donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents (e.g., Cas nuclease) described herein. In some embodiments, the invention comprises a method or use of modifying (e.g., creating a double strand break in) an albumin intron 1 region, such as a human albumin intron 1, comprising, administering or delivering to a host cell or population of host cells any one or more of the gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents (e.g., Cas nuclease) described herein. In some embodiments, the invention comprises a method or use of modifying (e.g., creating a double strand break in) a human safe harbor, such as liver tissue or hepatocyte host cell, comprising, administering or delivering to a host cell or population of host cells any one or more of the gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents (e.g., Cas nuclease) described herein. Insertion within a safe harbor locus, such as an albumin locus, allows overexpression of the Factor IX gene without significant deleterious effects on the host cell or cell population, such as hepatocytes or liver cells. In some embodiments, the invention comprises a method or use of modifying (e.g., creating a double strand break in) intron 1 of a human albumin locus comprising, administering or delivering to a host cell or population of host cells any one or more of the gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents (e.g., Cas nuclease) described herein. In some embodiments, the guide RNA comprises a guide sequence that contains at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides that bind within intron 1 of a human albumin locus (SEQ ID NO: 1). In some embodiments, the guide RNA comprises at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NO: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 34, 40, 45, 51, 60, 61, 63, 64, 65, 66, 72, 77, 83, 92, 93, 95, 96, and 97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is selected from the group consisting of SEQ ID NOS: 34-97. In some embodiments, the guide RNA comprises at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNA

comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the method is performed in vitro. In some embodiments, the method is performed in vivo. In some embodiments, the donor construct is a bidirectional construct that comprises a sequence encoding Factor IX. In some embodiments, the host cell is a liver cell, such as. In additional embodiments, the liver cell is a hepatocyte.

[0241] In some embodiments, the invention comprises a method or use of introducing a Factor IX nucleic acid to a host cell or population of host cells comprising, administering or delivering any one or more of the gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents (e.g., Cas nuclease) described herein. In some embodiments, the guide RNA comprises a guide sequence that contains at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides that are capable of binding to a region within intron 1 of human albumin locus (SEQ ID NO: 1). In some embodiments, the guide RNA comprises at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NO: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 34, 40, 45, 51, 60, 61, 63, 64, 65, 66, 72, 77, 83, 92, 93, 95, 96, and 97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17,

18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is selected from the group consisting of SEQ ID NOS: 34-97. In some embodiments, the guide RNA comprises at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is selected from the group consisting of SEQ ID NOS: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97. In some embodiments, the method is in vitro. In some embodiments, the method is in vivo. In some embodiments, the donor construct is a bidirectional construct that comprises a sequence encoding Factor IX. In some embodiments, the host cell is a liver cell, or the population of host cells are liver cells, such as hepatocyte.

[0242] In some embodiments, the invention comprises a method or use of expressing Factor IX in a host cell or a population of host cells comprising, administering or delivering any one or more of the gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents (e.g., Cas nuclease) described herein. In some embodiments, the guide RNA comprises a guide sequence that contains at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides that are capable of binding to a region within intron 1 of human albumin locus (SEQ ID NO: 1). In some embodiments, the guide RNA comprises at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NO: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected

from the group consisting of SEQ ID NOS: 34, 40, 45, 51, 60, 61, 63, 64, 65, 66, 72, 77, 83, 92, 93, 95, 96, and 97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is selected from the group consisting of SEQ ID NOS: 34-97. In some embodiments, the guide RNA comprises at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is selected from the group consisting of SEQ ID NOS: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97. In some embodiments, the method is *in vitro*. In some embodiments, the method is *in vivo*. In some embodiments, the donor construct is a bidirectional construct that comprises a sequence encoding Factor IX. In some embodiments, the host cell is a liver cell, or the population of host cells are liver cells, such as hepatocyte.

[0243] In some embodiments, the invention comprises a method or use of treating hemophilia (e.g., hemophilia B) comprising, administering or delivering any one or more of the gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents (e.g., Cas nuclease) described herein to a subject in need thereof. In some embodiments, the guide RNA comprises a guide sequence that contains at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides that are capable of binding to a region within intron 1 of human albumin locus (SEQ ID NO: 1). In some embodiments, the guide RNA comprises at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-33. In

some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NO: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2, 8, 13, 19, 28, 29, 31, 32, 33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 34, 40, 45, 51, 60, 61, 63, 64, 65, 66, 72, 77, 83, 92, 93, 95, 96, and 97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is selected from the group consisting of SEQ ID NOS: 34-97. In some embodiments, the guide RNA comprises at least 15, 16, 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNA comprises a sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence selected from the group consisting of SEQ ID NOS: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 95%, 90%, 85%, 80%, or 75% identical to a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 2-5, 10-17, 21-27, and 29-33. In some embodiments, the guide RNAs disclosed herein comprise a guide sequence that is selected from the group consisting of SEQ ID NOS: 34-37, 42-49, 53-59, 61-69, 74-81, 85-91, and 93-97. In some embodiments, the donor construct is a bidirectional construct that comprises a sequence encoding Factor IX. In some embodiments, the host cell is a liver cell, or the population of host cells are liver cells, such as hepatocytes.

[0244] As described herein, the donor construct (e.g., bidirectional construct) comprising a sequence encoding Factor IX, guide RNA, and RNA-guided DNA binding agent can be delivered using any suitable delivery system and method known in the art. The compositions can be delivered *in vitro* or *in vivo* simultaneously or in any sequential order. In some embodiments, the donor construct, guide RNA, and Cas nuclease can be delivered *in vitro* or *in vivo* simultaneously, e.g., in one vector, two vectors, individual vectors, one LNP, two LNPs, individual LNPs, or a combination

thereof. In some embodiments, the donor construct can be delivered in vivo or in vitro, as a vector and/or associated with a LNP, prior to (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more days) delivering the guide RNA and/or Cas nuclease, as a vector and/or associated with a LNP singly or together as a ribonucleoprotein (RNP). In some embodiments, the donor construct can be delivered in multiple administrations, e.g., every day, every two days, every three days, every four days, every week, every two weeks, every three weeks, or every four weeks. In some embodiments, the donor construct can be delivered at one-week intervals, e.g., at week 1, week 2, and week 3, etc. As a further example, the guide RNA and Cas nuclease, as a vector and/or associated with a LNP singly or together as a ribonucleoprotein (RNP), can be delivered in vivo or in vitro, prior to (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more days) delivering the construct, as a vector and/or associated with a LNP. In some embodiments, the albumin guide RNA can be delivered in multiple administrations, e.g., every day, every two days, every three days, every four days, every week, every two weeks, every three weeks, or every four weeks. In some embodiments, the the albumin guide RNA can be delivered at one-week intervals, e.g., at week 1, week 2, and week 3, etc. In some embodiments, the Cas nuclease can be delivered in multiple administrations, e.g., can be delivered every day, every two days, every three days, every four days, every week, every two weeks, every three weeks, or every four weeks. In some embodiments, the Cas nuclease can be delivered at one-week intervals, e.g., at week 1, week 2, and week 3, etc. In some embodiments, the guide RNA and Cas nuclease are associated with an LNP and delivered to the host cell or the population of host cells prior to delivering the Factor IX donor construct.

[0245] In some embodiments, the donor construct comprises a sequence encoding Factor IX, wherein the Factor IX sequence is wild type Factor IX, e.g., SEQ ID NO: 700. In some embodiments, the donor construct comprises a sequence encoding Factor IX, wherein the Factor IX sequence is wild type Factor IX, e.g., SEQ ID NO: 701. In some embodiments, the sequence encodes a variant of Factor IX. For example, the variant possesses increased coagulation activity than wild type Factor IX. For example, the variant Factor IX comprises one or more mutations, such as an amino acid substitution in position R338 (e.g., R338L), relative to SEQ ID NO: 701. In some embodiments, the sequence encodes a Factor IX variant that is 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, SEQ ID NO: 701, or SEQ ID NO: 702, having at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type Factor IX. In some embodiments, the sequence encodes a fragment of Factor IX, wherein the fragment possesses at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type Factor IX.

[0246] In one example, the Factor IX protein can comprise amino acid substitutions at positions L6 and V181. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6 and K265. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6 and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6 and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions

V181 and K265. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181 and an 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181 and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions K265 and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions K265 and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions 1383 and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, and K265. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, K265, and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, K265, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181, K265, and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181, K265, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181, 1383, and E186. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181, 1383, and E186. In another example, the Factor IX protein can comprise amino acid substitutions at positions K265, 1383, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, K265, and 1383. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, 1383, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions L6, K265, 1383, and E185. In another example, the Factor IX protein can comprise amino acid substitutions at positions V181, K265, 1383, and E185.

[0247] In some embodiments, the donor construct comprises a sequence encoding a Factor IX variant, wherein the Factor IX variant activates coagulation in the absence of its cofactor, Factor VIII (expression results in therapeutically relevant FVIII mimetic activity). Such Factor IX variants can further maintain the activity of wild type Factor IX. For example, such a Factor IX variant can comprise an amino acid substitution at position L6, V181, K265, 1383, E185, or a combination thereof relative to wild type Factor IX (e.g., relative to SEQ ID NO: 701). For example, such a Factor IX variant can comprise an L6F mutation, a V181I mutation, a K265A mutation, an I383V mutation, an E185D mutation, or a combination thereof relative to wild type Factor IX (e.g., relative to SEQ ID NO: 701).

[0248] In a specific example, the Factor IX protein can comprise amino acid substitutions at positions V181, K265, and 1383. In another specific example, the Factor IX protein can comprise amino acid substitutions at positions V181, K265, 1383, and E185. In another specific example, the Factor IX protein can comprise amino acid substitutions at positions L6, V181, K265, and 1383.

[0249] In one example, the Factor IX protein can comprise an L6F mutation and a V181I mutation. In another example, the Factor IX protein can comprise an L6F mutation and a K265A mutation. In another example, the Factor IX protein can comprise an L6F mutation and an I383V mutation. In

another example, the Factor IX protein can comprise an L6F mutation and an E185D mutation. In another example, the Factor IX protein can comprise a V181I mutation and a K265A mutation. In another example, the Factor IX protein can comprise a V181I mutation and an I383V mutation. In another example, the Factor IX protein can comprise a V181I mutation and an E185D mutation. In another example, the Factor IX protein can comprise a K265A mutation and an I383V mutation. In another example, the Factor IX protein can comprise a K265A mutation and an E185D mutation. In another example, the Factor IX protein can comprise an I383V mutation and an E185D mutation. In another example, the Factor IX protein can comprise an L6F mutation, a V181I mutation, and a K265A mutation. In another example, the Factor IX protein can comprise an L6F mutation, a V181I mutation, and an I383V mutation. In another example, the Factor IX protein can comprise an L6F mutation, a V181I mutation and an E185D mutation. In another example, the Factor IX protein can comprise an L6F mutation, a K265A mutation, and an I383V mutation. In another example, the Factor IX protein can comprise an L6F mutation, a K265A mutation, and an E185D mutation. In another example, the Factor IX protein can comprise an L6F mutation, an I383V mutation, and an E186D mutation. In another example, the Factor IX protein can comprise a V181I mutation, a K265A mutation, and an I383V mutation. In another example, the Factor IX protein can comprise a V181I mutation, an I383V mutation, and an E186D mutation. In another example, the Factor IX protein can comprise a K265A mutation, an I383V mutation, and an E185D mutation. In another example, the Factor IX protein can comprise an L6F mutation, a V181I mutation, a K265A mutation, and an I383V mutation. In another example, the Factor IX protein can comprise an L6F mutation, a V181I mutation, an I383V mutation, and an E185D mutation. In another example, the Factor IX protein can comprise a V181I mutation, a K265A mutation, an I383V mutation, and an E185D mutation.

[0250] In a specific example, the Factor IX protein can comprise a V181I mutation, a K265A mutation, and an I383V mutation. In another specific example, the Factor IX protein can comprise a V181I mutation, a K265A mutation, an I383V mutation, and an E185D mutation. In some embodiments, the Factor IX protein is at least 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, having at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type Factor IX. In certain embodiments, the Factor IX variant is at least 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, having at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity as compared to wild type Factor IX and comprises a V181I mutation, a K265A mutation, an I383V mutation, and/or an E185D mutation. In another specific example, the Factor IX protein can comprise an L6F mutation, a V181I mutation, a K265A mutation, and an I383V mutation. In certain embodiments, the Factor IX variant is at least 80%, 85%, 90%, 93%, 95%, 97%, 99% identical to SEQ ID NO: 700, having at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, 99%, 100%, or more, activity

as compared to wild type Factor IX and comprises a V181I mutation, a K265A mutation, and an I383V mutation.

[0251] In some embodiments, the host cell is a liver cell, or the population of host cells are liver cells. In some embodiments, the host cell is, or the population of host cells are, any suitable non-dividing cell. As used herein, a “non-dividing cell” refers to cells that are terminally differentiated and do not divide, as well as quiescent cells that do not divide but retains the ability to re-enter cell division and proliferation. Liver cells, for example, retain the ability to divide (e.g., when injured or resected), but do not typically divide. During mitotic cell division, homologous recombination is a mechanism by which the genome is protected and double-stranded breaks are repaired. In some embodiments, a “non-dividing” cell refers to a cell in which homologous recombination (HR) is not the primary mechanism by which double-stranded DNA breaks are repaired in the cell, e.g., as compared to a control dividing cell. In some embodiments, a “non-dividing” cell refers to a cell in which non-homologous end joining (NHEJ) is the primary mechanism by which double-stranded DNA breaks are repaired in the cell, e.g., as compared to a control dividing cell. Non-dividing cell types have been described in the literature, e.g. by active NHEJ double-stranded DNA break repair mechanisms. See, e.g. Iyama, DNA Repair (Amst.) 2013, 12(8): 620-636. In some embodiments, the host cell includes, but is not limited to, a liver cell, a muscle cell, or a neuronal cell. In some embodiments, the host cell, or the population of host cells are, is a hepatocyte, such as a mouse, cyno, or human hepatocyte. In some embodiments, the host cell is a myocyte, such as a mouse, cyno, or human myocyte. In some embodiments, provided herein is a host cell composition comprising any one or more guide RNA described herein, alone or in combination with an RNA-guided DNA binding protein. In some embodiments, provided herein is a host cell composition comprising any one or more of the vectors described herein.

[0252] In some embodiments, the donor construct (e.g., bidirectional construct) is administered in a nucleic acid vector, such as an AAV vector, e.g., AAV8. In some embodiments, the donor construct does not comprise a homology arm.

[0253] In some embodiments, the subject is a mammal. In some embodiments, the subject is human. In some embodiments, the subject is cow, pig, monkey, sheep, dog, cat, fish, or poultry.

[0254] In some embodiments, the donor construct (e.g., bidirectional construct) comprising a sequence encoding Factor IX, guide RNA, and RNA-guided DNA binding agent are administered intravenously. In some embodiments, the donor construct (e.g., bidirectional construct) comprising a sequence encoding Factor IX, guide RNA, and RNA-guided DNA binding agent are administered into the hepatic circulation.

[0255] In some embodiments, a single administration of a donor construct (e.g., bidirectional construct) comprising a sequence encoding Factor IX, guide RNA, and RNA-guided DNA binding agent is sufficient to increase expression of Factor IX to a desirable level. In other embodiments, more than one administration of a composition comprising a donor construct (e.g., bidirectional construct) comprising a sequence encoding Factor IX, guide RNA, and RNA-guided DNA binding agent may be beneficial to maximize therapeutic effects.

[0256] In some embodiments, the present disclosure includes combination therapies comprising any one or more of the gRNAs, donor construct (e.g., bidirectional construct comprising a sequence encoding Factor IX), and RNA-guided DNA binding agents (e.g., Cas nuclease) described herein together with an additional therapy suitable for treating hemophilia, as described above. For example, the methods of the present disclosure can be combined with the use of other hemostatic agents, blood factors, and medications. For example, the subject may be administered a therapeutically effective amount of one or more factors selected from the group consisting of factor XI, factor XII, prekallikrein, high molecular weight kininogen (HMWK), factor V, factor VII, factor VIII, factor X, factor XIII, factor II, factor VIIa, and von Willebrands factor.

[0257] In some embodiments, treatment may further comprise administering a procoagulant, such as an activator of the intrinsic coagulation pathway, including factor Xa, factor IXa, factor Xla, factor XIIa, and VIIIa, prekallekrein, and

high-molecular weight kininogen; or an activator of the extrinsic coagulation pathway, including tissue factor, factor VIIa, factor Va, and factor Xa.

[0258] This description and exemplary embodiments should not be taken as limiting. For the purposes of this specification and appended embodiments, unless otherwise indicated, all numbers expressing quantities, percentages, or proportions, and other numerical values used in the specification and embodiments, are to be understood as being modified in all instances by the term "about," to the extent they are not already so modified. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached embodiments are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the embodiments, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Human Factor IX Protein Sequence (SEQ ID NO: 700) NCBI Ref: NP_000124:
 MQRVNIMIAESPLGITICLLGYLSSAETVFLDHENAKILNRPKRYNSGKLEEFVQGNL
 ERECMEEKCSFEEAREVFENTERTTTEFWKQVYDGDQCESNPCLNGGCKDDINSYECWCP
 FGFGEGKNCELDVTCNIKNGRCEQFCCKNSADNKVVCSTEGYRLAENQKSCEPAVPPCGR
 VSVSQTSKLTRAETVFPDVYVNSTEAEILDNTQSTQSFNDFTRVGGEDAKPGQFPW
 QVVLNGKVDAGCGGSIVNEKWIVTAAHCVETGVKITVVAEHNIEETEHTEQKRNVIRII
 PHHNYNAAINKYNHDIALLELDEPLVLSNVYVTPICIADKEYTNIFLKFGSGYVSGWGRVF
 HKGRSLVLYQLRPLVDRATCLRSTKEPTIYNMFCAGFHEGGRDSQGDSGPHVTEVE
 GTSFLTGIIISWGEECAMKGKYIYTKVSRYNWIKEKTKL

Human Factor IX Nucleotide Sequence (SEQ ID NO: 706) NCBI Ref: NM_000133:
 1 accacttca caatctgcta gcaaaggta tgcagcgcgt gaacatgatc atggcagaat
 61 caccaggcct catcaccatc tgcccttttag gatatctact cagtgctgaa tgtacagtt
 121 ttcttgatca tggaaaacgc aacaaaattc tgaatcgcc aaagaggat aattcaggta
 181 aatttggaaa gttttgttcaa gggAACCTTG agagagaatg tatggaaagaa aagtgttagt
 241 ttgaaagaagc acggaaatgtt tttggaaaatc ctggaaaagac aactgaattt tggaaagcagt
 301 atgggtatgg agatcagtgt gagtccaaatc catgtttaa tggccggcagt tgcaggatg
 361 acattaattc ctatgaatgt tgggtccctt ttggatttga agggaaagaaac tggtaattag
 421 atgttaacatg taatctttag aatggcaatg ggcggcgtt ttgtaaaaat agtgtctgata
 481 acaagggtt ttgtctctgt ttgcggggat atcgacttgc agaaaaccag aagtccctgt
 541 aaccgcgtt gcattttcca tggtaaagag ttctgtttc acaaaacttc aagtcacc
 601 gtgtctgagac tttttttctt gatgtggact atgttaattt tactgaagct gaaaccattt
 661 tggataacatc actcaaaacatcat ttaatgactt cactccgggtt ttgggtggag
 721 aagatgcacca accaggcttta tttcccttggc aggttgggtttaa gatgtttaaa gttgtatc
 781 tctgttgagg ctctatcggtt aatggaaaat ggttggtaac tggtgttttccatc ttttttt
 841 ctgtgtttaa aatttacagtt gtgcgggtt aacataatat tgaggagaca aacatacac
 901 agcaaaaagc aaatgtgtt cgttatttttcc tccacaccaat ctacaatgc gctttaata
 961 agtacaacca tggatgttcc tttctggacat tggaccaacc cttagtgcata aacagctac
 1021 ttacacctat ttgcatgtt gacaaggaaat acacgaaatcat ttccctcaaa tttttgtatc
 1081 gctatgttaa tggctgggaa aggttctcc acaaaaggag atcagttta gtttttcat
 1141 accttagatgtt tcacttgcgtt gacccggccca catgttttcg atcttacaaag ttccatcat
 1201 atacaacatgtt gtttgcgtt ggttccatc aaggaggatg agtttcatgtt caaggagata
 1261 gtttttttttccatc ttttttttttccatc ttttttttttccatc ttttttttttccatc
 1321 ggggttggaaat gtttgcgtt gtttgcgtt gtttgcgtt gtttgcgtt gtttgcgtt
 1381 tcaactggat taaggaaaaaa acaaaatgttca cttatggaaat gatggatttc caaggtaat
 1441 tcatt
 1501 agatt
 1561 att
 1621 aatt
 1681 ctgtccatcat gatactatgg ttctccacta tggcaactaa ctcaacttcaat ttcccttcat
 1741 tagcggcatt ccattttccc gatctttttt gtttctccaa cccaaacatc aatgttttcat
 1801 agtttctgtt acatgttccatc ttatgtatc ttatgtatc ttatgtatc ttatgtatc
 1861 tggatgttcc ttt
 1921 ctt
 1981 ctcttt
 2041 catcatt
 2101 cgtatgttccatc ttatgtatc gatcatgtt atcatgtt atcaaaacccca gacttgcctt
 2161 ggaaaatgtt ttcttccatc ttt
 2221 taatataacaa tataaatata tagtgcgtt gttatgtatc ttatgtatc ttatgtatc
 2281 acacatataaa tggaaatgtt aagccatttctt aagatgttccatc atggatgttccatc
 2341 agggatgttccatc ttt
 2401 cccacacataa ttgttacttccatc ttt

-continued

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2461 ccgttcgttt gcaatctaca gctagtagag actttgagga agaattcaac agtgtgtctt
2521 caggcgtt cagagccaaq caagaqgtt aagtgccta gaccagggcataaagtatc
2581 atgtctccct taactagcat acccccgaat ggagaagggt gcagcggct caaaggcata
2641 agtcattcca atcagccaaq taagttgtcc tttctgggt tcgtgttac catggacat
2701 ttgattata gtaatcctt ctatcttcaa tcttcgtttag agttgtgtac caactgacgt
2761 atgtttccct ttgtgaatta ataaactggt gtctgggtt at

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Human Factor IX polypeptide (SEQ ID No: 701)
 YNSGKLEEFVQGNLERECMEEKSFEAREVFENTERTEFWKQYVDGDQCESNPCLNGGSCKDDINSYE
 CWCPFGFEGKNCEDVTCNIKGRCCEQFCCKNSADNKVVCSCTEGYRLAENQKSCPEAVPPPCGRVSVSQT
 SKLTRAETVFPDVYVNTEAETILDNITQSTQSFDTRVGGEDAKPQFPWQVVLNGKVDACCGGS
 VNEKWIVTAAHCVETGVKITVVAEGHNIETEHTEOKRNRVIRIIPHJHNYNAAINKYNHIALLEDEPLV
 LNSYVTPICIADKEYTNIFLKFGSGYVSGWGRVFHKGRSLALVQYLRVPLVDRATCLRSTKFTIYNNMFC
 AGFHEGGRDSCQDGGPHVTEVEGTSFLTGIISWGEECAMKGKYGIYTAKVSRVNWIEKTKLT

EXAMPLES

[0259] The following examples are provided to illustrate certain disclosed embodiments and are not to be construed as limiting the scope of this disclosure in any way.

Example 1—Materials and Methods

Cloning and Plasmid Preparation

[0260] A bidirectional insertion construct flanked by ITRs was synthesized and cloned into pUC57-Kan by a commercial vendor. The resulting construct (P00147) was used as the parental cloning vector for other vectors. The other insertion constructs (without ITRs) were also commercially synthesized and cloned into pUC57. Purified plasmid was digested with BglII restriction enzyme (New England Bio-Labs, cat #R0144S), and the insertion constructs were cloned into the parental vector. Plasmid was propagated in Stbl3™ Chemically Competent *E. coli* (Thermo Fisher, Cat #C737303).

AAV Production

[0261] Triple transfection in HEK293 cells was used to package genomes with constructs of interest for AAV8 and AAV-DJ production and resulting vectors were purified from both lysed cells and culture media through iodixanol gradient ultracentrifugation method (See, e.g., Lock et al., Hum Gene Ther. 2010 October; 21(10):1259-71). The plasmids used in the triple transfection that contained the genome with constructs of interest are referenced in the Examples by a “PXXXX” number, see also e.g., Table 9. Isolated AAV was dialyzed in storage buffer (PBS with 0.001% Pluronic F68). AAV titer was determined by qPCR using primers/probe located within the ITR region.

In Vitro Transcription (“IVT”) of Nuclease mRNA

[0262] Capped and polyadenylated *Streptococcus pyogenes* (“Spy”) Cas9 mRNA containing N1-methyl pseudo-U was generated by in vitro transcription using a linearized plasmid DNA template and T7 RNA polymerase. Generally, plasmid DNA containing a T7 promoter and a 100 nt poly (A/T) region was linearized by incubating at 37° C. with XbaI to complete digestion followed by heat inactivation of XbaI at 65° C. The linearized plasmid was purified from enzyme and buffer salts. The IVT reaction to generate Cas9 modified mRNA was incubated at 37° C. for 4 hours in the following conditions: 50 ng/µL linearized plasmid; 2 mM each of GTP, ATP, CTP, and N1-methyl pseudo-UTP (Trilink); 10 mM ARCA (Trilink); 5 U/µL T7 RNA polymerase (NEB); 1 U/µL Murine Rnase inhibitor (NEB); 0.004 U/µL Inorganic *E. coli* pyrophosphatase (NEB); and 1× reaction

buffer. TURBO Dnase (ThermoFisher) was added to a final concentration of 0.01 U/µL, and the reaction was incubated for an additional 30 minutes to remove the DNA template. The Cas9 mRNA was purified using a MegaClear Transcription Clean-up kit according to the manufacturer’s protocol (ThermoFisher). Alternatively, the Cas9 mRNA was purified using LiCl precipitation, ammonium acetate precipitation, and sodium acetate precipitation or using a LiCl precipitation method followed by further purification by tangential flow filtration. The transcript concentration was determined by measuring the light absorbance at 260 nm (Nanodrop), and the transcript was analyzed by capillary electrophoresis by Bioanalyzer (Agilent).

[0263] Cas9 mRNAs below comprise Cas9 ORF SEQ ID NO: 703 or SEQ ID NO: 704 or a sequence of Table 24 of PCT/US2019/053423 (which is hereby incorporated by reference).

Lipid Formulations for Delivery of Cas9 mRNA and gRNA

[0264] Cas9 mRNA and gRNA were delivered to cells and animals utilizing lipid formulations comprising ionizable lipid ((9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate), cholesterol, DSPC, and PEG2k-DMG.

[0265] For experiments utilizing pre-mixed lipid formulations (referred to herein as “lipid packets”), the components were reconstituted in 100% ethanol at a molar ratio of ionizable lipid:cholesterol:DSPC:PEG2k-DMG of 50:38:9:3, prior to being mixed with RNA cargos (e.g., Cas9 mRNA and gRNA) at a lipid amine to RNA phosphate (N:P) molar ratio of about 6.0, as further described herein.

[0266] For experiments utilizing the components formulated as lipid nanoparticles (LNPs), the components were dissolved in 100% ethanol at various molar ratios. The RNA cargos (e.g., Cas9 mRNA and gRNA) were dissolved in 25 mM citrate, 100 mM NaCl, pH 5.0, resulting in a concentration of RNA cargo of approximately 0.45 mg/mL.

[0267] For the experiments described in Example 2, the LNPs were formed by microfluidic mixing of the lipid and RNA solutions using a Precision Nanosystems NanoAssembler™ Benchtop Instrument, according to the manufacturer’s protocol. A 2:1 ratio of aqueous to organic solvent was maintained during mixing using differential flow rates. After mixing, the LNPs were collected, diluted in water (approximately 1:1 v/v), held for 1 hour at room temperature, and further diluted with water (approximately 1:1 v/v) before final buffer exchange. The final buffer exchange into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS) was

completed with PD-10 desalting columns (GE). If required, formulations were concentrated by centrifugation with Amicon 100 kDa centrifugal filters (Millipore). The resulting mixture was then filtered using a 0.2 m sterile filter. The final LNP was stored at -80° C. until further use. The LNPs were formulated at a molar ratio of ionizable lipid:cholesterol: DSPC:PEG2k-DMG of 45:44:9:2, with a lipid amine to RNA phosphate (N:P) molar ratio of about 4.5, and a ratio of gRNA to mRNA of 1:1 by weight.

[0268] For the experiments described in other examples, the LNPs were prepared using a cross-flow technique utilizing impinging jet mixing of the lipid in ethanol with two volumes of RNA solutions and one volume of water. The lipid in ethanol was mixed through a mixing cross with the two volumes of RNA solution. A fourth stream of water was mixed with the outlet stream of the cross through an inline tee (See WO2016010840 FIG. 2.). The LNPs were held for 1 hour at room temperature, and further diluted with water (approximately 1:1 v/v). Diluted LNPs were concentrated using tangential flow filtration on a flat sheet cartridge (Sartorius, 100kD MWCO) and then buffer exchanged by diafiltration into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS). Alternatively, the final buffer exchange into TSS was completed with PD-10 desalting columns (GE). If required, formulations were concentrated by centrifugation with Amicon 100 kDa centrifugal filters (Millipore). The resulting mixture was then filtered using a 0.2 m sterile filter. The final LNP was stored at 4° C. or -80° C. until further use. The LNPs were formulated at a molar ratio of ionizable lipid:cholesterol:DSPC:PEG2k-DMG of 50:38:9:3, with a lipid amine to RNA phosphate (N:P) molar ratio of about 6.0, and a ratio of gRNA to mRNA of 1:1 by weight.

Cell Culture and In Vitro Delivery of Cas9 mRNA, gRNA, and Insertion Constructs

Hepa1-6 cells

[0269] Hepa 1-6 cells were plated at density of 10,000 cells/well in 96-well plates. 24 hours later, cells were treated with LNP and AAV. Before treatment the media was aspirated off from the wells. LNP was diluted to 4 ng/ul in DMEM+10% FBS media and further diluted to 2 ng/ul in 10% FBS (in DMEM) and incubated at 37° C. for 10 min (at a final concentration of 5% FBS). Target MOI of AAV was 1e6, diluted in DMEM+10% FBS media. 50 µl of the above diluted LNP at 2 ng/ul was added to the cells (delivering a total of 100 ng of RNA cargo) followed by 50 µl of AAV. The treatment of LNP and AAV were minutes apart. Total volume of media in cells was 100 µl. After 72 hours post-treatment and 30 days post-treatment, supernatant from these treated cells were collected for human FIX ELISA analysis as described below.

Primary Hepatocytes

[0270] Primary mouse hepatocytes (PMH), primary cyno hepatocytes (PCH) and primary human hepatocytes (PHH) were thawed and resuspended in hepatocyte thawing medium with supplements (ThermoFisher) followed by centrifugation. The supernatant was discarded, and the pelleted cells resuspended in hepatocyte plating medium plus supplement pack (ThermoFisher). Cells were counted and plated on Bio-coat collagen I coated 96-well plates at a density of 33,000 cells/well for PHH and 50,000 cells/well for PCH and 15,000 cells/well for PMH. Plated cells were allowed to settle and adhere for 5 hours in a tissue culture incubator at

37° C. and 5% CO₂ atmosphere. After incubation cells were checked for monolayer formation and were washed thrice with hepatocyte maintenance prior and incubated at 37° C.

[0271] For experiments utilizing lipid packet delivery, Cas9 mRNA and gRNA were each separately diluted to 2 mg/ml in maintenance media and 2.9 µl of each were added to wells (in a 96-well Eppendorf plate) containing 12.5 µl of 50 mM sodium citrate, 200 mM sodium chloride at pH 5 and 6.9 µl of water. 12.5 µl of lipid packet formulation was then added, followed by 12.5 µl of water and 150 µl of TSS. Each well was diluted to 20 ng/µl (with respect to total RNA content) using hepatocyte maintenance media, and then diluted to 10 ng/µl (with respect to total RNA content) with 6% fresh mouse serum. Media was aspirated from the cells prior to transfection and 40 µl of the lipid packet/RNA mixtures were added to the cells, followed by addition of AAV (diluted in maintenance media) at an MOI of 1e5. Media was collected 72 hours post-treatment for analysis and cells were harvested for further analysis, as described herein.

Luciferase Assays

[0272] For experiments involving NanoLuc detection in cell media, one volume of Nano-Glo® Luciferase Assay Substrate was combined with 50 volumes of Nano-Glo® Luciferase Assay Buffer. The assay was run on a Promega Glomax runner at an integration time of 0.5 sec using 1:10 dilution of samples (50 µl of reagent+40 µl water+10 µl cell media).

[0273] For experiments involving detection of the HiBit tag in cell media, LgBiT Protein and Nano-GloR HiBiT Extracellular Substrate were diluted 1:100 and 1:50, respectively, in room temperature Nano-GloR HiBiT Extracellular Buffer. The assay was run on a Promega Glomax runner at an integration time of 1.0 sec using 1:10 dilution of samples (50 µl of reagent+40 µl water+10 µl cell media).

In Vivo Delivery of LNP and/or AAV

[0274] Mice were dosed with AAV, LNP, both AAV and LNP, or vehicle (PBS+0.001% Pluronic for AAV vehicle, TSS for LNP vehicle) via the lateral tail vein. AAV were administered in a volume of 0.1 mL per animal with amounts (vector genomes/mouse, "vg/ms") as described herein. LNPs were diluted in TSS and administered at amounts as indicated herein, at about 5 µl/gram body weight. Typically, mice were injected first with AAV and then with LNP, if applicable. At various times points post-treatment, serum and/or liver tissue was collected for certain analyses as described further below.

Human Factor IX (hFIX) ELISA Analysis

[0275] For in vitro studies, total human Factor IX levels secreted in cell media were determined using a Human Factor IX ELISA Kit (Abcam, Cat #ab188393) according to manufacturer's protocol. Secreted hFIX levels were quantitated off a standard curve using 4 parameter logistic fit and expressed as ng/ml of media.

[0276] For in vivo studies, blood was collected and the serum or plasma was isolated as indicated. The total human Factor IX levels were determined using a Human Factor IX ELISA Kit (Abcam, Cat #ab188393) according to manufacturer's protocol. Serum or plasma hFIX levels were quantitated off a standard curve using 4 parameter logistic fit and expressed as µg/mL of serum.

Next-Generation Sequencing (“NGS”) and Analysis for On-Target Cleavage Efficiency

[0277] Deep sequencing was utilized to identify the presence of insertions and deletions introduced by gene editing, e.g., within intron 1 of albumin. PCR primers were designed around the target site and the genomic area of interest was amplified. Primer sequence design was done as is standard in the field.

[0278] Additional PCR was performed according to the manufacturer’s protocols (Illumina) to add chemistry for sequencing. The amplicons were sequenced on an Illumina MiSeq instrument. The reads were aligned to the reference genome after eliminating those having low quality scores. The resulting files containing the reads were mapped to the reference genome (BAM files), where reads that overlapped the target region of interest were selected and the number of wild type reads versus the number of reads which contain an insertion or deletion (“indel”) was calculated.

[0279] The editing percentage (e.g., the “editing efficiency” or “percent editing”) is defined as the total number of sequence reads with insertions or deletions (“indels”) over the total number of sequence reads, including wild type.

In Situ Hybridization Analysis

[0280] BaseScope (ACDbio, Newark, CA) is a specialized RNA in situ hybridization technology that can provide specific detection of exon junctions, e.g., in a hybrid mRNA transcript that contains an insertion transgene (hFIX) and coding sequence from the site of insertion (e.g. exon 1 of albumin). BaseScope was used to measure the percentage of liver cells expressing the hybrid mRNA.

[0281] To detect the hybrid mRNA, two probes against the hybrid mRNAs that may arise following insertion of a bidirectional construct were designed by ACDbio (Newark, CA). One of the probes was designed to detect a hybrid mRNA resulting from insertion of the construct in one orientation, while the other probe was designed to detect a hybrid mRNA resulting from insertion of the construct in the other orientation. Livers from different groups of mice were collected and fresh-frozen sectioned. The BaseScope assay, using a single probe or pooled probes was performed according to the manufacturer’s protocol. Slides were scanned and analyzed by the HALO software. The background (saline treated group) of this assay was 0.58%.

Example 2—In Vitro Testing of Insertion Templates with and without Homology Arms

[0282] In this Example, Hepa1-6 cells were cultured and treated with AAV harboring insertion templates of various forms (e.g., having either a single-stranded genome (“ssAAV”) or a self-complementary genome (“scAAV”)), in the presence or absence of LNP delivering Cas9 mRNA and G000551 e.g., as described in Example 1 (n=3). The AAV and LNP were prepared as described in Example 1. Following treatment, the media was collected for human Factor IX levels as described in Example 1.

[0283] Hepa1-6 cells are an immortalized mouse liver cell line that continues to divide in culture. As shown in FIG. 2 (72 hour post-treatment time point), only the vector (scAAV derived from plasmid P00204) comprising 200 bp homology arms resulted in detectable expression of hFIX. Use of the AAV vectors derived from P00123 (scAAV lacking homol-

ogy arms) and P00147 (ssAAV bidirectional construct lacking homology arms) did not result in any detectable expression of hFIX in this experiment. The cells were kept in culture and these results were confirmed when re-assayed at 30 days post-treatment (data not shown).

Example 3—In Vivo Testing of Insertion Templates with and without Homology Arms

[0284] In this Example, mice were treated with AAV derived from the same plasmids (P00123, P00204, and P00147) as tested in vitro in Example 2. The dosing materials were prepared and dosed as described in Example 1. C57Bl/6 mice were dosed (n=5 for each group) with 3e11 vector genomes each (vg/ms) followed by LNP comprising G000551 (“G551”) at a dose of 4 mg/kg (with respect to total RNA cargo content). Four weeks post dose, the animals were euthanized and liver tissue and sera were collected for editing and hFIX expression, respectively.

[0285] As shown in FIG. 3A and Table 12, liver editing levels of ~60% were detected in each group of animals treated with LNP comprising gRNA targeting intron 1 of murine albumin. However, despite robust and consistent levels of editing in each treatment group, animals receiving the bi-directional vector without homology arms (ssAAV vector derived from P00147) in combination with LNP treatment resulted in the highest level of hFIX expression in serum (FIG. 3B and Table 13).

TABLE 12

Template	% Indel	
	Average Indel (%)	St. Dev Indel (%)
scAAV Blunt (P00123)	66.72	4.09
ssAAV Blunt (P00147)	68.10	2.27
ssAAV HR (P00204)	70.16	3.68
LNP only	68.24	6.47
Vehicle	0.28	0.08

TABLE 13

Template	Factor IX Levels	
	Average Factor IX (ug/mL)	St.Dev Factor IX (ug/mL)
scAAV Blunt (P00123)	0.75	0.28
ssAAV Blunt (P00147)	2.92	1.04
ssAAV HR (P00204)	0.96	0.35
LNP only	0	0
Vehicle	0	0

Example 4—In Vivo Testing of ssAAV Insertion Templates with and without Homology Arms

[0286] The experiment described in this example examined the effect of incorporating homology arms into ssAAV vectors *in vivo*.

[0287] The dosing materials used in this experiment were prepared and dosed as described in Example 1. C57Bl/6 mice were dosed (n=5 for each group) with 3e11 vg/ms followed by LNP comprising G000666 (“G666”) or G000551 (“G551”) at a dose of 0.5 mg/kg (with respect to total RNA cargo content). Four weeks post dose, the animals sera was collected for hFIX expression.

[0288] As shown in FIG. 4A and Table 14, use of the ssAAV vectors with asymmetrical homology arms (300/600 bp arms, 300/2000 bp arms, and 300/1500 bp arms for vectors derived from plasmids P00350, P00356, and P00362, respectively) for insertion into the site targeted by G551 resulted in levels of circulating hFIX that were below the lower limit of detection for the assay. However, use of the ssAAV vector (derived from P00147) without homology arms and having two hFIX open reading frames (ORF) in a bidirectional orientation resulted in detectable levels of circulating hFIX in each animal.

[0289] Similarly, use of the ssAAV vectors with asymmetrical homology arms (500 bp arms and 800 bp arms for vectors derived from plasmids P00353 and P00354, respectively) for insertion into the site targeted by G666 resulted in lower but detectable levels, as compared to use of the bidirectional vector without homology arms (derived from P00147) (see FIG. 4B and Table 15).

TABLE 14

Serum hFIX Levels		
AAV	Average Serum FIX (ug/mL)	St.Dev Serum FIX (ug/mL)
P00147	5.13	1.31
P00350	-0.22	0.08
P00356	-0.23	0.04
P00362	-0.09	0.16

TABLE 15

Serum hFIX Levels		
AAV	Average Serum FIX (ug/mL)	St.Dev Serum FIX (ug/mL)
P00147	7.72	4.67
P00353	0.20	0.23
P00354	0.46	0.26

Example 5—In Vitro Screening of Bidirectional Constructs Across Target Sites in Primary Mouse Hepatocytes

[0290] Having demonstrated that bidirectional constructs lacking homology arms outperformed vectors with other configurations, the experiment described in this Example examined the effects of altering the splice acceptors used to form the hybrid transcript between hFIX and exon 1 of albumin and altering the gRNAs for targeting CRISPR/Cas9-mediated insertion. These varied bidirectional constructs were tested across a panel of target sites utilizing 20 different gRNAs targeting intron 1 of murine albumin in primary mouse hepatocytes (PMH).

[0291] The ssAAV and lipid packet delivery materials tested in this Example were prepared and delivered to PMH as described in Example 1, with the AAV at an MOI of 1e5. Following treatment, isolated genomic DNA and cell media was collected for editing and transgene expression analysis, respectively. Each of the vectors comprised a reporter that can be measured through luciferase-based fluorescence detection as described in Example 1, plotted in FIG. 5C as relative luciferase units (“RLU”). The vectors comprised a HiBit peptide fused at the 3' ends of the hFIX ORF, which allows for sensitive detection of relative expression. Schematics of each vector tested are provided in FIG. 5A. The gRNAs tested are shown in FIGS. 5B and 5C, using a shortened number for those listed in Table 5 (e.g., where the leading zeros are omitted, for example where “G551” corresponds to “G000551” in Table 5).

[0292] As shown in FIG. 5B and Table 16, consistent but varied levels of editing were detected for each of the treatment groups across each combination tested. Transgene expression using various combinations of template and guide RNA is shown in FIG. 5C and Table 17. As shown in FIG. 5D, a significant level of indel formation did not necessarily result in more efficient expression of the transgenes. Using P00411- and P00418-derived templates, the R² values were 0.54 and 0.37, respectively, when guides with less than 10% editing are not included. The mouse albumin splice acceptor and human FIX splice acceptor each resulted in effective transgene expression.

TABLE 16

Guide ID	% Indel					
	P00411		P00418		P00415	
	Average Indel (%)	St. Dev Indel (%)	Average Indel (%)	St. Dev Indel (%)	Average Indel (%)	St. Dev Indel (%)
G000551	67.4	1.42	70.67	2.29	66.73	4.90
G000552	90.93	0.15	91.10	2.43	90.37	1.01
G000553	77.80	3.83	77.47	1.87	80.50	0.85
G000554	72.37	6.49	70.53	3.16	70.60	2.91
G000555	35.37	2.63	35.77	9.34	40.47	4.75
G000666	62.47	3.87	50.90	19.41	65.90	3.99
G000667	30.57	2.73	25.30	3.67	31.67	2.29
G000668	63.60	2.02	66.65	4.60	68.30	4.90
G000669	19.10	2.51	19.33	1.53	18.70	1.25
G000670	47.80	3.27	49.10	4.42	51.97	2.06
G011722	4.20	0.72	4.27	1.20	4.20	0.26
G011723	5.63	1.27	6.07	0.15	5.93	0.15
G011724	6.10	1.28	8.50	2.69	7.13	1.27
G011725	1.93	0.29	2.60	0.79	2.53	0.65
G011726	10.73	1.46	11.70	0.50	12.43	1.33
G011727	14.20	1.56	14.80	2.36	16.20	2.69
G011728	10.55	1.20	13.65	0.92	15.50	1.56
G011729	5.00	0.10	5.63	0.25	6.00	1.01

TABLE 16-continued

Guide ID	% Indel					
	P00411		P00418		P00415	
	Average Indel (%)	St. Dev Indel (%)	Average Indel (%)	St. Dev Indel (%)	Average Indel (%)	St. Dev Indel (%)
G011730	7.83	0.97	9.13	0.59	7.33	0.59
G011731	23.70	0.66	25.27	1.21	24.87	1.01
AAV Only	0.15	0.07	0.05	0.07	0.10	0.00

TABLE 17

Guide ID	Luciferase Levels					
	P00411		P00418		P00415	
	Average Luciferase (RLU)	St. Dev Luciferase (RLU)	Average Luciferase (RLU)	St. Dev Luciferase (RLU)	Average Luciferase (RLU)	St. Dev Luciferase (RLU)
G000551	58000.00	4331.28	41800.00	2165.64	78633.33	20274.70
G000552	95700.00	10573.08	80866.67	27911.35	205333.33	30664.86
G000553	205333.33	52993.71	177333.33	32929.22	471666.67	134001.00
G000554	125333.33	55949.38	91933.33	19194.10	232666.67	67002.49
G000555	59933.33	11566.04	77733.33	11061.80	155666.67	15947.83
G000666	88500.00	28735.87	93266.67	30861.19	313000.00	15394.80
G000667	75333.33	22653.11	68966.67	27222.11	153000.00	30805.84
G000668	164000.00	56320.51	133400.00	65111.29	429000.00	120751.80
G000669	28933.33	11636.29	22033.33	2413.16	46466.67	6543.19
G000670	162666.67	32959.57	200000.00	33867.39	424666.67	36473.73
G011722	16766.67	3384.28	8583.33	4103.10	24000.00	8915.16
G011723	22733.33	7252.82	17133.33	4905.44	26100.00	8109.87
G011724	17300.00	2400.00	28033.33	9091.94	30933.33	3365.02
G011725	8253.33	1163.20	8890.00	1429.27	20366.67	13955.05
G011726	12223.33	3742.54	11610.00	2490.44	14950.00	8176.03
G011727	35600.00	8128.35	36300.00	12301.22	86700.00	5023.94
G011728	14900.00	5011.99	22466.67	7130.45	38166.67	13829.08
G011729	10460.00	2543.95	11223.33	2220.28	26966.67	16085.50
G011730	14833.33	2307.24	21700.00	8681.59	41233.33	25687.03
G011731	16433.33	3274.65	22566.67	2205.30	20756.67	13096.20
AAV Only	217.00	15.56	215.00	15.56	207.00	1.41

Example 6—In Vivo Screening of Bidirectional Constructs Across Target Sites

[0293] The ssAAV and LNPs tested in this Example were prepared and delivered to C57Bl/6 mice as described in Example 1 to assess the performance of the bidirectional constructs across target sites in vivo. Four weeks post dose, the animals were euthanized and liver tissue and sera were collected for editing and hFIX expression, respectively.

[0294] In an initial experiment, 10 different LNP formulations containing 10 different gRNA targeting intron 1 of albumin were delivered to mice along with ssAAV derived from P00147. The AAV and LNP were delivered at 3e11 vg/ms and 4 mg/kg (with respect to total RNA cargo content), respectively (n=5 for each group). The gRNAs tested in this experiment are shown in FIG. 6 and tabulated in Table 18. As shown in FIG. 6 and as observed in vitro, a significant level of indel formation was not predictive for insertion or expression of the transgenes.

[0295] In a separate experiment, a panel of 20 gRNAs targeting the 20 different target sites tested in vitro in Example 5 were tested in vivo. To this end, LNP formulations containing the 20 gRNAs targeting intron 1 of albumin were delivered to mice along with ssAAV derived from P00147. The AAV and LNP were delivered at 3e11 vg/ms

and 1 mg/kg (with respect to total RNA cargo content), respectively. The gRNAs tested in this experiment are shown in FIGS. 7A and 7B.

[0296] As shown, in FIG. 7A and tabulated in Table 19, varied levels of editing were detected for each of the treatment groups across each LNP/vector combination tested. However, as shown in FIG. 7B and Table 20 and consistent with the in vitro data described in Example 5, higher levels of editing did not necessarily result in higher levels of expression of the transgenes in vivo, indicating a lack of correlation between editing and insertion/expression of the bidirectional hFIX constructs. Indeed, very little correlation exists between the amount of editing achieved and the amount of hFIX expression as viewed in the plot provided in FIG. 7D. In particular, an R² value of only 0.34 is calculated between the editing and expression data sets for this experiment, when those gRNAs achieving less than 10% editing are removed from the analysis. Interestingly, as shown in FIG. 7C, a correlation plot is provided comparing the levels of expression as measured in RLU from the in vitro experiment of Example 5 to the transgene expression levels in vivo detected in this experiment, with an R² value of 0.70, demonstrating a positive correlation between the primary cell screening and the in vivo treatments.

[0297] To assess insertion of the bidirectional construct at the cellular level, liver tissues from treated animals were assayed using an *in situ* hybridization method (BaseScope), e.g., as described in Example 1. This assay utilized probes that can detect the junctions between the hFIX transgene and the mouse albumin exon 1 sequence, as a hybrid transcript. As shown in FIG. 8A, cells positive for the hybrid transcript were detected in animals that received both AAV and LNP. Specifically, when AAV alone is administered, less than 1.0% of cells were positive for the hybrid transcript. With administration of LNPs comprising G011723, G000551, or G000666, 4.9%, 19.8%, or 52.3% of cells were positive for the hybrid transcript. Additionally, as shown in FIG. 8B, circulating hFIX levels correlated with the number of cells that were positive for the hybrid transcript. Lastly, the assay utilized pooled probes that can detect insertion of the bidirectional hFIX construct in either orientation. However, when a single probe was used that only detects a single orientation, the amount of cells that were positive for the hybrid transcript was about half that detected using the pooled probes (in one example, 4.46% vs 9.68%), suggesting that the bidirectional construct indeed is capable of inserting in either orientation giving rise to expressed hybrid transcripts that correlate with the amount of transgene expression at the protein level. These data show that the circulating hFIX levels achieved are dependent on the guide used for insertion.

TABLE 18

hFIX Serum Levels and % Indel				
Guide	Average Indel (%)	St. Dev Indel (%)	Average hFIX Serum Levels	St. Dev hFIX Serum Levels
G000551	75.02	1.27	3.82	3.38
G000555	51.18	1.19	32.56	9.05
G000553	62.78	2.64	25.07	4.04
G000667	52.96	4.96	32.03	6.74
G000554	55.24	2.28	29.48	7.34
G000552	67.56	1.73	14.79	5.34
G000668	43.14	5.78	26.72	7.97
G000669	50.68	2.97	10.70	4.43
G000666	64.62	1.34	26.19	5.56
G000670	55.90	1.30	30.96	8.44

TABLE 19

% Liver Editing		
Guide	Average Liver Editing (%)	St. Dev Liver Editing (%)
G000551	59.48	4.02
G000555	58.72	3.65
G000553	51.26	2.81
G000554	33.04	8.76
G000555	12.72	4.46
G000666	53.60	4.92
G000667	26.74	4.98
G000668	39.22	3.04
G000669	33.34	4.77
G000670	47.50	5.58
G011722	10.34	1.68
G011723	4.02	0.84
G011724	2.46	0.64
G011725	8.26	1.24
G011726	6.90	1.01
G011727	13.33	6.43
G011728	35.78	9.34

TABLE 19-continued

Guide	% Liver Editing	
	Average Liver Editing (%)	St. Dev Liver Editing (%)
G011729	4.62	1.46
G011730	12.68	3.14
G011731	26.70	1.86

TABLE 20

Guide	Serum hFIX Levels					
	Week 1		Week 2		Week 4	
	Average FIX (ug/mL)	St. Dev FIX (ug/mL)	Average FIX (ug/mL)	St. Dev FIX (ug/mL)	Average FIX (ug/mL)	St. Dev FIX (ug/mL)
G000551	10.88	2.74	10.25	2.51	9.39	3.48
G000555	13.34	2.09	12.00	2.75	12.43	2.57
G000553	17.64	4.34	20.27	6.35	15.31	2.43
G000554	12.79	4.99	14.29	6.09	12.74	4.93
G000555	11.94	5.79	11.99	5.76	8.61	4.02
G000666	21.63	1.32	20.65	1.55	17.23	0.62
G000667	16.77	2.86	12.35	2.85	12.57	5.60
G000668	21.35	1.51	18.20	3.18	17.72	2.25
G000669	5.76	2.10	6.72	2.93	3.39	0.78
G000670	18.18	2.17	19.16	3.05	15.49	3.61
G011722	8.07	1.74	7.74	2.41	8.07	1.74
G011723	2.11	0.28	1.65	0.28	2.11	0.28
G011724	0.92	0.43	0.60	0.30	0.92	0.43
G011725	1.75	0.77	1.14	0.67	1.75	0.77
G011726	0.59	0.30	1.01	0.64	0.59	0.30
G011727	6.71	2.80	6.90	3.68	6.71	2.80
G011728	11.77	3.12	12.29	3.43	11.77	3.12
G011729	0.94	0.35	0.89	0.29	0.94	0.35
G011730	5.93	1.77	6.33	1.73	5.93	1.77
G011731	3.56	0.87	3.78	0.50	3.56	0.87
AAV Only	0.00	0.00	0.00	0.00	0.00	0.00
Vehicle	0.00	0.00	0.00	0.00	0.00	0.00
Human Serum	3.63	0.32	3.61	0.35	3.28	0.03

Example 7—Timing of AAV and LNP Delivery In Vivo

[0298] In this Example, the timing between delivery of ssAAV comprising the bidirectional hFIX construct and LNP was examined in C57Bl/6 mice.

[0299] The ssAAV and LNPs tested in this Example were prepared and delivered to mice as described in Example 1. The LNP formulation contained G000551 and the bidirectional template was delivered as ssAAV derived from P00147. The AAV and LNP were delivered at 3e11 vg/ms and 4 mg/kg (with respect to total RNA cargo content), respectively (n=5 for each group). A “Template only” cohort received AAV only, and a “PBS” cohort received no AAV or LNP. One cohort received AAV and LNP sequentially (minutes apart) at day 0 (“Template+LNP day 0”); another cohort received AAV at day 0 and LNP at day 1 (“Template+LNP day 1”); and a final cohort received AAV at day 0 and LNP at day 7 (“Template+LNP day 7”). At 1 week, 2 weeks and 6 weeks, plasma was collected for hFIX expression analysis.

[0300] As shown in FIG. 9, hFIX was detected in each cohort at each time assayed, except for the 1 week timepoint for the cohort that received the LNP at day 7 post AAV delivery.

Example 8—Multiple Dosing of LNP Following Delivery of AAV

[0301] In this Example, the effects of repeat dosing of LNP following administration of ssAAV was examined.

[0302] The ssAAV and LNPs tested in this Example were prepared and delivered to C57Bl/6 mice as described in Example 1. The LNP formulation contained G000551 and the ssAAV was derived from P00147. The AAV and LNP were delivered at 3e11 vg/ms and 0.5 mg/kg (with respect to total RNA cargo content), respectively (n=5 for each group). A “Template only” cohort received AAV only, and a “PBS” cohort received no AAV or LNP. One cohort received AAV and LNP sequentially (minutes apart) at day 0 with no further treatments (“Template+LNP(1x)” in FIG. 10); another cohort received AAV and LNP sequentially (minutes apart) at day 0 and a second dose at day 7 (“Template+LNP(2x)” in FIG. 10); and a final cohort received AAV and LNP sequentially (minutes apart) at day 0, a second dose of LNP at day 7 and a third dose of LNP at day 14 (“Template+LNP(3x)” in FIG. 10). At 1, 2, 4 and 6 weeks post-administration of AAV, plasma was collected for hFIX expression analysis.

[0303] As shown in FIG. 10, hFIX was detected in each cohort at each time assayed, and multiple subsequent doses of LNP did not significantly increase the amount of hFIX expression.

Example 9—Durability of hFIX Expression In Vivo

[0304] The durability of hFIX expression over time in treated animals was assessed in this Example. To this end, hFIX was measured in the serum of treated animals post-dose, as part of a one-year durability study.

[0305] The ssAAV and LNPs tested in this Example were prepared and delivered to C57Bl/6 mice as described in Example 1. The LNP formulation contained G000551 and the ssAAV was derived from P00147. The AAV was delivered at 3e11 vg/ms and the LNP was delivered at either 0.25 or 1.0 mg/kg (with respect to total RNA cargo content) (n=5 for each group).

[0306] As shown in FIG. 11A and Table 21, hFIX expression was sustained at each time point assessed for both groups out to 41 weeks. A drop in the levels observed at 8 weeks is believed to be due to the variability of the ELISA assay. Serum albumin levels were measured by ELISA at week 2 and week 41, showing that circulating albumin levels are maintained across the study.

[0307] As shown in FIG. 11B and Table 22, hFIX expression was sustained at each time point assessed for both groups out to 52 weeks.

TABLE 21

Week	FIX Levels			
	Dose			
	0.25 mpk LNP		1 mpk LNP	
Week	Average hFIX (ug/mL)	StDev hFIX (ug/mL)	Average hFIX (ug/mL)	StDev hFIX (ug/mL)
2	0.48	0.21	2.24	1.12
4	0.55	0.18	2.82	1.67
8	0.40	0.17	1.72	0.77
12	0.48	0.20	2.85	1.34

TABLE 21-continued

Week	FIX Levels			
	Dose			
	0.25 mpk LNP		1 mpk LNP	
Week	Average hFIX (ug/mL)	StDev hFIX (ug/mL)	Average hFIX (ug/mL)	StDev hFIX (ug/mL)
20	0.48	0.27	2.45	1.26
41	0.79	0.49	4.63	0.95

TABLE 22

Week	FIX Levels			
	Dose			
	0.25 mpk LNP		1 mpk LNP	
Week	Average hFIX (ug/mL)	StDev hFIX (ug/mL)	Average hFIX (ug/mL)	StDev hFIX (ug/mL)
2	0.87	0.15	4.02	1.75
8	0.99	0.15	4.11	1.41
12	0.93	0.14	4.15	1.35
20	0.83	0.22	4.27	1.54
41	0.83	0.37	4.76	1.62
52	0.82	0.25	4.72	1.54

Example 10—Effects of Varied Doses of AAV and LNP to Modulate hFIX Expression In Vivo

[0308] In this Example, the effects of varying the dose of both AAV and LNP to modulate expression of hFIX was assessed in C57Bl/6 mice.

[0309] The ssAAV and LNPs tested in this Example were prepared and delivered to mice as described in Example 1. The LNP formulation contained G000553 and the ssAAV was derived from P00147. The AAV was delivered at 1e11, 3e11, 1e12 or 3e12 vg/ms and the LNP was delivered at 0.1, 0.3, or 1.0 mg/kg (with respect to total RNA cargo content) (n=5 for each group). Two weeks post-dose, the animals were euthanized. Sera were collected at two timepoints for hFIX expression analysis.

[0310] As shown in FIG. 12A (1 week), FIG. 12B (2 weeks) and Table 23, varying the dose of either AAV or LNP can modulate the amount of expression of hFIX in vivo.

TABLE 23

Timepoint	Serum hFIX				
	RNP Dose (mg/kg)	AAV Dose (MOI)	Mean FIX (ng/ml)	SD	N
Week 1	0.1	1E+11	0.08	0.02	2
		3E+11	0.11	0.04	5
		1E+12	0.41	0.15	5
		3E+12	0.61	0.17	5
	0.3	1E+11	0.36	0.14	5
		3E+11	0.67	0.26	5
	1E+12	1E+12	1.76	0.14	5
		3E+12	4.70	2.40	5

TABLE 23-continued

Serum hFIX					
Timepoint	RNP Dose (mg/kg)	AAV Dose (MOI)	Mean FIX (ng/ml)	SD	N
Week 2	1.0	1E+11	3.71	0.31	4
		3E+11	8.00	0.51	5
		1E+12	14.17	1.38	5
		3E+12	20.70	2.79	5
	0.1	Human serum 1:1000	6.62	—	1
		1E+11	0.12	0.01	2
		3E+11	0.26	0.07	5
		1E+12	0.83	0.24	5
	0.3	3E+12	1.48	0.35	5
		1E+11	0.70	0.26	4
		3E+11	1.42	0.37	5
		1E+12	3.53	0.49	5
	1.0	3E+12	8.94	4.39	5
		1E+11	5.40	0.47	4
		3E+11	12.31	2.45	5
		1E+12	17.89	1.95	5
	Human serum 1:1000	3E+12	25.52	3.62	5
			4.47	—	1

Example 11—In Vitro Screening of Bidirectional Constructs Across Target Sites in Primary Cynomolgus and Primary Human Hepatocytes

[0311] In this Example, ssAAV vectors comprising a bidirectional construct were tested across a panel of target sites utilizing gRNAs targeting intron 1 of cynomolgus (“cyno”) and human albumin in primary cyno (PCH) and primary human hepatocytes (PHH), respectively.

[0312] The ssAAV and lipid packet delivery materials tested in this Example were prepared and delivered to PCH and PHH as described in Example 1. Following treatment, isolated genomic DNA and cell media was collected for editing and transgene expression analysis, respectively. Each of the vectors comprised a reporter that can be measured through luciferase-based fluorescence detection as described in Example 1 (derived from plasmid P00415), plotted in FIGS. 13B and 14B as relative luciferase units (“RLU”). For example, the AAV vectors contained the NanoLuc ORF (in addition to GFP). Schematics of the vectors tested are provided in FIGS. 13B and 14B. The gRNAs tested are shown in each of the Figures using a shortened number for those listed in Table 1 and Table 7.

[0313] As shown in FIG. 13A for PCH and FIG. 14A for PHH, varied levels of editing were detected for each of the combinations tested (editing data for some combinations tested in the PCH experiment are not reported in FIG. 13A and Table 3 due to failure of certain primer pairs used for the amplicon based sequencing). The editing data shown in FIGS. 13A and 14A graphically, are reproduced numerically in Table 3 and Table 4 below. However, as shown in FIGS. 13B, 13C and FIGS. 14B and 14C, a significant level of indel formation was not predictive for insertion or expression of the transgenes, indicating little correlation between editing and insertion/expression of the bidirectional constructs in PCH and PHH, respectively. As one measure, the R² value calculated in FIG. 13C is 0.13, and the R² value of FIG. 14D is 0.22.

[0314] Additionally, ssAAV vectors comprising a bidirectional construct were tested across a panel of target sites utilizing single guide RNAs targeting intron 1 of human albumin in primary human hepatocytes (PHH).

[0315] The ssAAV and LNP materials were prepared and delivered to PHH as described in Example 1. Following treatment, isolated genomic DNA and cell media was collected for editing and transgene expression analysis, respectively. Each of the vectors comprised a reporter that can be measured through luciferase-based fluorescence detection as described in Example 1 (derived from plasmid P00415), plotted in FIG. 14D as relative luciferase units (“RLU”) and tabulated in Table 24 below. For example, the AAV vectors contained the NanoLuc ORF (in addition to GFP). Schematics of the vectors tested are provided in FIGS. 13B and 14B. The gRNAs tested are shown in FIG. 14D using a shortened number for those listed in Table 1 and Table 7.

TABLE 3

Albumin intron 1 editing data for sgRNAs delivered to primary cynomolgus hepatocytes		
GUIDE ID	Avg % Edit	Std Dev % Edit
G009867	25.05	0.21
G009866	18.7	3.96
G009876	14.85	4.88
G009875	12.85	2.33
G009874	28.25	6.01
G009873	42.65	5.59
G009865	59.15	0.21
G009872	48.15	3.46
G009871	46.5	5.23
G009864	33.2	8.34
G009863	54.8	12.45
G009862	44.6	7.21
G009861	28.65	0.21
G009860	33.2	7.07
G009859	0.05	0.07
G009858	14.65	1.77
G009857	23	0.99
G009856	14.8	0.99
G009851	1.5	0.42
G009868	12.15	2.47
G009850	63.45	13.93
G009849	57.55	8.27
G009848	33	5.37
G009847	66.75	7
G009846	61.85	5.02
G009845	54.4	7.5
G009844	47.15	2.05

TABLE 4

Albumin intron 1 editing data for sgRNAs delivered to primary human hepatocytes		
GUIDE ID	Avg % Edit	Std Dev % Edit
G009844	19.07	2.07
G009851	0.43	0.35
G009852	47.20	3.96
G009857	0.10	0.14
G009858	8.63	9.16
G009859	3.07	3.50
G009860	18.80	4.90
G009861	10.27	2.51
G009866	13.60	13.55
G009867	12.97	3.04
G009868	0.63	0.32
G009874	49.13	0.60
G012747	3.83	0.23
G012748	1.30	0.35
G012749	9.77	1.50
G012750	42.73	4.58
G012751	7.77	1.16

TABLE 4-continued

Albumin intron 1 editing data for sgRNAs delivered to primary human hepatocytes		
GUIDE ID	Avg % Edit	Std Dev % Edit
G012752	32.93	2.27
G012753	21.20	2.95
G012754	0.60	0.10
G012755	1.10	0.10
G012756	2.17	0.40
G012757	1.07	0.25
G012758	0.90	0.10
G012759	2.60	0.35
G012760	39.10	6.58
G012761	36.17	2.43
G012762	8.50	0.57
G012763	47.07	3.07
G012764	44.57	5.83
G012765	19.90	1.68
G012766	8.50	0.28

TABLE 24

hAlb Guide Screen Luciferase		
Guide	Average Luciferase (RLU)	St. Dev Luciferase (RLU)
G009844	3700000	509116.9
G009852	281000	69296.46
G009857	1550000	127279.2
G009858	551000	108894.4
G009859	1425000	77781.75
G009860	2240000	183847.8
G009861	663500	238295
G009866	274000	11313.71
G009867	44700	565.6854
G009874	2865000	431335.1
G012747	651000	59396.97
G012749	867000	93338.1
G012752	4130000	268700.6
G012753	1145000	162634.6
G012757	579000	257386.9
G012760	129000	36769.55
G012761	4045000	728320
G012762	2220000	127279.2
G012763	1155000	205061
G012764	11900000	1555635
G012765	1935000	134350.3
G012766	2050000	169705.6
LNP	8430	212.132

Example 12—In Vivo Testing of Factor IX Expression from an Alternative Safe Harbor Locus

[0316] In this Example, insertion of ssAAV comprising a bidirectional hFIX construct at an alternative safe harbor locus was evaluated. To test the insertion into an alternative safe harbor locus, AAV was prepared as described above. Mice were administered with AAVs at a dose of 3e11 vg/mouse immediately followed by administration of LNPs formulated with Cas9 mRNAs and guide RNAs at a dose of 0.3 mg/kg. Animals were sacrificed 4 weeks post-dose, and liver and blood samples were collected. Editing in the liver samples was determined by NGS. Human hFIX levels in the serum was determined by ELISA. The NGS and ELISA data showed effective insertion and expression of hFIX within the alternative safe harbor locus.

Example 13—In Vivo Testing of the Human Factor IX Gene Insertion in Non-Human Primates

[0317] In this example, an 8 week study was performed to evaluate the human Factor IX gene insertion and hFIX protein expression in cynomolgus monkeys through administration of adeno-associated virus (AAV) and/or lipid nanoparticles (LNP) with various guides. This study was conducted with LNP formulations and AAV formulations prepared as described above. Each LNP formulation contained Cas9 mRNA and guide RNA (gRNA) with an mRNA: gRNA ratio of 2:1 by weight. The ssAAV was derived from P00147.

[0318] Male cynomolgus monkeys were treated in cohorts of n=3. Animals were dosed with AAV by slow bolus injection or infusion in the doses described in Table 10. Following AAV treatment, animals received buffer or LNP as described in Table 10 by slow bolus or infusion.

[0319] Two weeks post-dose, liver specimens were collected through single ultrasound-guided percutaneous biopsy. Each biopsy specimen was flash frozen in liquid nitrogen and stored at -86 to -60°C. Editing analysis of the liver specimens was performed by NGS Sequencing as previously described.

[0320] For Factor IX ELISA analysis, blood samples were collected from the animals on days 7, 14, 28, and 56 post-dose. Blood samples were collected and processed to plasma following blood draw and stored at -86 to -60°C until analysis.

[0321] The total human Factor IX levels were determined from plasma samples by ELISA. Briefly, Reacti-Bind 96-well microplate (VWR Cat #PI15041) were coated with capture antibody (mouse mAB to human Factor IX antibody (HTI, Cat #AHIX-5041)) at a concentration of 1 µg/ml then blocked using 1xPBS with 5% Bovine Serum Albumin. Test samples or standards of purified human Factor IX protein (ERL, Cat #HFIX 1009, Lot #HFIX4840) diluted in Cynomolgus monkey plasma were next incubated in individual wells. The detection antibody (Sheep anti-human Factor 9 polyclonal antibody, Abcam, Cat #ab128048) was adsorbed at a concentration of 100 ng/ml. The secondary antibody (Donkey anti-Sheep IgG pAbs with HRP, Abcam, Cat #ab97125) was used at 100 ng/mL. TMB Substrate Reagent set (BD OptEIA Cat #555214) was used to develop the plate. Optical density was assessed spectrophotometrically at 450 nm on a microplate reader (Molecular Devices i3 system) and analyzed using SoftMax pro 6.4.

[0322] Indel formation was detected, confirming that editing occurred. The NGS data showed effective indel formation. Expression of hFIX from the albumin locus in NHPs was measured by ELISA and is depicted in Table 11 and FIG. 15. Plasma levels of hFIX reached levels previously described as therapeutically effective (George, et al., NEJM 377(23), 2215-27, 2017).

[0323] As measured, circulating hFIX protein levels were sustained through the eight week study (see FIG. 15, showing day 7, 14, 28, and 56 average levels of ~135, ~140, ~150, and ~110 ng/mL, respectively), achieving protein levels ranging from ~75 ng/mL to ~250 ng/mL. Plasma hFIX levels were calculated using a specific activity of ~8 fold higher for the R338L hyperfunctional hFIX variant (Simioni et al., NEJM 361(17), 1671-75, 2009) (which reports a protein-specific activity of hFIX-R338L of 390±28 U per milligram, and a protein-specific activity for wild-type factor IX of 45±2.4 U per milligram). Calculating the func-

tionally normalized Factor IX activity for the hyperfunctional Factor IX variant tested in this example, the experiment achieved stable levels of human Factor IX protein in the NHPs over the 8 week study that correspond to about 20-40% of wild type Factor IX activity (range spans 12-67% of wild type Factor IX activity).

TABLE 10

Editing in liver					
Animal ID	Guide ID	F9-AAV (vg/kg)	F9-AAV Volume (mL/kg)	LNP (mg/kg)	LNP Volume (mL/kg)
4001	G009860	3E+13	1	3	2
4002	G009860	3E+13	1	3	2
4003	G009860	3E+13	1	3	2
5001	TSS	3E+13	1	0	0
5002	TSS	3E+13	1	0	0
5003	TSS	3E+13	1	0	0
6001	G009862	0	0	3	2
6002	G009862	0	0	3	2
6003	G009862	0	0	3	2

#ab128048), and donkey anti-Sheep IgG pAbs with HRP (Abcam, Cat #ab97125), as described in Example 13. Human FIX protein levels >3 fold higher than those achieved in the experiment of Example 13 were obtained from the bidirectional template using alternative CRISPR/Cas9 LNP. In the study, ELISA assay results indicate that circulating hFIX protein levels at or above the normal range of human FIX levels (3-5 ug/mL; Amiral et al., Clin. Chem., 30(9), 1512-16, 1984) were achieved using G009860 in the NHPs by at least the day 14 and 28 timepoints. Initial data indicated circulating human FIX protein levels of ~3-4 g/mL at day 14 after a single dose, with levels sustained through the first 28 days (~3-5 g/mL) of the study. The human FIX levels were measured at the conclusion of the study by the same method and data are presented in the Table 25. Additional guides G009847, G009862, and G009864 were also tested and shown to facilitate insertion of a FIX-expressing template in the NHP study.

TABLE 25

Serum human Factor IX protein levels—ELISA Method of Example 13									
	Day 7	Day 14	Day 28	Day 42	Day 56		Day 7	Day 14	Day 28
	FIX ng/mL	STD DEV	FIX ng/mL						
3001	2532.8	145.6	2562.6	99.0	3011.7	62.7	2936.7	72.4	2748.5
3002	2211.4	95.8	2958.5	119.2	3350.2	98.4	3049.7	112.7	3036.7
3003	3195.1	475.6	4433.9	238.7	3367.2	157.7	3746.1	95.6	3925.0
									86.0
									90.6
									157.4

TABLE 11

hFIX expression				
Animal ID	Day 7 Factor IX (ng/mL)	Day 14 Factor IX (ng/mL)	Day 28 Factor IX (ng/mL)	Day 56 Factor IX (ng/mL)
4001	122.84/+2.85	94.93/+0.56	105.65/+1.94	97.31/+1.49
4002	149.77/+13.5	222.92/+9.61	252.49/+6.46	152.05/+7.46
4003	134.06/+6.17	107.04/+6.46	95.30/+3.18	74.23/+3.53
5001	ND	ND	ND	ND
5002	ND	ND	ND	ND
5003	ND	ND	ND	ND
6001	ND	ND	ND	ND
6002	ND	ND	ND	ND
6003	ND	ND	ND	ND

Example 14 In Vivo Testing of Factor IX Insertion in Non-Human Primates

[0324] In this example, a study was performed to evaluate the Factor IX gene insertion and hFIX protein expression in cynomolgus monkeys following administration of ssAAV derived from P00147 and/or CRISPR/Cas9 lipid nanoparticles (LNP) with various guides including G009860 and various LNP components.

[0325] Indel formation was measured by NGS, confirming that editing occurred. Total human Factor IX levels were determined from plasma samples by ELISA using a mouse mAB to human Factor IX antibody (HTI, Cat #AHIX-5041), sheep anti-human Factor 9 polyclonal antibody (Abcam, Cat

[0326] Circulating albumin levels were measured by ELISA, indicating that baseline albumin levels are maintained at 28 days. Tested albumin levels in untreated animals varied ±~15% in the study. In treated animals, circulating albumin levels changed minimally and did not drop out of the normal range, and the levels recovered to baseline within one month.

[0327] Circulating human FIX protein levels were also determined by a sandwich immunoassay with a greater dynamic range. Briefly, an MSD GOLD 96-well Streptavidin SECTOR Plate (Meso Scale Diagnostics, Cat. L15SA-1) was blocked with 1% ECL Blocking Agent (Sigma, GERPN2125). After tapping out the blocking solution, biotinylated capture antibody (Sino Biological, 11503-R044) was immobilized on the plate. Recombinant human FIX protein (Enzyme Research Laboratories, HFIX 1009) was used to prepare a calibration standard in 0.5% ECL Blocking Agent. Following a wash, calibration standards and plasma samples were added to the plate and incubated. Following a wash, a detection antibody (Haematologic Technologies, AHIX-5041) conjugated with a sulfo-tag label was added to the wells and incubated. After washing away any unbound detection antibody, Read Buffer T was applied to the wells. Without any additional incubation, the plate was imaged with an MSD Quick Plex SQ120 instrument and data was analyzed with Discovery Workbench 4.0 software package (Meso Scale Discovery). Concentrations are expressed as mean calculated concentrations in ug/m. For the samples, N=3 unless indicated with an asterisk, in which case N=2.

Expression of hFIX from the albumin locus in the treated study group as measured by the MSD ELISA is depicted in Table 26.

TABLE 26

Serum human Factor IX protein levels—MSD ELISA					
Mean Calc. Conc. (μg/mL)					
	3001	3002	3003		
Time Point	Conc.	Inter-Assay CV	Conc.	Inter-Assay CV	Inter-Assay CV
Day 7	7.85	20%	5.63	14%	11.20
Day 14	8.65	15%	11.06	18%	14.70
Day 28	9.14	7%	14.12	7%	10.85
Day 42	9.03	10%	33.12*	0%	13.22
Day 56	10.24	13%	16.72	12%	33.84*

Example 15—Off-Target Analysis of Albumin Human Guides

[0328] A biochemical method (See, e.g., Cameron et al., *Nature Methods*. 6, 600-606; 2017) was used to determine potential off-target genomic sites cleaved by Cas9 targeting Albumin. In this experiment, 13 sgRNA targeting human Albumin and two control guides with known off-target profiles were screened using isolated HEK293 genomic DNA. The number of potential off-target sites detected using a guide concentration of 16 nM in the biochemical assay were shown in Table 27. The assay identified potential off-target sites for the sgRNAs tested.

TABLE 27

Off-Target Analysis			
sgRNA ID	Target	Guide Sequence (SEQ ID NO.:)	Off-Target Site Count
G012753	Albumin	GACUGAAACUUCACAGAAUA (SEQ ID NO: 20)	62
G012761	Albumin	AGUGCAAUGGAUAGGUUUU (SEQ ID NO: 28)	75
G012752	Albumin	UGACUGAAACUUCACAGAAU (SEQ ID NO: 19)	223
G012764	Albumin	CCUCACUCUUGUCUGGGCAA (SEQ ID NO: 31)	3985
G012763	Albumin	UGGGCAAGGGAAGAAAAAAA (SEQ ID NO: 30)	5443
G009857	Albumin	AUUUAUGAGAUCAACAGCAC (SEQ ID NO: 5)	131
G009859	Albumin	UUAAAUAAGCAUAGUGCAA (SEQ ID NO: 7)	91
G009860	Albumin	UAAAGCAUAGUGCAAUGGAU (SEQ ID NO: 8)	133
G012762	Albumin	UGAUUCCUACAGAAAAACUC (SEQ ID NO: 29)	68

TABLE 27-continued

Off-Target Analysis			
sgRNA ID	Target	Guide Sequence (SEQ ID NO.:)	Off-Target Site Count
G009844	Albumin	GAGCAACCUCACUCUUGUCU (SEQ ID NO: 2)	107
G012765	Albumin	ACCUCACUCUUGUCUGGGCA (SEQ ID NO: 32)	41
G012766	Albumin	UGAGCAACCUCACUCUUGUC (SEQ ID NO: 33)	78
G009874	Albumin	UAAUAAAUAUCAAACAUCCU (SEQ ID NO: 13)	53
G000644	EMX1	GAGUCCGAGCAGAAGAAGAA (SEQ ID NO: 1129)	304
G000645	VEGFA	GACCCCCUCCACCCGCCUC (SEQ ID NO: 1130)	1641

[0329] In known off-target detection assays such as the biochemical method used above, a large number of potential off-target sites are typically recovered, by design, so as to “cast a wide net” for potential sites that can be validated in other contexts, e.g., in a primary cell of interest. For example, the biochemical method typically overrepresents the number of potential off-target sites as the assay utilizes purified high molecular weight genomic DNA free of the cell environment and is dependent on the dose of Cas9 RNP used. Accordingly, potential off-target sites identified by these methods may be validated using targeted sequencing of the identified potential off-target sites.

Example 16. Use of Humanized Albumin Mice to Screen Guide RNAs for Human F9 Insertion In Vivo

[0330] We aimed to identify effective guide RNAs for hF9 insertion into the human albumin locus. To this end, we utilized mice in which the mouse albumin locus was replaced with the corresponding human albumin genomic sequence, including the first intron (ALB^{hu/hu} mice). This allowed us to test the insertion efficiency of guide RNAs targeting the first intron of human albumin in the context of an adult liver in vivo. Two separate mouse experiments were set up using the ALB^{hu/hu} mice to screen a total of 11 guide RNAs, each targeting the first intron of the human albumin locus. All mice were weighed and injected via tail vein at day 0 of the experiment. Blood was collected at weeks 1, 3, 4, and 6 via tail bleed, and plasma was separated. Mice were terminated at week 7. Blood was collected via the vena cava, and plasma was separated. Livers and spleens were dissected as well.

[0331] In the first experiment, 6 LNPs comprising Cas9 mRNA and the following guides were prepared as in Example 1 and tested: G009852, G009859, G009860, G009864, G009874, and G012764. LNPs were diluted to 0.3 mg/kg (using an average weight of 30 grams) and co-injected with AAV8 packaged with the bi-directional hF9 insertion template at a dose of 3E11 viral genomes per mouse. Five ALB^{hu/hu} male mice between 12 and 14 weeks old were injected per group. Five mice from same cohort were injected with AAV8 packaged with a CAGG promoter

operably linked to hF9, which leads to episomal expression of hF9 (at 3E11 viral genomes per mouse). There were three negative control groups with three mice per group that were injected with buffer alone, AAV8 packaged with the bi-directional hF9 insertion template alone, or LNP-G009874 alone.

[0332] In the experiment, the following LNPs comprising Cas9 mRNA and the following guides were prepared as in Example 1 and tested: G009860, G012764, G009844, G009857, G012752, G012753, and G012761. All were diluted to 0.3 mg/kg (using an average weight of 40 grams) and co-injected with AAV8 packaged with the bi-directional hF9 insertion template at a dose of 3E11 viral genomes per mouse. Five ALB^{hu/hu} male mice 30 weeks old were injected per group. Five mice from same cohort were injected with AAV8 packaged with a CAGG promoter operably linked to hF9, which leads to episomal expression of hF9 (at 3E11 viral genomes per mouse). There were three negative control groups with three mice per group that were injected with buffer alone, AAV8 packaged with the bi-directional hF9 insertion template alone, or LNP-G009874 alone.

[0333] For analysis, an ELISA was performed to measure levels of hFIX circulating in the mice at each timepoint. Human Factor IX ELISA Kits (ab188393) were used for this purpose, and all plates were run with human pooled normal plasma from George King Bio-Medical as a positive assay control. Human Factor IX expression levels in the plasma samples in each group at week 6 post-injection are shown in FIG. 16A and FIG. 16B. Consistent with the in vitro insertion data, low to no Factor IX serum levels were detected when guide RNA G009852 was used. Consistent with the lack of an adjacent PAM sequence in human albumin, Factor IX serum levels were not detectable when guide RNA G009864 was used. Factor IX expression in the serum was observed for the groups using guide RNAs G009859, G009860, G009874, and G0012764.

[0334] Spleens and a portion of the left lateral lobe of all livers were submitted for next-generation sequencing (NGS) analysis. NGS was used to assess the percentage of liver cells with insertions/deletions (indels) at the humanized albumin locus at week 7 post-injection with AAV-hF9 donor and LNP-CRISPR/Cas9. Consistent with the lack of an adjacent PAM sequence in human albumin, no editing was detectable in the liver when guide RNA G009864 was used. Editing in the liver was observed for the groups using guide RNAs G009859, G009860, G009874, and G012764 (data not shown).

[0335] The remaining liver was fixed for 24 hours in 10% neutral buffered formalin and then transferred to 70% ethanol. Four to five samples from separate lobes were cut and shipped to HistoWisz and were processed and embedded in paraffin blocks. Five-micron sections were then cut from each paraffin block, and BASESCOPE™ was performed on the Ventana Ultra Discovery (Roche) using the universal BASESCOPE™ procedure and reagents by Advanced Cell Diagnostics and a custom designed probe that targets the unique mRNA junction formed between the human albumin signal sequence from the first intron of the ALB^{hu/hu} albumin locus and the hF9 transgene when successful integration and transcription is achieved. HALO imaging software (Indica Labs) was then used to quantify the percentage of positive cells in each sample. The average of percentage positive cells across the multiple lobes for each animal was then correlated to the hFIX levels in the serum at week 7. The

results are shown in FIG. 17 and Table 28. The week 7 serum levels and the 00 positive cells for the hALB-hF9 mRNA strongly correlated ($r=0.89$; $R^2=0.79$).

TABLE 28

Week 7 hFIX and BASESCOPE™ Data.					
Mouse	Guide	hFIX ug/mL (Week 7)	% mRNA Probe (4-5 Sections)	STD % mRNA Probe	Total Cells Counted
1	Buffer	ND	0.09	0.03	152833
4	AAV	ND	0.53	0.67	351084
7	Only	ND	0.48	0.33	75160
7	LNP	ND	0.48	0.33	75160
10	CAG F9	211.8	0.20	0.22	190277
15	G009852	ND	0.30	0.09	144518
20	G009859	0.5	0.82	0.45	143817
21	G009859	0.5	0.88	0.43	160172
22	G009859	2.3	1.71	1.54	26015
23	G009859	3.8	2.74	0.59	183085
24	G009859	0.6	2.78	1.96	152424
25	G009860	5.6	12.46	5.80	78935
26	G009860	10.6	13.76	5.32	112252
27	G009860	9.7	14.80	5.45	201592
28	G009860	2.1	3.32	0.76	84710
29	G009860	3.0	1.52	0.35	203277
30	G009864	ND	1.94	1.78	145807
35	G009874	1.7	2.42	1.14	126665
36	G009874	1.5	1.08	0.53	195861
37	G009874	2.1	1.02	1.29	181679
38	G009874	5.5	0.40	0.43	175359
39	G009874	1.5	0.44	0.18	205417
40	G012764	15.7	28.85	7.11	167824
41	G012764	19.6	19.17	8.23	70081
42	G012764	1.9	1.95	1.79	154742
43	G012764	7.7	4.38	0.68	114060
44	G012764	3.0	1.64	1.04	238623
43	DapB(-)	—	0.12	0.07	144730

Example 17—Use of Humanized Albumin Mice
Crossed with F9 Knockout Mice to Assess
Functionality of Inserted Human F9 In Vivo

[0336] For a next study, functionality of inserted hF9 was tested in male ALB^{ms/hu}xF9^{-/-} mice. LNPs comprising Cas9 mRNA and the following guides were prepared as in Example 1 and tested: G009860 (targeting the first intron of the human albumin locus) and G000666 (targeting the first intron of the mouse albumin locus). G009860 was diluted to 0.3 mg/kg, and G000666 was diluted to 1.0 mg/kg (using an average weight of 31.2 grams), and both were co-injected with AAV8 packaged with the bi-directional hF9 insertion template at a dose of 3E11 viral genomes per mouse. Five ALB^{ms/hu}xF9^{-/-} male mice (16 weeks old) were injected per group. Five mice from same cohort were injected with AAV8 packaged with a CAGG promoter operably linked to hF9, which leads to episomal expression of hF9 (at 3E11 viral genomes per mouse). There were six negative control animals with one mouse per group that was injected with buffer alone or AAV8 packaged with the bi-directional hF9 insertion template alone, and two mice per group that were injected with LNP-G009860 or LNP-G000666 alone at 0.3 mg/kg and 1.0 mg/kg, respectively.

[0337] For analysis, an ELISA was performed to measure levels of hFIX circulating in the mice at each timepoint. Human Factor IX ELISA Kits (ab188393) were used for this purpose, and all plates were run with human pooled normal plasma from George King Bio-Medical as a positive assay

control. Spleens and a portion of the left lateral lobe of all livers were submitted for NGS analysis.

[0338] Human Factor IX expression levels in the plasma samples in each group at weeks 1, 2, and 4 post-injection are shown in FIG. 18 and in Table 29. In addition, NGS results showing insertion and deletion (indel) levels at the albumin locus in the liver and spleen are shown in Table 29. As shown in FIG. 18 and Table 29, hFIX was detected in the plasma of treated Alb^{+hu/F9^{-/-} mice at 1, 3, and 4 weeks, with ELISA showing expression values of 0.5-10 g/mL at 1, 3 and 4 weeks}

TABLE 29

Sample	Week 1 (μ g/mL)	Week 3 (μ g/mL)	Week 4 (μ g/mL)	INDEL Liver	INDEL Spleen
S1 PBS	BLD	BLD	BLD	6.12	0.12
S18 AAV8 only	BLD	BLD	BLD	0.73	0.10
S2 G000666 only	BLD	BLD	BLD	37.48	0.92
S4 G000666 only	BLD	BLD	BLD	30.67	1.17
S19 G009860 only	BLD	BLD	BLD	12.25	0.31
S20 G009860 only	BLD	BLD	BLD	10.73	0.45
S10 CAG	42.60	129.83	117.74	1.45	0.12
S14 CAG	35.55	82.25	100.95	0.08	0.11
S15 CAG	37.30	115.51	107.26	0.10	0.05
S16 CAG	36.39	81.27	116.24	0.05	0.10
S17 CAG	40.50	101.38	124.15	0.16	0.06
S5 AAV8 + G000666	2.90	5.00	8.79	41.46	1.43
S6 AAV8 + G000666	4.67	6.11	10.29	33.81	1.59
S7 AAV8 + G000666	2.88	3.15	3.01	33.47	1.04
S8 AAV8 + G000666	0.94	1.61	No sample	36.54	1.34
S9 AAV8 + G000666	7.14	7.53	7.23	30.63	1.38
S11 AAV8 + G009860	0.73	0.62	0.86	11.15	0.52
S12 AAV8 + G009860	0.52	0.43	0.47	7.05	0.39
S13 AAV8 + G009860	1.71	1.89	0.93	18.38	0.57
S21 AAV8 + G009860	1.21	2.79	0.59	13.44	0.22
S22 AAV8 + G009860	2.06	1.03	2.37	18.06	0.19
Human	4.00	3.91	4.12	N/A	N/A

[0339] The remaining liver was fixed for 24 hours in 1000 neutral buffered formalin and then transferred to 700% ethanol. Four to five samples from separate lobes were cut and shipped to HistoWiz and were processed and embedded in paraffin blocks. Five-micron sections were then cut from each paraffin block for analysis via BASESCOPE™ on the Ventana Ultra Discovery (Roche) using the universal BASESCOPE™ procedure and reagents by Advanced Cell Diagnostics and a custom designed probe that targets the unique mRNA junction formed between either the human or the mouse albumin signal sequence from the first intron of each respective albumin locus in the ALB^{ms/hu} mouse and the hF9 transgene when successful integration and transcription is achieved. HALO imaging software (Indica Labs) is used to quantify the percentage of positive cells in each sample.

[0340] Next, terminal blood was used for assessment of functional coagulation activity by activated partial thromboplastin time (aPTT) and Thrombin Generation Assay (TGA). Activated partial thromboplastin time (aPTT) is a clinical measurement of intrinsic pathway clotting activity in plasma. Plasma is induced to clot by the addition of ellagic acid or kaolin, both of which activate coagulation factor XII in the intrinsic pathway (as known as the contact pathway)

of coagulation, that subsequently results in the generation of fibrin from fibrinogen once thrombin is activated. The aPTT assay provides an estimation of an individual's ability to generate a clot, and this information can be used to determine risk of bleeding or thrombosis. To test aPTT, a semi-automated benchtop system (Diagnostica Stago STart 4) with an electro-mechanical clot detection method (viscosity-based detection system) was used to assess clotting in plasma. To each cuvette with a steel ball, 50 μ L of citrated plasma was added and incubated at 37° C. for 5 min, and then clotting was triggered with the addition of 50 μ L of ellagic acid (final concentration of 30 μ M) at 37° C. for 300 seconds. Following final activation of clotting by adding 50 μ L of 0.025 M calcium chloride (final concentration of 8 mM) to each cuvette, the steel ball began to oscillate back and forth between the two drive coils. The movement of the ball was detected by the receiver coil. The generation of fibrin increased plasma viscosity until the ball ceased to move, which was recorded as the clotting time. The only parameter measured was clotting time. Runs were conducted in duplicate.

[0341] Thrombin generation assay (TGA) is a non-clinical assessment of the kinetics of thrombin generation in activated plasma. Thrombin generation is an essential process of coagulation because thrombin is responsible for activation of other coagulation factors and propagation of additional thrombin (via FXI activation) for the conversion of fibrinogen to fibrin. Thrombin generation assay provides an estimation of an individual's ability to generate thrombin, and this information can be used to determine risk of bleeding or thrombosis. To perform the TGA, a calibrated automated thrombogram was used to assess thrombin generation levels in a spectrophotometer (Thrombinograph™, Thermo Scientific). For high throughput experimentation, 96-well plates (Immulon II HB) were used. To each well, 55 μ L of citrated plasma (4x diluted with saline for mouse plasma) was added and incubated at 37° C. for 30 min. Thrombin generation is triggered with the addition of 15 μ L of 2 M ellagic acid (final concentration of 0.33 μ M) at 37° C. for 45 min. Thrombin generation was determined following the automated injection of 15 μ L of the fluorogenic substrate with 16 mM CaCl₂ (FluCa; Thrombinoscope BV) into each well. The fluorogenic substrate reacted with the generated thrombin, which was measured continuously in the plasma every 33 sec for 90 min at 460 nm. The fluorescence intensity was proportional to the proteolytic activity of thrombin. The main parameters measured in the tracing were lag time, peak thrombin generation, time to peak thrombin generation, and endogenous thrombin potential (ETP). The lag time provides an estimation of time required for initial detection of thrombin in plasma. The peak is the maximum amount of thrombin generated at a given time after activation. Time to peak thrombin generation is time from initiation of the coagulation cascade to the peak generation of thrombin. ETP is the total amount of thrombin generated during the 60 minutes measured. Runs were conducted in duplicate.

[0342] As shown in FIG. 19 and Table 30, insertion of the hF9 transgene using for example G000666 showed recovered clotting function in the aPTT assay. AAV only and LNP only negative control samples showed prolonged aPTT times of 45-60 seconds in saline. The positive control CAGG and test samples AAV8+LNP were closer to the normal human aPTT of 28-34 seconds.

TABLE 30

aPTT and TGA-EA.

Sample #	I.V. Injection	Week 4 F9 μg/mL	Average aPTT (sec)	TGA-EA Peak (nM)
1	PBS	BLD	40.2	11.13
18	AAV Only	BLD	62.5	-1
2	LNP g666 only	BLD	53.9	-1
4	LNP g666 only	BLD	65.0	2.45
19	LNP G009860 only	BLD	34.1	42.83
20	LNP G009860 only	BLD	56.7	18.07
10	AAV + CAGG F9	117.74	41.1	42.65
14	AAV + CAGG F9	100.95	34.1	49.96
15	AAV + CAGG F9	107.26	42.2	49.49
16	AAV + CAGG F9	116.24	37.9	44.46
17	AAV + CAGG F9	124.15	44.1	38.02
5	AAV + g666	8.79	31.3	72.11
6	AAV + g666	10.29	32.6	90.14
7	AAV + g666	3.01	33.5	58.33
8	AAV + g666	no sample	NA	NA
9	AAV + g666	7.23	25.9	67.23
11	AAV + G009860	0.86	36.8	56.92
12	AAV + G009860	0.47	37.7	45.16
13	AAV + G009860	0.93	35.3	60.45
21	AAV + G009860	0.59	36.1	47.44
22	AAV + G009860	2.37	>300	Clots in tube

[0343] As shown in FIG. 20A, FIG. 20B, and FIG. 21 and in Table 30, insertion of the hF9 transgene using for example G000666 showed increased thrombin generation in TGA-EA analysis. Thrombin concentrations were higher in the positive control CAGG and AAV8+LNP as compared to the negative control samples.

[0344] In conclusion, hFIX was detected in the plasma of Alb^{+/hu}/F9^{-/-} mice at 1, 3, and 4 weeks, and the expressed

hFIX-R338L was found to be functional since thrombin was generated in a TGA assay, and aPTT clotting time was improved.

Human albumin intron 1:

(SEQ ID NO: 1)

GTAAGAAATCCATTTCATTGTTCAACTTTTATTCTATTCCCAG
 TAAAATAAGTTTAGTAAACTCTGCATCTTAAAGAATTATTTGGC
 ATTTATTTCTAAATGCCATAGTATTGTATTGAAAGTCTTACAA
 GGTTATCTTATAATAAAATTCAAACATCCTAGGTAAAAAAAAAAAA
 GGTCAAGATTGTTAGTGACTGTAATTCTTTGCGCACTAAGGAAA
 GTGCAAAGTAACCTAGAGTGACTGAAACTCACAGAATAGGGTGAAG
 ATTGAAATTCAACTATCCAAAGACCTATCCATTGCACTATGCTTTA
 TTTAAAAACACAAAACCTGTGCTGTGATCTCATAAATAGAACTTGT
 ATTTATTTATTTCTATTAGTCTGCTTCTGGTGCTGTTGATA
 GACACTAAAAGAGTATTAGATATTCTAAGTTGAATATAAGGCTAT
 AAATATTTAATAATTAAAGTATTCTGGTAATTGAATTATTC
 TTCTGTTAAAGGCAAGAGAAATAATTGAACATCATCCTGAGTTTC
 TGAGGAATCAGAGCCAATATTGAAACAAATGCATAATCTAAGTC
 AAATGGAAAGAAATATAAAAGTAACATTATTACTTCTGTTCTTC
 AGTATTTAACAACTCTTTCTTCTGCCAG

TABLE 5

Mouse albumin guide RNA			
Guide ID	Guide Sequence	Mouse Genomic Coordinates (mm10)	SEQ ID NO:
G000551	AUUUGCAUCUGAGAACCUU	chr5:90461148-90461168	98
G000552	AUCGGGAACUGGCAUCUUCA	chr5:90461590-90461610	99
G000553	GUUACAGGAAAUCUGAAGG	chr5:90461569-90461589	100
G000554	GAUCGGGAACUGGCAUCUUC	chr5:90461589-90461609	101
G000555	UGCAUCUGAGAACCCUUAGG	chr5:90461151-90461171	102
G000666	CACUCUUGUCUGUGGAAACA	chr5:90461709-90461729	103
G000667	AUCGUUACAGGAAAUCUGA	chr5:90461572-90461592	104
G000668	GCAUCUUCAGGGAGUAGCUU	chr5:90461601-90461621	105
G000669	CAAUCUUUAUUUAUGUUGUG	chr5:90461674-90461694	106
G000670	UCACUCUUGUCUGUGGAAAC	chr5:90461710-90461730	107
G011722	UGCUUGUAUUUUUCUAGUAA	chr5:90461039-90461059	108
G011723	GUAAAUAUCUACUAAGACAA	chr5:90461425-90461445	109
G011724	UUUUUCUAGUAAUGGAAGCC	chr5:90461047-90461067	110
G011725	UUUAUUAUUGAUUAUUUU	chr5:90461174-90461194	111
G011726	GCACAGAUAAAACACUAAA	chr5:90461480-90461500	112

TABLE 5-continued

Mouse albumin guide RNA			
Guide ID	Guide Sequence	Mouse Genomic Coordinates (mm10)	SEQ ID NO:
G011727	CACAGAUUAACACUUUAC	chr5:90461481-90461501	113
G011728	GGUUUUAAAAAUAAUAAAUGU	chr5:90461502-90461522	114
G011729	UCAGAUUUCCUGUAACGAU	chr5:90461572-90461592	115
G011730	CAGAUUUCCUGUAACGAUC	chr5:90461573-90461593	116
G011731	CAAUGGUAAAUAAGAAAUA	chr5:90461408-90461428	117
G013018	GGAAAAUCUGAAGGUGGCAA	chr5:90461563-90461583	118
G013019	GGCGAUCUCACUCUUGUCUG	chr5:90461717-90461737	119

TABLE 6

Mouse albumin sqRNAs and modification pattern			
Guide ID	Full Sequence	SEQ ID NO: Full Sequence Modified	SEQ ID NO:
G000551	AUUUGCAUCUGAGAACCUUUG UUAGAGCUAGAAUAGCAAGU UAAAUAAGCUAGGCCUUAU CAACUUGAAAAGUGCACC GUCGGGCGUUU	120 mA*mU*mU*UGCAUCUGAGAACCCUUGUUUAGAm GmCmUmAmGmAmAmUmAmGmCAAGUUAAAAAU AAGGCUAGUCGUUAUCAmCmUmUmGmAmAm AmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	142
G000552	AUCGGGAACUGGCACUUCA GUUUUAGAGCUAGAAAAGC AAGUAAAUAAGCUAGUC CGUUUACUACUUGAAAAGU GGCACCGAGUCGGGCGUUU	121 mA*mU*mC*GGGACUGGCACUUCAGUUUAGAm GmCmUmAmGmAmAmUmAmGmCAAGUUAAAAAU AAGGCUAGUCGUUAUCAmCmUmUmGmAmAm AmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	143
G000553	GUUACAGGAAAUCUGAAGG GUUUUAGAGCUAGAAAAGC AAGUAAAUAAGCUAGUC CGUUUACUACUUGAAAAGU GGCACCGAGUCGGGCGUUU	122 mG*mU*mU*ACAGGAAAUCUGAAGGGUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAAU UAAGGCUAGUCGUUAUCAmCmUmUmGmAmAm mAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmG GmGmUmGmCmU*mU*mU*mU	144
G000554	GAUCGGGAACUGGCACUUUC GUUUUAGAGCUAGAAAAGC AAGUAAAUAAGCUAGUC CGUUUACUACUUGAAAAGU GGCACCGAGUCGGGCGUUU	123 mG*mA*mU*CGGGACUGGCACUUCGUUUUAGAm GmCmUmAmGmAmAmUmAmGmCAAGUUAAAAAU AAGGCUAGUCGUUAUCAmCmAmCmUmUmGmAmAm AmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	145
G000555	UGCAUCUGAGAACCCUUAGG GUUUUAGAGCUAGAAAAGC AAGUAAAUAAGCUAGUC CGUUUACUACUUGAAAAGU GGCACCGAGUCGGGCGUUU	124 mU*mG*mC*AUCUGAGAACCCUUAGGGUUUAGAm GmCmUmAmGmAmAmUmAmGmCAAGUUAAAAAU AAGGCUAGUCGUUAUCAmCmAmCmUmUmGmAmAm AmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	146
G000666	CACCUUUGUCUGGGAAACA GUUUUAGAGCUAGAAAAGC AAGUAAAUAAGCUAGUC CGUUUACUACUUGAAAAGU GGCACCGAGUCGGGCGUUU	125 mC*mA*mC*UCUUGUCUGGGAAACAGUUUAGAm GmCmUmAmGmAmAmUmAmGmCAAGUUAAAAAU AAGGCUAGUCGUUAUCAmCmAmCmUmUmGmAmAm AmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	147
G000667	AUCGUUACAGGAAAUCUGA GUUUUAGAGCUAGAAAAGC AAGUAAAUAAGCUAGUC CGUUUACUACUUGAAAAGU GGCACCGAGUCGGGCGUUU	126 mA*mU*mC*GUUACAGGAAAUCUGAGUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAAU UAAGGCUAGUCGUUAUCAmCmUmUmGmAmAm mAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	148

TABLE 6 - continued

Mouse albumin sgRNAs and modification pattern			
Guide ID	Full Sequence	SEQ NO: Full Sequence Modified	SEQ ID NO:
G000668	GCAUCUUCAGGGAGUAGCUU GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	127 mG*mC*mA*UCUUUCAGGGAGUAGCUUGUUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAA UAAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	149
G000669	CAAUCUUAAAUAUGUUGUG GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	128 mC*mA*mA*UCUUUAAAUAUGUUGUGGUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAA UAAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	150
G000670	UCACUCUUGUCUGUGGAAAC GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	129 mU*mC*mA*CUCUUUCUGUGGAAACGUUUUAGA GmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAA AAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	151
G011722	UGCUUGUAUUUUUUCUAGUAA GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	130 mU*mG*mC*UUGUAUUUUUUCUAGUAGUUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAA UAAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	152
G011723	GUAAAUAUCUACUAAAGACAA GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	131 mG*mU*mA*AAUAUCUACUAAAGACAAGUUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAA UAAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	153
G011724	UUUUUCUAGUAAUGGAAGCC GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	132 mU*mU*mU*UUCUAGUAAUGGAAGCCGUUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAA UAAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	154
G011725	UUUAUUAUUGUAAUAAA GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	133 mU*mU*mA*UAUUAUUGUAAUAAAUGUUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAA UAAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	155
G011726	GCACAGAUAAAACACUUAA GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	134 mG*mC*mA*CAGAUAAAACACUUAAAGUUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAA UAAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	156
G011727	CACAGAUAAAACACUUAC GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	135 mC*mA*mC*AGAUAAAACACUUAAAGUUUUAGA GmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAA AAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	157
G011728	GGUUUUAAAUAUAUAUGU GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	136 mG*mG*mU*UUUUAAAUAUAUGGUUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAA UAAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	158
G011729	UCAGAUUUCCUGUACGAU GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGGAGCUUUU	137 mU*mC*mA*GAUUUUCUGUACGAUGUUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAA UAAGGCCUAGUCGGUUAUCAmAmCmUmUmGmAmA mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	159

TABLE 6 -continued

Mouse albumin sqRNAs and modification pattern					
Guide ID	Full Sequence	SEQ NO:	SEQ ID NO:	Full Sequence Modified	SEQ ID NO:
G011730	CAGAUUUUCUGUAACGAUC GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	138	160	mC*mA*mG*AUUUUCCUGUAACGAUCGUUUUAGAm GmCmUmAmGmAmAmUmAmGmCAAGUUAAAAAU AAGGCUGAGUCGUUAUCAmAmCmUmUmGmAmAm AmAmAmGmUmGmCmAmCmCmGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	
G011731	CAAUGGUAAAUAAGAAAUA GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	139	161	mC*mA*mA*UGGUAAAUAAGAAAUAAGUUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAAU UAAGGCUGAGUCGUUAUCAmAmCmUmUmGmAmAm mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	
G013018	GGAAAAACUGAAGGGGGCAA GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	140	162	mG*mG*mA*AAACUGAAGGGGGCAAGUUUUAGA mGmCmUmAmGmAmAmUmAmGmCAAGUUAAAAAU UAAGGCUGAGUCGUUAUCAmAmCmUmUmGmAmAm mAmAmAmGmUmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU	
G013019	GGCGAUACUCACUCUUGUCUG GUUUUAGAGCUAGAAAUGC AAGUUAAAUAAGGUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	141	163	mG*mG*mC*GAUCACACUUCUUGUCUGGUUUUAGAm GmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAAU AAGGCUGAGUCGUUAUCAmAmCmUmUmGmAmAm AmAmAmGmUmGmCmAmCmCmGmAmGmUmCmG mGmUmGmCmU*mU*mU*mU	

TABLE 7

Cyno albumin guide RNA			
Guide ID	Guide Sequence	Cyno Genomic Coordinates (mf5)	SEQ ID NO:
G009844	GAGCAACCUCACUCUUGUCU	chr5:61198711-61198731	2
G009845	AGCAACCUCACUCUUGUCUG	chr5:61198712-61198732	165
G009846	ACCUCACUCUUGUCUGGGGA	chr5:61198716-61198736	166
G009847	CCUCACUCUUGUCUGGGGAA	chr5:61198717-61198737	167
G009848	CUCACUCUUGUCUGGGGAAG	chr5:61198718-61198738	168
G009849	GGGGAAAGGGGAGAAAAAAA	chr5:61198731-61198751	169
G009850	GGGAAGGGGAGAAAAAAA	chr5:61198732-61198752	170
G009851	AUGCAUUUGUUUCAAAAU	chr5:61198825-61198845	3
G009852	UGCAUUUGUUUCAAAAU	chr5:61198826-61198846	172
G009853	UGAUUCCUACAGAAAAAGUC	chr5:61198852-61198872	173
G009854	UACAGAAAAAGUCAGGAUA	chr5:61198859-61198879	174
G009855	UUUCUUCUGCCUUAAAACAG	chr5:61198889-61198909	175
G009856	UUAUAGUUUUAUUCAAC	chr5:61198957-61198977	176
G009857	AUUUAUGAGAACAGCAC	chr5:61199062-61199082	5
G009858	GAUCAACAGCACAGGUUUG	chr5:61199070-61199090	6
G009859	UUAAAUAAGCAUAGUGCAA	chr5:61199096-61199116	7
G009860	UAAAAGCAUAGUGCAAUGGAU	chr5:61199101-61199121	8
G009861	UAGUGCAAUGGAUAGGUUU	chr5:61199108-61199128	9

TABLE 7-continued

Cyno albumin guide RNA			
Guide ID	Guide Sequence	Cyno Genomic Coordinates (mf5)	SEQ ID NO:
G009862	AGUGCAAUGGAUAGGUUUUA	chr5:61199109-61199129	182
G009863	UUACUUUGCACUUUCCUUAG	chr5:61199186-61199206	183
G009864	UACUUUGCACUUUCCUUAGU	chr5:61199187-61199207	184
G009865	UCUGACCUUUUAAAAUACCU	chr5:61199238-61199258	185
G009866	UACUUAAAACUUUAAAAUACU	chr5:61199367-61199387	10
G009867	AAAGUUGAACAAUAGAAAAA	chr5:61199401-61199421	11
G009868	AAUGCAUAAUCUAAGUAAA	chr5:61198812-61198832	2
G009869	AUUAUCCUGACUUUUUCUGU	chr5:61198860-61198880	189
G009870	UGAAUUAUUUCUCUGUUUAA	chr5:61198901-61198921	190
G009871	UAAUUUUUUCCCCACUA	chr5:61199203-61199223	191
G009872	AAAAGGUCAGAAUUGUUUAG	chr5:61199229-61199249	192
G009873	AACAUCUAGGUAAAUAAAA	chr5:61199246-61199266	193
G009874	UAAAUAUUUCAACAUCCU	chr5:61199258-61199278	13
G009875	UUGUCAUGUAUUUCUAAAUA	chr5:61199322-61199342	195
G009876	UUUGUCAUGUAUUUCUAAAA	chr5:61199323-61199343	196

TABLE 8

Cyno sqRNA and modification patterns			
Guide ID	Full Sequence	SEQ ID NO:	SEQ ID NO:
G009844	GAGCAACCUCACUCUUGUCU GUUUUAGAGCUAGAAAAGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	34	mG*mA*mG*CAACCUCACUCUUGUCUGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCGUUAUCAmCmUmUm GmAmAmAmAmGmUmGmGmCmAmCmGm AmGmUmCmGmUmGmCmUmU*mU*mU*mU
G009845	AGCAACCUCACUCUUGUCUG GUUUUAGAGCUAGAAAAGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	198	mA*mG*mC*AACCUCACUCUUGUCUGGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUA AAAUAAGGCUAGUCGUUAUCAmCmUmUm GmAmAmAmAmGmUmGmGmCmAmCmGm AmGmUmCmGmUmGmCmUmU*mU*mU*mU
G009846	ACCUCACUCUUGUCUGGGGA GUUUUAGAGCUAGAAAAGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	199	mA*mC*mC*UCACUCUUGUCUGGGGAGUUUU AGAmGmCmUmAmGmAmAmUmAmGmCAA GUUAAAUAAGGCUAGUCGUUAUCAmCmAmCm UmUmGmAmAmAmAmGmUmGmGmCmAmCm CmGmAmGmUmCmGmUmGmCmUmU*mU*mU*mU
G009847	CCUCACUCUUGUCUGGGGA GUUUUAGAGCUAGAAAAGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	200	mC*mC*mU*CACUCUUGUCUGGGGAAGUUUU GAmGmCmUmAmGmAmAmUmAmGmCAAGU UAAAUAAGGCUAGUCGUUAUCAmCmAmCm UmGmAmAmAmAmGmUmGmGmCmAmCm GmAmGmUmCmGmUmGmCmUmU*mU*mU*mU
G009848	CUCACUCUUGUCUGGGGAAG GUUUUAGAGCUAGAAAAGC AAGUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	201	mC*mU*mC*ACUCUUGUCUGGGGAAGGUUUU AGAmGmCmUmAmGmAmAmUmAmGmCAA GUUAAAUAAGGCUAGUCGUUAUCAmCmAmCm UmUmGmAmAmAmAmGmUmGmGmCmAmCm CmGmAmGmUmCmGmUmGmCmUmU*mU*mU*mU

TABLE 8 -continued

Cyno sgRNA and modification patterns			
Guide ID	Full Sequence	SEQ ID NO: Full Sequence Modified	SEQ ID NO:
G009849	GGGGAAAGGGGAGAAAAAAA GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	202 mG*mG*GAAGGGGAGAAAAAAAAGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	235
G009850	GGGAAGGGGAGAAAAAAA GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	203 mG*mG*GAAGGGGAGAAAAAAAAGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	236
G009851	AUGCAUUUGUUUCAAAAUU GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	35 mA*mU*mG*CAUUGUUUCAAAAUAGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	67
G009852	UGCAUUUGUUUCAAAAUU GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	36 mU*mG*mC*AUUUGUUUCAAAAUAGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	68
G009853	UGAUUCCUACAGAAAAAGUC GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	206 mU*mG*mA*UUCCUACAGAAAAAGUCGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	239
G009854	UACAGAAAAAGUCAGGAUAA GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	207 mU*mA*mC*AGAAAAAGUCAGGAUAGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	240
G009855	UUUCUUUCUGCCUUUAAAAG GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	208 mU*mU*mU*CUCUCGCCUUUAAAACAGGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	241
G009856	UUUAAGUUUUAUUCAAC GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	209 mU*mU*mA*UAGUUUUAUUCAACAGGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	242
G009857	AUUUAUGAGAUCAACAGCAC GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	37 mA*mU*mU*UAUGAGAUCAACAGCACGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	69
G009858	GAUCAACAGCACAGGUUUUG GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	38 mG*mA*mU*CAACAGCACAGGUUUUGGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	70
G009859	UUAAAUAAGCAUAGUGCAA GUUUUAGAGCUAGAAAUAGC AAGUUAACAUAGGCUGAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	39 mU*mU*mA*AAUAAAGCAUAGUGCAAGUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUGAGCCGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	71

TABLE 8 -continued

Cyno sgRNA and modification patterns			
Guide ID	Full Sequence	SEQ ID NO: Full Sequence Modified	SEQ ID NO:
G009860	UAAAAGCAUAGUGCAAUGGAU GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	40 mU*mA*mA*AGCAUAGUGCAAUGGAUGUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	72
G009861	UAGUGCAAUGGAUAGGUUU GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	41 mU*mA*mG*UGCAAUGGAUAGGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	73
G009862	AGUGCAAUGGAUAGGUUU GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	215 mA*mG*mU*GCAAUGGAUAGGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	248
G009863	UUACUUUGCACUUUCCUUA GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	216 mU*mU*mA*CUUUGCACUUUCCUUAGGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	249
G009864	UACUUUGCACUUUCCUUA GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	217 mU*mA*mC*UUUGCACUUUCCUUAGGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	250
G009865	UCUGACCUUUUAUUUACCU GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	218 mU*mC*mU*GACCUUUUAUUUACCUAGGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	251
G009866	UACAAAACUUUUAUUUACU GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	42 mU*mA*mC*UAAAACUUUUAUUUACGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	74
G009867	AAAGUUGAACAAUAGAAAAA GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	43 mA*mA*mA*GUUGAACAAUAGAAAAAGUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	75
G009868	AAUGCAUAAUCUAAAGUAAA GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	44 mA*mA*mU*GCAUAAUCUAAAGUCAAAGUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	76
G009869	AUUAUCCUGACUUUUCUGU GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	222 mA*mU*mU*AUCUCGACUUUUCUGUGUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	255
G009870	UGAAAAUUUCCUCUGUUUAA GUUUUAGAGCUAGAAAUAGC AAGUUAAAAUAAGGCUAGUC CGUUAUCAACUJGAAAAGU GGCACCGAGUCGGUGCUUUU	223 mU*mG*mA*AUUAUCCUCUGUUUAGUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU	256

TABLE 8 -continued

Cyno sgRNA and modification patterns								
Guide ID	Full Sequence	SEQ NO:	SEQ ID	SEQ NO:	SEQ ID	SEQ NO:	SEQ ID	SEQ NO:
Full Sequence	Modified							
G009871	UAUUUUUCUUUUGCCCACUA GUUUUAGAGCUAGAAAUGC AAGUAAAAAUAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	224	mU*mA*mA*UUUUCUUUUGCCCACUAGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUm GmAmAmAmAmGmUmGmGmCmAmCmCmGm AmGmUmCmGmGmUmGmCmU*mU*mU*mU		257			
G009872	AAAAGGUAGAGAAUUGUUUAG GUUUUAGAGCUAGAAAUGC AAGUAAAAAUAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	225	mA*mA*mA*AGGUAGAGAAUUGUUUAGGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU		258			
G009873	AACAUCUAGGUAAAAAAA GUUUUAGAGCUAGAAAUGC AAGUAAAAAUAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	226	mA*mA*mC*AUCCUAGGUAAAAAAAAGUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU		259			
G009874	UAUAAAUAUCAACAUCCU GUUUUAGAGCUAGAAAUGC AAGUAAAAAUAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	45	mU*mA*mA*UAUAAAUAUCAACAUCCUGUUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU		77			
G009875	UUGUCAUGUAAAUCUAAAA GUUUUAGAGCUAGAAAUGC AAGUAAAAAUAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	228	mU*mU*mG*UCAUGUAAAUCUAAAAGUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU		261			
G009876	UUUGUCAUGUAAAUCUAAAA GUUUUAGAGCUAGAAAUGC AAGUAAAAAUAGGCUAGUC CGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU	229	mU*mU*mU*GUCAUGUAAAUCUAAAAGUUUAG AmGmCmUmAmGmAmAmUmAmGmCAAGUUAA AAUAAGGCUAGUCGGUUAUCAmAmCmUmUmGm AmAmAmAmGmUmGmGmCmAmCmCmGmAmGm UmCmGmGmUmGmCmU*mU*mU*mU		262			

TABLE 9

Vector Components and Sequences								
Plasmid ID	5' ITR	1 st orientation			2 nd orientation			3' ITR
		Splice Acceptor	Transgene	Poly-A	Poly-A	Transgene	Splice Acceptor	
P00147	(SEQ ID NO: 263)	Mouse Splice (R338L)- Acceptor (SEQ ID NO: 265) NO: 264)	Human Factor IX (R338L)- Acceptor (SEQ ID NO: 265)	SEQ ID NO: 266	SEQ ID NO: 267	Human Factor IX (R338L)- Acceptor (SEQ ID NO: 268)	Mouse Splice (R338L)- Acceptor (SEQ ID NO: 269)	(SEQ ID NO: 270)
P00411	(SEQ ID NO: 263)	Human Factor IX Splice (R338L)- Acceptor HiBit (SEQ ID NO: 272)	Human Factor IX (R338L)- Acceptor HiBit (SEQ ID NO: 273)	SEQ ID NO: 266	SEQ ID NO: 267	Human Factor IX Splice (R338L)- Acceptor HiBit (SEQ ID NO: 274)	Human Factor IX Splice (SEQ ID NO: 270)	(SEQ ID NO: 270)

TABLE 9-continued

Plasmid ID	5' ITR	Vector Components and Sequences							
		1 st orientation				2 nd orientation			
		Splice Acceptor	Transgene	Poly-A	Poly-A	Splice Acceptor	Transgene	Poly-A	3' ITR
P00415	(SEQ ID NO: 263)	Mouse Albumin Splice Acceptor (SEQ ID NO: 275) (SEQ ID NO: 264)	Nluc-P2A- GFP (SEQ ID NO: 266)	SEQ ID NO: 266	SEQ ID NO: 267	Nluc-P2A- GFP (SEQ ID NO: 276)	Mouse Albumin Splice Acceptor (SEQ ID NO: 270) (SEQ ID NO: 269)	(SEQ ID NO: 270)	
P00418	(SEQ ID NO: 263)	Mouse Albumin Splice Acceptor (SEQ ID NO: 272)	Human Factor IX (R338L)- HiBit (SEQ ID NO: 273)	SEQ ID NO: 266	SEQ ID NO: 267	Human Factor IX (R338L)- HiBit (SEQ ID NO: 273)	Mouse Albumin Splice Acceptor (SEQ ID NO: 270) (SEQ ID NO: 269)	(SEQ ID NO: 270)	

5' ITR Sequence (SEQ ID NO: 263) :

TTGGCCACTCCCTCTCGCGCCTCGCTCACTGAGGCCGGGACCAAAGGTC

GCCCGACGCCGGGCTTGCCGGCGGCCTCAGTGAGCGAGCGCGCAGAGA

GGGAGTGGCCAATCCATCACTAGGGGTTCT

Mouse Albumin Splice Acceptor (1st orientation) (SEQ ID NO: 264) :

TAGGTCAGTGAAGAGAAGAACAAAAGCAGCATATTACAGTTAGTGTCTTCATCA

ATCTTTAAATATGTTGTGGTTCTCTCCCTGTTCCACAG

Human Factor IX (R338L), 1st Orientation (SEQ ID NO: 265) :

TTTCTTGATCATGAAACGCCAACAAAATTCTGAATCGGCAAAGAGGTATAATTCA

GGTAAATGGAAAGAGTTGTTCAAGGGAACCTTGAGAGAGAATGTATGGAAGAAAA

GTGTAGTTTGAGAAGCACGAGAAGTTTGAAAACACTGAAAGAACAACTGAAT

TTTGGAAAGCAGTATGTTGATGGAGATCAGTGTGAGTCCAATCCATGTTAAATGGCG

GCAGTTGCAAGGATGACATTAATTCTATGAATGTTGGTGTCCCTTGGATTGAAAG

GAAAAGACTGTGAATTAGATGTAACATGTAACATTAAGAATGGCAGATGCGAGCAG

TTTGTTAAAATAGTGTGATAACAAGGTGGTTGCTCCTGTACTGAGGGATATCGA

CTTGCAGAAAACCAGAAGTCCTGTGAACCAGCAGTGCCATTCCATGTGGAAGAGTT

TCTGTTTACAAACTCTAAGCTCACCGTGCTGAGACTGTTTCTGATGTGGACT

ATGTAATTCTACTGAAGCTGAAACCATTGGATAACATCACTCAAAGCACCAAT

CATTAAATGACTCACTGGGTGTTGGAGAAGATGCCAACCAGGTCAATTCC

CTTGGCAGGTTGTTGAATGGTAAAGTTGATGCATTCTGTGAGGCTCTATCGTTA

ATGAAAAATGGATTGTAACTGCTGCCACTGTGTTGAAACTGGTGTAAAATTACAG

TTGTCGAGGTGAAACATAATATTGAGGAGACAGAACATACAGAGCAAAGCGAAAT

GTGATTGCAATTATTCCCTCACCAACATACAATGCACTTAAAGTACAACCAT

GACATTGCCCTCTGGAACGGACGAACCTTAGTGCTAACAGCTACGTTACACCT

ATTTGCATTGCTGACAAGGAATACACGAACATCTTCTCAATTGGATCTGGCTAT

GTAAGTGGCTGGGAAGAGTCTCCACAAAGGGAGATCAGCTTAGTTCTCAGTAC

CTTAGAGTTCCACTGTTGACCGAGCCACATGTCCTATCTACAAAGTTACCATCT

ATAACAAACATGTTCTGTGCTGGCTTCCATGAAGGAGGTAGAGATTGTCAGGAG

- continued

ATAGTGGGGACCCATGTTACTGAAGTGGAAAGGGACCAGTTCTTAACGTGGAAATTAA

TTAGCTGGGTGAAGAGTGTGCAATGAAAGGCAAATATGGAATATATAACCAAGGTA

TCCCGGTATGTCAACTGGATTAAGGAAAAAACAAAGCTCACTTAA

Poly-A (1st orientation) (SEQ ID NO: 266) :

CCTCGACTGTGCCCTAGTGCAGCCATCTGTTGCCCCCTCCCCGTGCCCTTC

CTTGACCCCTGGAAGGTGCCACTCCCACTGTCCTTCTAATAAAATGAGGAATTGC

ATCGCATTGTCAGTAGGTGTCATTCTATTCTGGGGGTGGGGTGGGCAGGACAG

CAAGGGGGAGGATTGGAAGACAATAGCAGGCATGCTGGGATGCGGTGGCTA

TGGCTTCTGAGGCGGAAAGAACAGCTGGGCTCTAGGGGTATCCCC

Poly-A (2nd orientation) (SEQ ID NO: 267) :

AAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGT

TGTTAACCTGTTATTGCAGCTTAAATGGTACAAATAAGCAATAGCATCACAAA

TTTCACAAATAAACGATTTTCACTGCATTCTAGTTGTGGTTGTCCAAACTCATC

AATGTATCTTATCATGTCTG

Human Factor IX (R338L), 2nd Orientation (SEQ ID NO: 268) :

TTAGGTGAGCTTAGTCTTTCTTTATCCAATTCACTGAGCGAGACCTTCGTATAG

ATGCCATATTCCTTCATCGCACATTCCCTCCCCAACTTATTATCCGGTCAAGA

AACTTGTCTCGACTTCAGTGACGTGTGGTCCACCTGAATCACCTGGCATGAGTC

GCGACCGCCCTCGTAAACCCAGCACAAACATGTTATTGAAATCGTAAATTCTG

GGACAGAAGACAGGTGCGCTATCGACCAACGGGACGCGCAAATATTGAGACGA

GGCTGATCGACCTTGTGGAAGACCCGCCCCCACCACCTCACATATCCGCTCCAA

ATTTCAAGAAGATATTGTATATTCTTATCGGCTATACAAATCGGGTAACATAGG

AGTTAAGTACGAGTGGCTCGTCCAGCTCCAGGAGGCTATATCATGGTTACTGT

TTATAGCGCATTAAATTGTGATGGGTATGATCCTGATAACATTCTTTCTGTC

AGTATGCTCAGTTCTTCAATGTTGTTGCCAGCCACGACGTAATCTAACCCCC

GTCTCGACACAGTGTGCCGGCTTACAATCCACTTCAATTGACTATGGAGCCCCA

CAAAACCGCTGACTTTCCGTTGAGCACCACTGCCATGAAATTGCCAGGTTA

GCGTCCTCGCCCCGACAACCCTAGTAAAGTCATTAAATGACTGTGGATTGT

ATATTATCAAGAATCGTTCGCTTCAGTAGAGTTAACGTAGTCCACATCGGGAAA

ACTGTCTCGCCCTGTCAACTTGATGTCAGTGGACACACTTACCGACCGCACGG

AAGGGCACCGCCGGTCACAGCTCTTGATTCAGCGAGCCGGTAGCCCTCAGTG

CAACTACACACAACCTTGTTGCGCGAATTTCACAGAATTGCTCGCATCGTCCA

TTTTAATGTTGAGGTGACGTCAACTCGCAGTTTCTCCTCAAAACCAAAAGGG

CACCAACACTCGTAGGAATTATCGTCTTACAACCTCCCCCATTGAGACATGGA

TTAGATTGCACTGGTCCCCATCGACATATTGCTTCCAGAACACTGAGTGGTCCGTTCTG

- continued

TATTCTCAAACACCTCGCGCCTTCTCAAAAAGTCATTTCCCTCCATAACACTCTCG

CTCCAAGTCCCTTGACGAATTCTCAAGCTTCCTGAGTTACCTTTAGGCCGG

TTAAGTATCTTATTCGCGTTTCGTGGTCCAGAAA

Mouse Albumin Splice Acceptor (2nd orientation) (SEQ ID NO: 269) :
CTGTGGAACAGGGAGAAAAACCACAAACATATTAAAGATTGATGAAGACAA

CTAACTGTAATATGCTTTGTTCTTCACTGACCTA

3' ITR Sequence (SEQ ID NO: 270) :

AGGAACCCCTAGTGATGGAGTGGCACTCCCTCTGCGCGCTCGCTCACTG

AGGCCGCCGGCAAAGCCCGGGCGTCGGCGACCTTGGTCGCCCGGCCTCAGTG

AGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA

Human Factor IX Splice Acceptor (1st Orientation) (SEQ ID NO: 271) :
GATTATTTGGATTTAAACAAAGACTTCTTAAGAGATGTAAATTTCATGATGTT

TTCTTTTTGCTAAAATAAAGAATTATTCTTTACATTCAG

Human Factor IX (R338L)-HiBit (1st Orientation) (SEQ ID NO: 272) :
TTCTTGATCATGAAAAGCCAACAAATTCTGAATCGGCAAAGAGGTATAATTCA

GGTAAATTGAAAGAGTTGTTCAAGGGAACCTTGAGAGAGAATGTATGAAAGAAAA

GTGTAGTTGAAAGAACGACGAGAAGTTTGAAAACACTGAAAGAACAACTGAAT

TTTGGAACAGTATGTTGATGGAGATCAGTGTGAGTCCAATCCATGTTAAATGGCG

GCAGTTGCAAGGATGACATTAATTCTATGAATGTTGGTGTCCCTTGATTGAAAG

GAAAGAACTGTGAATTAGATGTAACATGTAACATTAAGAATGGCAGATGCGAGCAG

TTTGTAAAAATAGTGCTGATAACAAGGTGGTTGCTCCTGACTGAGGGATATCGA

CTTGCAGAAAACCAGAAGTCCTGTGAACCAGCAGTGCCATTCCATGTGAAAGAGTT

TCTGTTCACAAACTCTAACGCTCACCGTGCTGAGACTGTTTCTGATGTGGACT

ATGTAATTCTACTGAAGCTGAAACCATTGGATAACATCACTCAAAGCACCAAT

CATTTAATGACTTCACTCGGGTTGTTGGAGAAGATGCCAACCCAGGTCAATTCC

CTTGGCAGGTTGTTGAAATGGAAAGTTGATGCATTCTGTGAGGGCTATCGTTA

ATGAAAATGGATTGTAACTGCTGCCACTGTGTTGAAACTGGTGTAAAATTACAG

TTGTCGAGGTGAAACATAATTGAGGAGACAGAACATACAGAGCAAAGCGAAAT

GTGATTGAAATTATTCTCACACAAACTACAATGCAGCTATTAAATAAGTACAACCAT

GACATTGCCCTCTGAACTGGACGAACCCCTAGTGCTAACAGCTACGTTACACCT

ATTTGCATTGCTGACAAGGAATACACGAACATCTTCTCAAAATTGGATCTGGCTAT

GTAAGTGGCTGGGGAGAGTCCTCCACAAAGGGAGATCAGCTTAGTTCTCAGTAC

- continued

CTTAGAGTCCACTTGTTGACCGAGGCCACATGTCTTCTATCTACAAAGTTACCATCT

ATAACAACATGTTCTGTGCTGGCTTCATGAAGGAGGTAGAGATTATGTCAAGGAG

ATAGTGGGGACCCATGTTACTGAAGTGGAAAGGGACCAGTTCTTAAGTGGAAATTA

TTAGCTGGGTGAAGAGTGTGCAATGAAGGAAATATGGAATATATACCAAGGTC

TCCCGGTATGTCAACTGGATTAAGGAAAAAACAAAGCTCACTGTCAGCGGATGGAG

ACTGTTCAAGAAGATCAGCTAA

Human Factor IX (R338L) -HiBit (2nd Orientation) (SEQ ID NO: 273) :
TTAGGAAATCTCTTAAACAGCCGCCAGCGCTCACGGTGAGCTTAGTCTTTCTTT

ATCCAATTTCACGTAGCGAGAGACCTTCGTATAGATGCCATATTCCCCTTCATCGCA

CATTCCCTCCCCAACTTATTATCCGGTCAAGAAACTTGTCTTCGACTTCAGTGA

CGTGTGGTCCACCTGAATCACCTGGCATGAGTCGCACCGCCCTCGTGAAACCCAG

CACAAAACATGTTATTGTAATCGTAAATTCTGTGGACAGAAAGACAGGTGCGCTAT

CGACCAACGGGACGCGCAAATATTGAGAACGAGGGCTGATGACCTTGTTGGAAG

ACCCGCCCTCACACTCACATATCCGCTCCAAATTCAAGAAGATATTGTATAT

TCTTATCGGCTATAACAAATCGGGTAACATAGGAGTTAAGTACGAGTGGCTCGTCC

AGCTCCAGGAGGGCTATATCATGGTTGACTTGTATAGCGGCATTATAATTGTGA

TGGGTATGATCTGATAACATTCTTTCTGTCACTGCTAGTATGCTCAGTTCTTCATGT

TGTGTTGCCAGCCACGACCGTAATCTAACCCCCGTCGACACAGTGTGCGGCCG

TTACAATCCACTTTCTATTGACTATGGAGCCCCACAAAACCGTCGACTTTCCGTT

GAGCACACCTGCCATGGAAATTGGCAGGTTAGCGTCTCGCCCCGACAACCC

AGTAAAGTCATTAATGACTGTGTTGTTATATTCAAGAATCGTTGCGC

TTCAGTAGAGTTAACGTAGTCCACATGGGAAAAACTGTCTGGCCCTGTCAACTT

TGATGTCTGGCACACTTACCCGACCGCACGGGAAGGGCACCGCGTTCACAGC

TCTTTGATTCTCAGCGAGCGGTAGCCCTAGTGCAACTACACAAACTTGTGTC

GGCGGAATTTACAGAATTGCTCGCATCGTCATTAAATGTTGAGGTGACGTCC

AACTCGCAGTTTCCTCAAAACAAAAGGGCACCAACACTCGTAGGAATTATA

TCGTCTTACAACCTCCCCCATTGACATGGATTAGATTGCACTGGTCCCCATCGA

CATATTGCTTCCAGAACTCAGTGGTCTGTTCTCAACACCTCGCGCGCTTC

TTCAAAACTGCATTTCTCCATACACTCTGCTCCAAGTCCCTGACGAATTCT

TCAAGCTTCCTGAGTTACCTTTAGGCCGGTTAAGTATCTTATTGCGTTTGT

GGTCCAGAAA

Human Factor IX Splice Acceptor (2nd Orientation) (SEQ ID NO: 274) :
CTGAAATGTAAGAATAATTCTTTAGTTAGCAAAAAAGAAAACATCATGAAAA

TTTACATCTCTTAAGAAAGTCTTGTGTTAATCCAAATAATC

Nluc-P2A-GFP (1st Orientation) (SEQ ID NO: 275) :

TTCTTGATCATGAAACGCCAACAAATTCTGAATCGGCCAAGAGGTATAATTCA

GGTAAATTGGAAGAGTTGTTCAAGGGAACCTTGAGAGAGAAATGTATGGAAGAAA

GTGTAGTTTGAGAAGAACGAGTATTCACTTGGAGGACTTGTGGTACTGGAGGCA

AACCGCTGGTTATAATCTGACCAAGTACTGGAACAGGGCGGGTAAGTCCCTCTT

TCAGAATTGGGTGTAAGCGTCACACCAATCCAGCGATTGTTGCTGGAGAGAA

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CGGACTAAAATTGACATCCATGTTATCATTCCATATGAAGGTCTCAGTGGAGACCA
AATGGGGCAGATCGAGAAGATTTCAAGGTAGTTACCCAGTCGACGATCACCACTT
CAAAGTCATTCTCCACTATGGCACACTTGTATCGACGGAGTAACCTAATATGAT
TGATTACTTGGTCGCCGTATGAGGGCATCCAGTGTGATGGCAAAAGATCAC
CGTAACAGGAACGTTGTGGAATGGGAACAAGATAATCGACGAGAGATTGATAAAC
CAGACGGGTCACTCCTGTTCAAGGTTACAATTAAACGGCGTACAGGATGGAGACTCT
GTGAACGAATACTGGCCACAAATTTCACTCCTGAAGCAGGCCGGAGACGTGGAG
GAAAACCCAGGGCCCGTGAGCAAGGGCGAGGAGCTGTTCACCGGGTGGTGCCTCAT
CCTGGTCAGCTGGACGGCGACGTAACGGCCACAAGTTCAAGCGTGTCCGGCGAGG
GCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGCACCACCGC
AAGCTGCCGTGCCCTGGCCACCCCTCGTGAACCCCTGACCTACGGCGTGCAGTGC
TTAGGCCGCTACCCCGACCATGAAGCAGCACGACTTCTCAAGTCCGCATGCC
GAAGGCTACGTCAGGAGCGCACCATCTTCTCAAGGACGACGGCAACTACAAGAC
CCCGGCCGAGGTGAAGTTCGAGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGG
GCATCGACTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTAC
AACAGCCACAACGTCTATATCATGGCGACAAGCAGAAGAACGGCATCAAGGTGAA
CTTCAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTGCCGACCAACTACCC
AGCAGAACACCCCCATGGCGACGGCCCGTGTGCTGCCGACAACCACACTACCTG
AGCACCCCAGTCGCCCTGAGCAAAGACCCCAAGAGAACGGCGATCACATGGTCT
GCTGGAGTCGTGACGCCGCCGGGATCACTCTGGCATGGACGAGCTGTACAAGG
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Nluc-P2A-GFP (2nd Orientation) (SEQ ID NO: 276) :

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TTACACCTTCCTCTTCTTCTGGGCTGCCCGCCCTGTACAGCTCGCCATGCC
AGGGTGATGCCGGCGCGGTCAAGAACTCCAGCAGCACCATGTGGTCCCTCTCG
TTGGGTCTTGCTCAGGGCGCTCTGGTGCTCAGGTAGTGGTGTGCGGAGCAGC
ACGGGGCGTCGCCGATGGGGTGTCTGCTGGTAGTGGTGTGCGCAGCTGCACGCTG
CCGTCCTCGATGTTGTGCCTGATCTTGAAGTTCACCTGATGCCGTTCTGCTTGT
CGGCATGATGTACAGTTGTGCTGTTGAGTGTACTCCAGCTGTGGCCAGGA
TGTGCGTCCTCTTGAAGTCGATGCCCTCAGCTCGATCTGTCACCAGGGTGTG
GCCCTCGAACCTCACCTGGCCCTGGCTTGTAGTTGCCGTCGCTTGAAGAACGAT
GGTCCTCTCCTGCCACGTAGGCCCTGGGATGGCGCTCTTGAAGAACGCTGTGCTG
CATGTGGTCGGGTACCTGCTGAAGCACTGCACGCCGTAGGTAGGGTGGTCACCA
GGGTGGGCCAGGGCACGGGCAGCTGCCGGTGGTGCAGATGAACCTCAGGGTCA
TGCCGTTAGGGCGTCGCCCTGCCCTGCCCTGCCGTCACGCTGAACCTGTGGCGT
ACGTCGCCGTCAGCTCCACCAAGGATGGGCACCCAGGCCGTGAACAGCTCCCGC
CTTGCCTCACGGGCCGGGTTCTCCTCCACGTCGCCGGCTGCTTCAAGCAGGTGAA
GTTGGTGGCCAGGATCCTCTCGCACAGCCTCCAGCCGGTACGCCGTTGATGGTCAC
CCTGAAACAGCAGGCTGCCGTGGGTTGATCAGCCTCTCGTCGATGATCTGTTGCC
GTTCCACAGGGTGCCTCACGGTGTCTTCTGCGTCGAACACGGCGATGCCCTC
GTAGGGCCTGCCGAAGTAGTCGATCATGTTGGGGTCAGGCCGTGATCACCAGGG

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TGCCGTAGTGCAGGATCACCTGAAGTGGTGTGTCGACGGGGTACACCACCTGA
AAATCTTCTCGATCTGGCCCATCTGGTCGCCGCTCAGGCCCTCGTAGGGATGATCA
CGTGGATGTCGATCTCAGGCCGTTCTGCCGCTCAGCACGATCCTCTGGATGGGG
TCACGCTCACGCCAGGTTCTGGAACAGGCTGCTCACGCCGCTCAGCACCT
GGTCCAGGTTGAGCCGGCTGCCTCCAGTCGCCACGAAGTCCTCCAGGGTGA
ACACGGCCTCTCGAAGCTGCACCTCTCCATGCACTCCCTCCAGGTTGCCCTG
CACGAACCTCCAGCTTGCCGTGTTGACCTCTGGCCTGTTAGGATCTGTTG
GCGTTCTCGTGGTCCAGGAA

P00147 full sequence (from ITR to ITR) :

(SEQ ID NO: 277)

TGGGCCACTCCCTCTCGCGCCTCGCTCACTGAGGCCGGCAGCAAAGGTC
GCCCGACGCCGGCTTGCCGGCGGCCTCAGTGAGCGAGCGCGCAGAGA
GGGAGTGGCCAATCCATCACTAGGGTTCTAGATCTCTTAGGTCAGTGAAGAGA
AGAACAAAAGCAGCATATTACAGTTAGTTGTCATCAATTTAAATATGTTGT
GTGGTTTTCTCCCTGTTTACAGTTTCTTGATCATGAAAACGCCAACAAAAT
TCTGAATCGGCCAAAGGGTATAATTCAAGGAAATTGGAAGAGTTGTCAGAGA
ACCTTGAGAGAGAATGTATGGAAGAAAAGTAGTTGAAAGAACGACGAGAAGTT
TTGAAAACACTGAAAGAACACTGAATTGGAAGCAGTAGTTGATGGAGATCA
GTGTGAGTCCAATCCATGTTAAATGGCGCAGTTGCAAGGATGACATTAATTCTA
TGAATGTTGGTGCCTTGGATTGAAAGGAAAGACTGTGAATTAGATGTAACATG
TAACATTAAGAATGGCAGATGCGAGCAGTTGTAAGGATAGTGTGATAACAAGG
TGGTTGCTCTGACTGAGGGATATCGACTTGCAGAAAACCAGAACGCTGTGAAAC
CACCACTGCCATTCCATGGAAGAGTTCTGTTCACAAACTCTAACGCTCACCC
GTGCTGAGACTGTTTCTGATGTGGACTATGTAATTCTACTGAAGCTAACCA
TTTGATAACACTCAAAGCACCAATCTTAATGACTCAGTGGTTGTTG
GTGGAGAAGATGCCAACCAGGTCATCCCTGGCAGGTTGTTGAATGGTAAAG
TTGATGCATTCTGGAGGCTATGTTAATGAAAAATGGATTGTAAGTGTGCCCC
ACTGTGTTGAAACTGGTAAATTACAGTTGTCGAGGTAAACATAATTGAGG
AGACAGAACATACAGAGCAAAGCGAAATGTGATTGAAATTCTCACCAAC
TACAATGCACTTAAATAAGTACAACCATGACATTGCCCTCTGGAACGGACGAA
CCCTTAGTGCCTAACAGCTACGTTACACCTATTGCAATTGCTGACAAGGAATACAG
AACATCTCTCAAATTGGATCTGGCTATGTAAGTGGCTGGGAAGAGTCTCCAC
AAAGGGAGATCAGCTTAGTTCTCAGTACCTAGAGTCCACTTGTGACCGAGCC
ACATGTCTTCTACAAAGTCACCATCTATAACAACATGTTCTGTGCTGGCTTCC
ATGAAGGAGGATGAGATTGTCAGGAGATAGTGGGGACCCATGTTACTGAA
GTGGAAAGGGACCAAGTTCTTAAGGAAATTAGCTGGGTGAAGAGTGTGCAAT
GAAAGGCAAATATGGAATATACCAAGGTATCCCGGTATGTCACGGATTAAGG
AAAAAACAAAGCTACTTAACCTGACTGTCCTCTGACCCCTGGAAGGTGCCAC
TTGCCCTCCCCGTGCCTCTTGACCCCTGGAAGGTGCCACCTCCACTGTCCTTCC
TAATAAAATGAGGAAATTGCATCGCATTGTCAGTAGGTGTCATTCTATTCTGGGG

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GGTGGGGTGGGCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCATG
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 AGGGGGTATCCCCAAAAACCTCCACACCTCCCCTGAACCTGAAACATAAAATG
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 GTCCAAAATCTCATCAATGTATCTTATCATGTCGTAGGTGAGCTTAGTCTTTCTTT
 ATCCAATTCACGTAGCGAGAGACCTCGTATAGATGCCATATTCCCTTCATCGCA
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 CGTGTGGTCCACCTGAATCACCTGGCATGAGTCGCACCGCCCTCGTGAACCCAG
 CACAAAACATGTTATTGTAATCGTAAATTCTGTGGACAGAACAGGTCGCTCTAT
 CGACCAACGGGACGCGAAATATTGCAGAACGAGGCTGATCGACCTTGTTGGAAG
 ACCCGCCCCCACCACACATATCCGCTCCAAATTCAAGAAGATATTGTATAT
 TCTTTATCGGCTATAAAATCGGGTAACATAGGAGTTAAGTACGAGTGGCTCGTCC
 AGCTCCAGGAGGGCTATATCATGGTTGACTTGTATAGCGGCATTATAATTGTGA
 TGGGGTATGATCCTGATAAACATTCTTTCTGTCAGTATGCTCAGTTCTCAATGT
 TGTGTTGCCAGCCACGACCGTAATCTAACCCCCGTCTGACACAGTGTGCGGCC
 TTACAATCCACTTTCACTGACTATGGAGCCCCACAAAACCGTCGACTTCCGTT
 GAGCACCACCTGCCATGAAATTGCCAGGTTAGCGTCCTGCCCGACAACCC
 AGTAAAGTCATTAATGACTGTGTTGATTGTATATTCAAGAACGTTGCGC
 TTCAGTAGAGTTAACGTAGTCCACATGGGAAAAACTGTCGGCCCTGTCAACTT
 TGATGTCGGCACACTTACCGACCGCACGGGAAGGGCACCGCCGTTACAGC
 TCTTTGATTCTCAGCGAGCCCGTAGCCCTCACTGCAACTACACACAATTGTTGTC
 GGCGGAATTTCACAGAATTGCTCGCATCGTCATTGTTAATGTTGCAAGGTGACG
 AACTCGCAGTTTCCTCAAACACAAAAGGGCACCAACACTCGTAGGAATTATA
 TCGTCTTACAACCCCCCATTCAAGACATGGATTAGATTCGATTGGTCCCCATCGA
 CATATTGCTTCAGAACACTGAGTGGCCGTTCTGTATTCTCAAAACACCTCGCGC
 TTCAAAAACGCTTTCCATACACTCTCGCTCCAAGTCCCTGCAAGAATTCT
 TCAAGCTTCCTGAGTTACCTTTAGGCCGTTAAGTATCTTATTGCGTTTC
 GGTCCAGAAAATGTTGAAACAGGGAGAGAAAACCACACAAATTTAAAGA
 TTGATGAAGAACAACTAATGCTGCTTTGTTCTCTTCACTGACCTAA
 GAGATCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTGCGCGCTCG
 CTCACTGAGGCCGCCGGCAAAGCCGGCGTGGCGACCTTGTCGCCCGC
 CTCAGTGAGCGAGCGAGCGCAGAGAGGGAGTGGCCAA
 P00411 full sequence (from ITR to ITR) :
 (SEQ ID NO: 278)
 TTGGCCACTCCCTCTGCGCGCTCGCTCACTGAGGCCGGGACCAAAGGTC
 GCCCGACGCCGGCTTGCCGGCGGCCTCAGTGAGCGAGCGCAGAGA
 GGGAGTGGCCAATCCATCACTAGGGGTTCTAGATCTCTGATTATTGATTAAA
 ACAAAAGACTTCTTAAGAGATGAAAATTTCATGATGTTCTTTGCTAAA
 AAAGAATTATTCTTTACATTCACTGAGTTCTGATCATGAAAACGCCAACAAATTC

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TGAATCGCCAAAGAGGTATAATTCAAGTAAATTGGAAGAGTTGTCAGGGAAC
CTTGAGGAGAAATGTATGGAAGAAAAGTGTAGTTTGAGAAGCAGAGAAGTT
TGAAAACACTGAAAGAACAACTGAATTGGAGCAGTATGTTGATGGAGATCAGT
GTGAGTCCAATCATGTTAATGGCGCAGTTGCAAGGGATGACATTAATTCTATG
AATGTTGGTGTCCCTTGGATTGAGAAGAAAGACTGTGAATTAGATGTAACATGTA
ACATTAAGAATGGCAGATGCGAGCAGTTGTAAGGAAATAGTGTGATAACAAGGTG
GTTTGCTCCTGACTGAGGGATATGCAGTTGCAGAAAACCAGAAGTCCTGTGAACCA
GCAGTGCCATTCCATGTGGAAGAGTTCTGTTCACAAACTCTAACGTCACCCGT
GCTGAGACTGTTTCTGATGTTGACTATGTAATTCTACTGAAAGCTGAAACCA
TGGATAACATCACTAAAGCACCCAACTCATTTAATGACTTCACTCGGTTGTTG
GAGAAGATGCCAACCCAGGTCAATTCCCTGGCAGGTTGTTGAATGGTAAAGTTG
ATGCATTCTGTGGAGGCTCATGTTAATGAAAAATGGATTGTAAGTGTGCTGCCACT
GTGTTGAAACTGGTGTAAAATTACAGTTGTCGCAGGTGAAACATAATTGAGGAGA
CAGAACATACAGAGCAAAGCGAAATGTGATTGAAATTCTCACCACAACTAC
AATGCAGCTATTAAATAAGTACAACCATGACATTGCCCTCTGGAACGGAAACCGAAC
TTAGTGCTAACAGCTACGTTACACCTATTGCTTGACTGACAAGGAATACACGAAC
ATCTCCCAAAATTGGATCTGGCTATGTAAGTGGCTGGGAAGAGTCTTCACAA
GGGAGATCAGCTTAGTTCTCAGTACCTAGAGTTCCACTGTTGACCGAGCCACA
TGTCTTATCTACAAAGTTCACCATCTATAACACATGTTCTGCTGGCTTCCATG
AAGGAGGTAGAGATTGTCAGGAGATAGTGGGGACCCATGTTACTGAAGTG
GAAGGGACCAGTTCTTAACGGAATTATTAGCTGGGTGAAGAGTGTGCAATGAA
AGGCAAATATGAAATATACCAAGGTCTCCCGTATGTCACGGATTAAAGGAA
AAACAAAGCTCACTGTCAGCGGATGGGAGACTGTTCAAGAAGATCAGCTAACCTCGA
CTGTGCCTCTAGTTGCCAGCCATCTGTTGTTGCCCTCCCCGTGCCTTCCATG
CCTGGAAAGGTGCCACTCCCACGTCCCTTCCTAATAAAATGGGAATTGCAATCGCA
TTGCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGTGGGCAGGACAGCAAGG
GGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGATGCGGTGGCTATGGCT
TCTGAGGCGGAAAGAACAGCTGGGCTCTAGGGGTATCCCCAAAAACCTCCA
CACCTCCCCGTAACACTGAAACATAAAATGAATGCAATTGTTGTTAACTGTT
ATTGCACTTAAATGGTTACAAATAAGCAATAGCATCACAAATTTCACAAATAA
GCATTTTTCACTGCATTCTAGTTGTTGTCACAAACTCATCAATGTTACTTATC
ATGTCGTTAGGAAATCTCTTAAACAGCCGCCAGCGCTCACGGTGAGCTAGTCT
TTCTTTTATCCAATTACGTCAGCGAGAGACCTTGTATAGATGCCATTTCCTT
CATCGCACATTCTCCCCCAACTTATTATCCGGTCAAGAAACTGTTCTCGACT
TCAGTGACGTGTTGGTCCACCTGAATCACCTGGCATGAGTCGCGACCGCCCTCGTGA
AACCCAGCACAAACATGTTATTGTAATGTAATTCTGTGGACAGAACAGAGT
CGCTCTATCGACCAACGGGACCGCAGCGAAATATTGCAAGAACGAGGGCTGATCGACCTT
TGTGGAAGACCCGCCCCACCCACTCACATATCCGCTCCAAATTCAAGAAGATAT
TTGTATATTCTTATCGGCTATACAAATCGGGTAACATAGGAGTTAAGTACGAGTG

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GCTCGTCCAGCTCAGGAGGGTATATCATGGTGACTTGTATAGCGCATTAT
AATTGTGATGGGGTATGATCCTGATAACATTCTTTCTGTTAGTGTCACTTTC
TTCAATGTTGTTGCCAGCACGACCGTAATCTAACCCCCGTCGACACAGTG
TGCAGCCGTTACAATCCACTTTCAATTGACTATGGAGCCCCACAAAACGCGTCGAC
TTTCGTTGAGCACCACCTGCCATGGAAATTGGCCAGGTTAGCGTCCTGCC
GACAACCCTAGTAAAGTCATTAATGACTGTGGAATTGTGTATATTCAAGAAT
CGTTTGGCTTCAGTAGAGTTAACGTAGTCCACATGGGAAAAACTGTCTGCC
TGTCAACTTGATGTCGGACACACTAACCGACCGCACGGGAAGGGACCGCC
GTTCACAGCTCTTGATTCAGCGAGGCCAGCCAGTGCACACTACACAA
CTTGTGTCGGCGAATTTACAGAATTGTCGCATGTCATTTAATGTTGCA
GGTGAACGCCACTCGCAGTTTCCAAACAAAAGGGCACCAACACTCGTA
GGAATTATATCGTCTTACAACCTCCCCCATTCAAGACATGGATTAGATTGCAATTGG
TCCCCATCGACATATTGCTCCAGAACTCAGGGTCCGTTCTGTATTCTCAAACACCT
CGCGCGCTTCTCCTCAGCTTCCATACACTCTCGCTCCAAGTCCCCTG
CAGAATTCTCAAGCTTCTGAGTTACCTTTAGGCCGGTTAAGTATCTTATTC
GCGTTTCTGTCAGAAAAGTAAATGAAAAGAATAATTCTTAGTTAGCA
AAAAAGAAAACATCATGAAATTACATCTTAAAGAAAGCTTTAGTTAATCC
AAATAATCAGAGATCTAGGAACCCCTAGTGATGGAGTTGCCACTCCCTCTCGC
GCTCGCTCGCTCACTGAGGCCGCCGGCAAGCCGGCGTCGGCGACCTTGG
TCGCCCGGCTCAGTGAGCGAGCGAGCGCAGAGAGGGAGTGGCAA

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P00415 full sequence (from ITR to ITR) :

(SEQ ID NO: 279)

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TTGCCACTCCCTCTCGCGCTCGCTCACTGAGGCCGGCACAAAGTC
GCCGACGCCGGCTTGCCTGGCGCCTAGTGAGCGAGCGCAGAGA
GGGAGTGGCCAACCCATCACTAGGGTTCTAGATCTCTAGTCAGTGAAGAGA
AAACAAAAGCAGCATATTACAGTTAGTTGTCATCAATTAAATGTTGT
GTGGTTTCTCCCTGTTCCACAGTTCTGATCATGAAACGCCAACAAAAT
TCTGAATGCCAAAGAGGTATAATTAGGAAATTGGAAGAGTTGTCAGAGGA
ACCTTGAGAGAGATGTATGGAAGAAAAGTGTAGTTGAAGAAGCAGTATTCA
TTGGAGGACTTGTGCGTGACTGGAGGCAACCGCTGGTTATAATCTGACCAAGTA
CTGGAACAGGGGGGGTAAGTCCCTCTTCAAGAATTGGGTAAAGCGTCACACCA
ATCCAGCGATTGTGTTCTGGAGAGAACGGACTCAAATTGACATCCATGTTAC
ATTCCATATGAAGGTCTCAGTGGAGACCAATGGGCAGATCGAGAAGATTTCAA
GGTAGTTACCCAGTCGACGATCACCACCTCAAAGTCATTCTCCACTATGGCACACT
TGTTATCGACGGAGTAACCTAAATGATTGATTACTTGGTCGCCGTATGAGGG
CATCGCAGTGGATGGCAAAAGATCACCGTAACAGGAACGTTGTGGAATGGGA
ACAAGATAATCGACGAGAGATTGATAAATCCAGACGGTCACCTGTCAGGGTT
ACAATTAACGGCGTACAGGATGGAGACTCTGTGAAACGAATACTGGCCACAAATT
TTCACTCTGAAGCAGGCCGGAGACGTGGAGGAAACCCAGGGCCCGTGAGCAAGG
GCGAGGAGCTGTTCACCGGGGGGTGCCATCTGGTCAGCTGGACGGCGACGTA

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AACGGCCACAAGTTCA CGTGTCCGGCGAGGGCGATGCCACCTACGGCAA
GCTGACCCCTGAAGTCATCTGCACCACCGCAAGCTGCCGTGCCCTGCCAACCT
CGTGACCACCTGACCTACGGCGTGCAGTGCTCAGCGTACCCGACCACATGAA
GCAGCACGACTTCTCAAGTCCGCCATGCCGAAGGCTACGTCAGGAGCGCACCA
TCTTCTCAAGGACGACGGCAACTACAAGACCCGCGCGAGGTGAAGTTCGAGGG
GACACCCCTGGTGAAACCGCATCGAGCTGAAGGGCATCGACTCAAGGAGGACGGCAA
CATCCTGGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGG
CCGACAAGCAGAAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCGAG
GACGGCGCGTGCAGCTGCCGACCACTACCGCAGAACACCCCCATGGCGACGG
CCCCGTGCTGCTGCCGACAACCAACTACCTGAGCACCCAGTCCGCCCTGAGCAAAG
ACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCTGTGACCGCCGCCGG
ATCACTCTCGGCATGGACGAGCTGTACAAGGGAGGAGGAAGCCCGAAGAAGAAGA
GAAAGGTCTAACCTCGACTGTGCTTAGTTGCCAGCCATCTGTTGTTGCCCTCC
CCCGTGCTCTCTTGACCTGGAAGGTGCCACTCCACTGTCTTTCTAATAAAATG
AGGAAATTGCATCGCATTGTCAGTAGGTGTCATTCTATTCTGGGGGTGGGTGG
GGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGATGC
GGTGGGCTCTATGGCTCTGAGGCGGAAAGAACCCAGCTGGGCTCTAGGGGTATC
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CCTTGACAGCTCGTCCATGCCAGGGTGATGCCGCCGGGTACGAACCTCAGCA
GCACCATGTGGCCCTCTCTGCTGGGTCTCAGGGCGCTCTGGGTGCTCAG
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AGTGGTCGGCCAGCTGCACGCTGCCGTCTCGATGTTGCTGATCTGAAGTTCA
CCTTGATGCCGTTCTCTGCTTGCGGCATGATGTACACGTTGGCTGTTGTTAGTT
GTACTCCAGCTGTGGCCAGGATGTTGCCGTCTCTGTAAGTCGATGCCCTCAG
CTCGATCTGTTACCAGGGTGTGCCCTCGAACCTCACCTGGCCCTGGTCTGTAG
TTGCCGTCGTCCTTGAAAGAAGATGGCTCTCTGACGTAAGCCCTGGGATGGCG
CTCTTGAAAGAAGTCGTGCTGTTCATGTTGGTGGGGTACCTGCTGAAGCACTGCAC
CCGTAAGGTGCTGGTCAACAGGGTGGGCCAGGGCACGGGAGCTTGGCTGGGGTGG
GCAGATGAACCTCAGGGTCAGCTTGCGTAGGTGGCGTCGCCCTGCCCTGCC
CACGCTGAACATTGTCGGCGTTCACGTCGCCGTCCAGCTCCACCAAGGATGGCAC
GCCGGTGAACAGCTCCTGCCCTGCTCACGGGCCGGGTTCTCTGCCACGTC
GCCCTGCTTCAGCAGGCTGAAGTTGGTGGGCCAGGATCTCTCGCACAGCCTCAG
GGTCACGCCGTTGATGGTCACCTGAACAGCAGGCTGCCGTGGGGTTGATCAGC
CTCGTCGATGATCTTGTGCCGTTCCACAGGGTGGCGTCACGGTGATCTTGC
TCGAACACGGCGATGCCCTCGTAGGGCTGCCGAAGTAGTCGATCATGTTGGGGTC
ACGCCGTCGATCACCAAGGGTGGCGTAGTGCAGGATCACCTGAAGTGGTGGTC

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ACGGGGTACACCACCTGAAAATCTTCGACTGCCCATCTGGTCGCCGCTCAGG
CCCTCGTAGGGATGATCACGTGGATGTCGATCTTCAGGCGTTCTCGCCGCTCAGC
ACGATCCTCTGGATGGGGTACGCTCACGCCAGGTTCTGGAACAGGCTGCTCAGC
CCGCCTGCTCCAGCACCTGGCCAGGTTGAGCCGGCGGTCTGCCTCAGTCGCC
ACGAAGTCCTCAGGGTAACACGCCCTCCTCGAAGCTGCACCTCTCCATGCAC
TCCCTCTCCAGGTTGCCCTGCAGAACCTCCAGCTGCCGTGTTGACCTCTTGG
GCCTGTTCAAGGATCTTGGTGGCGTTCTCGTGGTCCAGGAA

P00418 full sequence (from ITR to ITR) :

(SEQ ID NO: 280)

TTGGCCACTCCCTCTCGCGCCCTCGCTCACTGAGGCCGGCGACCAAAGGTC
GCCCGACGCCGGCTTGCCGGCGGCCTCAGTGAGCGAGCGCAGCAGAGA
GGGAGTGCCAATCCATCACTAGGGTTCTAGATCTCTTAGGTCAAGAGA
AGAACAAAAAGCAGCATATTACAGTTAGTTGCTTCATCAATTTAAATATGTTGT
GTGGTTTTCTCCCTGTTCCACAGTTTCTTGATCATGAAACGCCAACAAAAT
TCTGAATCGGCCAAGAGGTATAATTCAAGGTAATTGGAAGAGTTGTTCAAGGGA
ACCTTGAGAGAGAATGTATGGAAGAAAAGTGTAGTTGAAAGAACGACGAGAAGTT
TTGAAAACACTGAAAGAACACTGAATTGGAAAGCAGTATGTTGATGGAGATCA
GTGTGAGTCCAATCCATGTTAAATGGCGGCAGTTGCAAGGATGACATTAAATCCTA
TGAATGTTGGTGTCCCTTGGATTGAAAGGAAAGAACGTGTGAATTAGATGTAACATG
TAACATTAAGAATGGCAGATGCGAGCAGTTGTAAGGAAACTAGTGTGATAAACAGG
TGGTTTGCTCCTGACTGAGGGATATCGACTTCAGAAAACCAGAACGTCCTGTGAAAC
CAGCAGTGCCATTCCATGTGGAAGAGTTCTGTTCACAAACTCTAACGTCACCC
GTGCTGAGACTGTTTCTGATGTGACTATGTAATTCTACTGAAGCTGAAACCA
TTTGGAATAACACTCAAAGCACCCAACTATTAATGACTCAGTCCGGTTGTTG
GTGGAGAAGATGCCAACCCAGGTCAATTCCCTGGCAGGTTGTTGAATGGTAAAG
TTGATGCATTCTGTGGAGGCTCTATGTTAATGAAAAATGGATTGTAAGTGTGCCC
ACTGTGTTGAAACTGGTGTAAAATTACAGTTGCGCAGGTAACTGAACTATTGAGG
AGACAGAACATACAGAGCAAAGCGAAATGTGATTGAAATTCTCACCACAC
TACAATGCAGCTATTAAAGTACAACCATGACATTGCCCTCTGGAACGGACGAA
CCCTTAGTGCTAACAGCTACGTTACACCTATTGCTGCTGACAAGGAATACAG
AACATCTCCTCAAATTGGATCTGGCTATGTAAGTGGCTGGGAAGAGTCTTCCAC
AAAGGGAGATCAGCTTAGTTCTCAGTACCTTAGAGTTCCACTTGTGACCGAGCC
ACATGTCTTCTATCTACAAAGTCACCATCTATAACACATGTTCTGTGCTGGCTTCC
ATGAAGGAGGTAGAGGATTGATGTCAAGGAGATAGTGGGGACCCATGTTACTGAA
GTGGAAGGGACCAAGTTCTTAAGTGAATTAGCTGGGGTGAAGAGTGTGCAAT
GAAAGGCAAATATGGAATATACCAAGGTCTCCCGGTATGTCAACTGGATTAAAGG
AAAAAACAAAGCTACTGTCAGCGGATGGAGACTGTTCAAGAAGATCAGCTAACCT
CGACTGTGCCCTAGTTGCCCCGATCTGTTGTTGCCCTCCCCGTGCTTCC
GACCCCTGGAAGGTGCCACTCCACTGTCCTTCTAATAAAATGAGGAAATTGCA
GCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCA

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AGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGCTCATG
GCTTCTGAGGCCAAGAACAGCTGGGCTCTAGGGGTATCCCCAAAAACCTC
CCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTAACTTG
TTTATTGCAGCTTATAATGGTACAAAATAAGCAATAGCATCACAAATTCACAAAT
AAAGCATTTTTCACTGCATTCTAGTTGTTGTCACACTCATCAATGTATCTT
ATCATGTCTGTTAGGAAATCTCTAAACAGCGCAGCCGCTCACGGTGAGCTTAG
TCTTTCTTTATCCAATTACGTAGCGAGAGACCTCGTATAGATGCCATATTC
CTTCATCGCACATTCCCTCCCCAACTTATTATCCCGTCAAGAAACTGTTCC
ACTTCAGTGACCTGTTGGTCCACCTGAATCACCTGGCATGAGTCGACCC
TGAAACCCAGCACAAAATGTTATTGTAATCGTAAATTCTGTGGACAGAAAGACA
GGTCGCTCTATCGACCAACGGACGCGCAAATATTGAGACAGGAGCTGATCGAC
CTTGTTGGAAGACCCGCCCCACCCACTCACATATCCGCTCCAAATTCAAGAAGA
TATTGTATATTCTTATCGGTATACAAATCGGGTAACATAGGAGTTAAGTACGA
GTGGCTCGTCCAGCTCCAGGAGGGCTATATCATGGTTGTTAGCGGAT
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CGGTTCACAGCTTTGATTCTCAGCGAGCCGTTAGCCCTCAGTCAACTACAC
AACTTTGTTGCGCGGAATTTCAGAATTGCTCGCATCGCCATTAAATGTT
CAGGTGACGTCAAACCGCAGTTTCTTCAAAACCAAAAGGGACCAACACTCG
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GGTCCCCATCGACATATTGCTTCAAGACTCAGTGGTCCGTTCTGATTCT
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ATATTTAAAGATTGATGAAGACAACACTGTAATATGCTGCTTTGTTCT
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GCGCTCGCTCGCTCACTGAGGCCGCCGGCAAAGCCC
GGCGACGGCTCAGTGAGCGAGCGCAGAGAGGGAGTGGCCAA
P00123 full sequence (from ITR to ITR) :
(SEQ ID NO: 281)
GGCCACTCCCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGCGAC
CCGACGCCGGCTTGCCGGCGCTCAGTGAGCGAGCGCAGAGAG
GAGTGGCCAACCTCCATCACTAGGGGTTCTGGAGGGTGGAGTC
TGAAGAGAAGAACAAAAGCAGCATATTACAGTTAGTGT
TATGTTGTTGAGTGGTTCTCCCTGTTCCACAGTTTCTGAT
CATGAAACGCCA

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ACAAAATTCTGAATCGGCCAAAGAGGTATAATTCAAGGTAATTGGAAAGAGTTGTC
 AAGGGAACCTTGAGAGAGAATGTATGGAAAGAAAAGTGTAGTTTGAAGAACACGA
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 AACATGTAACATTAAGAATGGCAGATGCGAGCAGTTGTAAGGAAAGAACGTGATA
 ACAAGGTGGTTGCTCCTGTACTGAGGGATATCGACTTGCAGAAAACCAGAACGCT
 GTGAACCAGCAGTGCCATTCCATGTGGAAGAGTTCTGTTCACAAACTCTAAGC
 TCACCCGTGCTGAGACTGTTCCATGTGACTATGTAATTCTACTGAAGCTGA
 AACCATTTGGATAACATCACTCAAAGCACCCAACTATTAATGACTTCACTCGGGT
 TGTTGGTGGAGAAGATGCCAACCCAGGTCAATTCCCTGGCAGGTGTTGAATGG
 TAAAGTTGATGCAATTCTGTGGAGGCTCTATGTTAATGAAAAATGGATTGTAAGTGC
 TGCCCACGTGTTGAAACTGGTAAATTACAGTTGTCGCAGGTGAACATAATAT
 TGAGGAGACAGAACATAAGAGCAGGAAATGTGATTGAAATTCTCACC
 ACAACTACAATGCAAGCTATTAAAGTACAACCATGACATTGCCCTCTGGAACACTGG
 ACGAACCCCTAGTGCTAACAGCTACGTTACACCTATTGCAATTGCTGACAAGGAAT
 ACACGAACATCTCCTCAAATTGGATCTGGCTATGTAAGTGGCTGGGAAGAGTCT
 TCCACAAAGGGAGATCAGCTTAGTTCTCAGTACCTTAGAGTTCCACTGTTGACC
 GAGCCACATGCTTCTATCTACAAAGTCACCATCTATAACACATGTTCTGCTG
 GCTTCCATGAAGGAGGTAGAGATTGTCAGGAGATAGTGGGGACCCATGTT
 ACTGAAGTGGAGGGACCAAGTCTTAACGTTGAAATTAGTGGCTGGGTGAAGAGTG
 TCGCAATGAAAGGAAATATGGAATATACCAAGGTATCCCGTATGTCACGTTG
 TTAAGGAAAAAAACAAAGTCACCTAACCTCGACTGTCCTCTAGTTGCCAGCCATC
 TGTTGTTGCCCTCCCCGTGCCCTTGACCCCTGGAAGGTGCCACTCCACTGTC
 CTTTCCTAATAAAATGAGGAATTGCACTGCAATTGCTGAGTAGGTGTCATTCTATT
 TGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAG
 GCATGCTGGGGATGCGGTGGGCTCTATGGCTCTGAGGCGAAAGAACAGCTGG
 GCTCTAGGGGTATCCCCACTAGTCCACTCCCTCTGCGCGCTCGCTCGCTACTG
 AGGCCGGCGACCAAAGTCGCCCCGACGCCCGGGCTTGCCGGCGCCTCAGTG
 AGCGAGCGAGCGCCAGAGAGGGA

P00204 full sequence (from ITR to ITR) :

(SEQ ID NO: 282)

GGCCACTCCCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGCGACCAAAGGTGCG
 CGACGCCGGCTTGCCGGCGCTCAGTGAGCGAGCGAGCGCGAGAGAG
 GAGTGGCCAACCTCATCACTAGGGGTTCTGGAGGGGTGGAGTCGTGACCTAGGTC
 GTCTCCGGCTCTGCTTTTCCAGGGGTGTGTTCGCCGAGAACGCACTGTAAGAGTTT
 ATGTTTTTCATCTGCTGTATTCTAGTAATGGAAGCCTGGTATTAAAATA
 GTTAAATTTCCTTAGTGCTGATTTCTAGATTATTACTGTTGTTGTTATTAT
 TGTCATTATTCATCTGAGAACCTAGGTCAGTGAAGAGAAGAACAAAAGCAGCAT
 ATTACAGTTAGTGTCTCATCAATCTTAAATATGTTGTTGTTCTCCCTGT

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TTCCACAGTTTCTTGATCATGAAAACGCCAACAAATTCTGAATCGGCCAAGAG
 GTATAATTCAAGGAAATTGGAGAGAGTTGTTCAAGGGAACCTTGAGAGAGAAATGTA
 TGGAGAAAAGTGTAGTTGAAGAACGAGAAGTTTGAAAACACTGAAAGA
 ACAACTGAATTGGAGCAGTATGTTGATGGAGATCAGTGTGAGTCCAATCCATGT
 TAAATGGCGCAGTTGCAAGGATGACATTAATTCTATGAATGTTGGTGTCCCTT
 GGATTTGAAGGAAAGAACGTGAAATTAGATGTAACATGTAACATTAAGAATGGCAG
 ATGCGAGCAGTTGAAAAATAGTGTGATAACAAGGTGGTTGCTCCTGTACTGA
 GGGATATCGACTTGAGAAAACCAGAAGTCCTGTGAACCAGCAGTGCCATTTCCAT
 GTGGAGAGTTCTGTTCACAAACTCTAAGCTCACCCGTGCTGAGACTGTTTCC
 TGATGTGGACTATGTAATTCTACTGAAGCTGAAACCATTGGATAACATCACTCA
 AACGCACCAATTTAATGACTTCACTCGGGTTGTTGGAGAGATGCCAAACC
 AGGTCAATTCCCTGGCAGGTTGTTGAATGGTAAAGTTGATGCATTCTGGAGG
 CTCTATCGTTAATGAAAATGGATTGTAACTGCTGCCACTGTGTTGAAACTGGTGT
 TAAAATTACAGTTGTCGAGGTGAAACATAATTGAGGAGACAGAACATACAGAGC
 AAAAGCGAAATGTGATTGAAATTCTCACACAAACTACAATGCAGCTATTAA
 AGTACAACCATGACATTGCCCTCTGAACTGGACGAACCCCTAGTGCTAACAGCT
 ACCTTACACCTATTGCAATTGCTGACAAGGAATACACGAACATCTCTCAAATTG
 GATCTGGCTATGTAAGTGGCTGGGAAGAGTCTTCCACAAAGGGAGATCAGCTTA
 GTTCTTCAGTACCTTAGAGTTCCACTTGTGACCGAGCCACATGTTCTATCTACAA
 AGTTCAACCATCTATAACAAACATGTTCTGCTGGCTTCCATGAAGGGAGTAGAGATT
 CATGTCAGGAGATACTGGGGACCCATGTTACTGAAGTGGAGGGACAGTTCT
 TAACTGAAATTATTAGCTGGGTGAAGAGTGTGCAATGAAAGGAAATATGGAAT
 ATATACCAAGGTATCCCGTATGTCAACTGGATTAAAGGAAAAACAAAGCTCACTT
 AACCTCGACTGTGCCCTCTAGTTGCCAGCCATCTGTTGTTGCCCTCCCCGTGCC
 TCCTTGACCTGGAAAGGTGCCACTCCACTGTCCTTCTAATAAAATGAGGAATT
 GCATCGCATTGTCGAGTAGGTGTCATTCTATTCTGGGGGGGGGGGGCAGGAC
 AGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGATGCCGGGGCT
 CTATGGCTTCTGAGGCGGAAAGAACCCAGCTGGGCTCTAGGGGTATCCCCCTAG
 GTGGTTATTATTGATATTTGGTATCTTGATGACAATAATGGGGATTTGA
 AAAGCTTAGCTTAAATTCTTTAATTAAAAAAATGCTAGGCAGAATGACTCAA
 TTACGTTGGATACTGGTGAATTATTACGGCTCATAGGGCCCTGCTCGACCAT
 GCTATACTAAAAATTAAAAGTGTACTAGTCCACTCCCTCTGCGCGCTCGCTCG
 CACTGAGGCCGGCGACCAAGGTGCCCCGACGCCGGCTTGCCGGCGCCT
 CAGTGAGCGAGCGAGCGCGCAGAGAGGG

P00353 full sequence (from ITR to ITR) :

(SEQ ID NO: 283)

TTGGCCACTCCCTCTGCGCGCTGCTCGCTCACTGAGGCCGGCGACCAAAGGTC
 GCGCGACGCCGGCTTGCCGGCGCCCTAGTGAGCGAGCGAGCGCAGAGA
 GGGAGTGGCCAACCTCCATCACTAGGGGTTCTAGATCTGATTGAAAGCTTAGCT
 TAAATTCTTTAATTAAAAAAATGCTAGGCAGAATGACTCAAATTACGTTGGAT

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ACAGTTGAATTATTACGGCTCATAGGGCTGCCCTGCTGACCATGCTATACTAAA
AATTAAAAGTGTGTTACTAATTTATAATGGAGTTCCATTATTTACCTTTA
TTCTTATTACCATTGTCTTAGATATTACAAACATGACAGAAACACTAAATCT
TGAGTTGAATGCACAGATATAAACACTTAACGGGTTTAAAAATAATGTTGGT
GAAAAAAATAACTTGAGTGTAGCAGAGAGAACATTGCCACCTTCAGATTTCC
TGTAACGATCGGAACTGGCATCTTCAGGGAGTAGCTTAGGTAGTGAAGAGAAGA
ACAAAAAGCAGCATATTACAGTTAGTTGTCTCATCAATCTTAAATATGTTGTG
GTTTTCTCTCCCTGTTCCACAGTTTCTTGATCATGAAAACGCCAACAAATTCT
GAATCGGCCAAGAGGTTCTTGATCATGAAAACGCCAACAAATTCTGAATCGGC
CAAAGAGGTATAATTCAAGGTTAGGAAAGAGTTGTTCAAGGGAACCTTGAGAGA
GAATGTATGGAAGAAAAGTGTAGTTTGAGAAGACGAGAAGAGTTTGAAAACAC
TGAAAGAACAACTGAATTGGAAGCAGTATGTTGATGGAGATCAGTGTGAGTCCA
ATCCATGTTAAATGGCGCAGTTGCAAGGATGACATTAATTCTATGAATGTTGGT
GTCCTTGGATTGAGGAAAGAAACTGTGAATTAGATGTAACATGTAACATTAAGA
ATGGCAGATCGCAGCTTTGTAAGGAAACTGCTGATAACAAGGTGGTTGCTCCT
GTACTGAGGGATATCGACTTGCAAGAAACAGAAGTCCTGTGAACCAGCAGTGCCA
TTCCATGTGGAAGAGTTCTGTTCAAAACTCTAAGCTCACCGTGCTGAGACTG
TTTCCTGATGTGACTATGTAATTCTACTGAAGCTGAAACCATTGGATAACAT
CACTCAAAGCACCAACTATTAAATGACTTCACTCGGGTTGTTGGAGAAGATGC
CAAACCAGGTCAATTCCCTGGCAGGTTGTTGAATGGTAAAGTTGATGCATTCTG
TGGAGGCTCTATGTTAAATGGATTGTAAGTGTGCTGCCACTGTGTTGAAAC
TGGTGTAAATTACAGTTGTCGCAGGTGAACATAATTGAGGAGACAGAACATA
CAGAGCAAAGCAGGAAATGTGATTGCAATTCTCACCACAACATGCAAGCT
ATTAATAAGTACAACCATGACATTGCCCTCTGGAACGGACAACTTAGTGCTA
AACAGCTACGTTACACCTATTGCTGACAGGAAACACGAACATCTCCTC
AAATTGGATCTGGCTATGTAAGTGGCTGGGAAGAGCTTCCACAAAGGGAGATC
AGCTTTAGTTCTCAGTACCTAGTTGACTTGTGACCGAGCCACATGTCTTCTA
TCTACAAAGTTACCATCTATAACACATGTTCTGTGCTGGCTTCCATGAAGGAGGT
AGAGATTCTGCAAGGAGATGTTGGGACCCATGTTACTGAAGTGGAAAGGGAC
CAGTTCTTAACGGATTATTAGCTGGGTGAAGAGTGTGCAATGAAAGGCAAAT
ATGGAATATACCAAGGTATCCGGTATGCAACTGGATTAGGAAAAACAAAG
CTCACTTAACCTCGACTGTGCCCTCTAGTTGCCAGCCATCTGTTGCCCCCTCCC
CGTCCTCTGACCCCTGGAGGTGCCACTCCACTGCTTCTCTAAATAAAATGA
GGAAATTGCATCGCATTGCTGAGTAGGTGTCAATTCTATTCTGGGGGTGGGTGG
GCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGATGCG
GTGGGCTCTATGGCTCTGAGGCGGAAAGAACAGCTGGGCTCTAGGGGTATCC
CCGTGAGATCGCCCATCGGTATAATGATTGGGAGAACACATTCAAAGGCTGTA
AGTTATAATGCTGAAAGCCCCTTAATTCTGGTAGTATTAGTTAAAGTTAAA
ACACCTTTCCACCTTGAGTGTGAGAATTGTAGAGCAGTGCTGTCCAGTAGAAATG

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TGTGCATTGACAGAAAAGACTTGGATCTGTCTGACAATGGCAGGCCAGAGATC
ACAAGGCTATCAAGCACTTGACATGGCAAGTGTAACTGAGAAGCACACATTCAA
ATAATAGTTAATTAAATTGAATGTATCTAGCCATGTGTGGCTAGTAGCTCCCTTCCT
GGAGAGAGAATCTGGAGGCCACATCTAACTTGTAAAGTCTGGAATCTTATTTTAT
TTCTGGAAAGGTCTATGAACATAGTTGGGGCAGCTCAGTTACTAACTTTAAT
GCAATAAGAACATCTCATGGTATCTTGAGAACATTATTTGTCTTTGTAGATCTAGGA
ACCCCTAGTGTGGAGTTGCCACTCCCTCTCGCGCGCTCGCTCGCTCACTGAGGC
CGCCCGGGCAAAGCCCCGGCGTCGGCGACCTTGGTCGCCCGCCTCAGTGAGCG
AGCGAGCGCGAGAGAGGGAGTGGCAA

P00354 full sequence (from ITR to ITR) :

(SEQ ID NO: 284)

TTGGCCACTCCCTCTCGCGCGCTCGCTCACTGAGGCCAGCGAGA
GCCCGACGCCCGGGCTTGCCGGCGGCCTAGTGAGCGAGCGAGA
GGGAGTGGCCAATCCATCACTAGGGTTCTAGATCTTAGCCTGGCAAATGAA
GTGGGTAACCTTCTCCCTCTCGTCTCGGCTCTGCTTTCCAGGGGTGTG
TTCGCGAGAACGACGTAAGAGTTATGTTTCTAGCTCTGCTGTATTTCAG
TAATGGAAGCCTGGTATTTAAATAGTTAAATTCCCTTAGTGCTGATTCTAGAT
TATTATTACTGTGTGTTATTATTGTATTGTCATTGACATCTGAGAACCTTAGGTG
GTTTATTTGATATATTTGGTATTTGATGACAATAATGGGGATTGAAAG
CTTAGCTTAAATTCTTTAAATTAAAAAAATGCTAGGAGAACGACTCAAATTA
CGTTGGATACAGTTGAATTATTACGGTCTCATAGGGCTGCCCTGCTGACCAGCT
ATACTAAAAATAAAAGTGTGTACTAATTATAATGGAGTTCCATTATATT
TACCTTATTCTTATTACCATTTGCTTAGAGATATTACAAACATGACAGAAC
CTAAATCTGAGTTGAATGCACAGATATAAACACTTAACGGTTTAAATA
ATGTTGGTAAAAAATAACTTGAGTGTAGCAGAGAGGAACCTGCCACCTCA
GATTTCCTGAAACGATCGGGACTGGCATCTCAGGGAGTAGCTTAGGTAGTGAA
GAGAAGAACAAAAAGCAGCATATTACAGTTAGTGCTTCATCAATTTAAATAG
TTGTGTGGTTTCTCTCCCTGTTCCACAGTTCTTGATCATGAAACGCCAAC
AAATTCTGAATCGGCCAAGAGGTATAATTCAAGTAAATTGGAAGAGTTGTTCAA
GGGAACTCTGAGAGAGAATGTGGAAGAAAAGTGTAGTTGAGAACGACGAGA
AGTTTTGAAAACACTGAAAGAACAACTGAATTGGAAAGCAGTATGTTGATGGAG
ATCAGTGTGAGTCCAATCCATGTTAAATGGGGCAGTTGCAAGGATGACATTAA
CCTATGAATGTTGGTGTCCCTTGGATTGAGGAAAGAACGACTGTGAATTAGATGAA
CATGTAACATTAAGAATGGCAGATGCGAGCAGTTGTAAGGAAACTGTGATAAAC
AAGGTGGTTGCTCTGTAAGGGATATCGACTTGCAAGAACCCAGAACGACT
GAACCAAGCAGTGCCATTCCATGTGGAAGAGTTCTGTTCAAAACTCTAAGCTC
ACCCGTGCTGAGACTGTTTCTGATGTGGACTATGTAATTCTACTGAGCTGAA
ACCATTTGGATAACATCACTCAAAGCACCAATCATTAAATGACTTCAGTGGTT
GTTGGTGGAGAAGATGCCAACCCAGGTCATTCCCTGGCAGGTTGTTGAATGGT
AAAGTTGATGCATTCTGTGGAGGCTCTATCGTTAATGAAAATGGATTGTAAGCT

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GCCCACTGTGTTGAAACTGGTAAAATTACAGTTGTCGAGGTGAAACATAATATT
 GAGGAGACAGAACATACAGAGCAAAGCGAAATGTGATTGCAATTATTCCCTCACCA
 CAACTACAATGCAGCTATTAATAAGTACAACCAGCATTGCCCTCTGGAACCTGGA
 CGAACCTTAGTGTCAAACAGCTACGTTACACCTATTGCTGACAAGGAATA
 CACGAACATCTCCTCAAATTGGATCTGGCTATGTAAGTGGCTGGGAAGAGTCTT
 CCACAAAGGGAGATCAGCTTAGTCTCAGTACCTTAGAGTCCACTTGTGACCG
 AGCCACATGTCTTCTATCTACAAAGTCACCATCTATAACACATGTTCTGCTGGC
 TTCCATGAAGGAGGTAGAGATTCAATGTCAGGAGATAGTGGGGACCCATGTTAC
 TGAAGTGGAAAGGAGCAGTTCTTAACTGGAATTAGCTGGGTGAAGAGTGTG
 CAATGAAAGGCAAATATGGAATATACCAAGGTATCCGGTATGTCACACTGGATT
 AAGGAAAAAAACAAAGCTCACTAACCTCGACTGTGCCTCTAGTTGCCAGCCATCTG
 TTGTTGCCCTCCCCGTGCCCTTGACCTGGAAAGGTGCCACTCCACTGTCCT
 TTCCCTAATAAAATGAGGAATTGCATGCATTGCTGAGTAGGTGTCATTCTATTCT
 GGGGGTGGGTGGGGCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGG
 CATGCTGGGATGCGGTGGCTATGGCTTGAGGGCGAAAGAACCCAGCTGGGG
 CTCTAGGGGTATCCCGTGAGATGCCATCGGTATAATGATTGGAGAACACA
 TTCAAAAGGCTGTAAGTTATAATGCTGAAAGCCCACCTTAATATTCTGGTAGTATT
 AGTTAAAGTTAAACACCTTTCCACCTTGAGTAGTGAGAATTGAGACAGTGC
 TGCCAGTAGAAATGTCATTGACAGAAAGACTGTGGATCTGCTGAGCAATGT
 GGCAAGGAGATCACAAGGCTATCAAGCACTTGACATGGCAAGTGTAACTGAG
 AACACACATTCAAATAATGTTAATTGAAATGTATCTAGCCATGTGTGGCT
 AGTAGCTCTTCTGGAGAGAGAATCTGGAGGCCACATCTAACTGTTAAGTCTGG
 AATCTTATTTTATTCGGAAAGGTCTATGAACTATAGTTGGGGCAGCTCACT
 TACTAACTTTAATGCAATAAGAATCTCATGGTATCTTGAGAACATTATTTGTCT
 TTGTTAGTACTGAAACCTTACATGTAAGTAAGGGCTATGAACTTAAAGTCACATCT
 CCAACCTTAGTAATGTTAATGTTAGTAAAAAAATGAGTAATTAAATTATTTAGA
 AGGTCAATAGTATCATGTTACCTAAACAGAGGTATATGGTTAGAAAAAGAAC
 ATTCAAAGGACTTATATAATCTAGCCTGACAATGAATAATTAGAGAGTAGTT
 TGCCCTGTTGCCATGTTACATGACACATATGTGCTCTGCACCTCAGC
 ATGGTAGAGTCATATTCAAGATCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCC
 TCTCTGCGCCTCGCTCGCTCACTGAGGCCGCCGGCAAAGCCGGCGTGGC
 GACCTTGGTCGCCCGCCTCAGTGAGCGAGCGAGCGCAGAGAGGGAGTGGCCA

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P00350: The 300/600 bp HA F9 construct (for G551) (SEQ ID NO: 285)
 TTGGCCACTCCCTCTGCGCGCTCGCTCACTGAGGCCGGCGACCAAAGGTC
 GCCCGACGCCGGCTTGCCGGCGGCCTCAGTGAGCGAGCGAGCGCAGAGA
 GGGAGTGGCCAATCCATCACTAGGGGTTCTAGATCTAAGTATATTAGAGCGAGTC
 TTTCTGACACAGATCACCTTCTATCAACCCACTAGCCTCTGGCAAAATGAAGT
 GGGTAACCTTCTCCTCCTCTCGTCTCGGCTCTGCTTTCCAGGGTGTGTTT

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CGCCGAGAAGCACGTAAGAGTTTATGTTTTCATCTCTGCCTGTATTTCTAGTA
ATGGAAGCCTGGTATTTAAATAGTTAAATTCCCTTAGTGCTGATTTCTAGATTA
TTATTACTGTTGTTGTTATTATGTCATTATTCATCTGAGAACCTTTCTTGA
TCATGAAAACGCCAACAAAATTCTGAATCGGCCAACAGAGGTATAATTCAAGTAAAT
TGGAAAGAGTTGTTCAAGGGAACCTTGAGAGAGAATGTATGGAAGAAAAGTGTAGT
TTGAAAGAACGAGAACAGTTGAAAACACTGAAAGAACAACTGAATTGGAA
GCAGTATGTTGATGGAGATCAGTGTGAGTCCAATCCATGTTAAATGGCGCAGTTG
CAAGGATGACATTAATTCTATGAATGTTGGTCCCTTGATTTGAAGGAAAGAA
CTGTGAATTAGATGTAACATGTAACATTAAGAATGGCAGATGGCAGCAGTTGTAA
AAATAGTGTGATAACAAGGTGGTTGCTCCTGTACTGAGGGATATCGACTTGCAGA
AAACCAGAAGTCTGTGAACCAGCAGTGCCATTCCATGTGGAAGAGTTCTGTTTC
ACAAACTCTAACGCTCACCGTGTGAGACTGTTTCCATGTGACTATGTAAA
TTCTACTGAAGCTGAAACCATTGGATAACATCACTCAAAGCACCAATCATTAA
TGACTTCACTCGGGTTGTTGGAGAACGATGCCAACCCAGGTCAATTCCCTGGCA
GGTGTTTGAATGGAAAGTTGATGCCATTGTGAGGGCTATCGTTAATGAAAA
ATGGATTGTAACTGCTGCCACTGTGTTGAAACTGGTGTAAATTACAGTTGCGC
AGGTGAACATAATTGAGGAGACAGAACATACAGAGCAAAGCGAAATGTGATT
GAATTATTCCCTACCCACAACATGCAAGCTATTAAAGTACAACCATGACATTG
CCCTCTGGAACGGACGAACCTTAGTGCTAACAGCTACGTTACACCTATTGCA
TTGCTGACAAGGAATACACGAACATCTCCTCAAATTGGATCTGGTATGTAAGT
GCTGGGGAAAGAGTCTTCACAAAGGGAGATCAGCTTAGTTCTCAGTACCTAGAG
TTCCACTTGTGACCGAGCCACATGCTTCTATCTACAAAGTCACCATCTATAACAA
CATGTTCTGTGCTGGCTTCATGAAGGAGGTAGAGATTGTCAGGAGATAGTGG
GGGACCCATGTTACTGAAAGTGGAAAGGGACAGTTCTTAAGTGGATTATTAGCTG
GGGTGAAGAGTGTGCAATGAAAGGAAATATGGAATATACCAAGGTATCCCGT
ATGTCAACTGGATTAAGGAAAAACAAAGCTACTAACCTCGACTGTGCCTCTAG
TTGCCAGCCATCTGTTGTTGCCCTCCCCGTGCCTCCTTGACCCGTGAAGGTGCC
ACTCCCACGTGCTTCTTAATAAAATGAGGAAATTGATCGCATTGCTGAGTAGG
TGTCAATTCTATTCTGGGGGTGGGTGGGCAGGACAGCAAGGGGGAGGATTGGGA
AGACAATAGCAGGCATGCTGGGATGCGGTGGGCTATGGCTTGAGGCGAAA
GAACCAGCTGGGCTCTAGGGGTATCCCCCTAGGTGGTTATTGATATT
TTGGTATCTTGATGACAATAATGGGGATTGAAAGCTAGCTTAAATTCTT
TAATTAAAAAAATGCTAGGCAGAATGACTCAAATTACGTTGATACAGTTGAAT
TTATTACGGTCTCATAGGGCCTGCCTGCTGACCAGTGTACACTAAAAATTAAAAGT

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GTGTGTTACTAATTATAAAATGGAGTTCCATTATTTACCTTATTCTTATT
CCTTGCTTAGATATTACAAACATGACAGAACACTAAAGATCTAGGAACCC
CTAGTGATGGAGTTGCCACTCCCTCTGCGCGCTCGCTCGCTACTGAGGCC
CGGGCAAAGCCGGCGTCGGCGACCTTGGTCGCCCGCCTAGTGAGCGAGCG
AGCGCGCAGAGAGGGAGTGGCAA

P00356: The 300/2000 bp HA F9 construct (for G551) (SEQ ID NO: 286)
TTGGCCACTCCCTCTGCGCGCTCGCTCGCTACTGAGGCCGGCACAAAGGTC
GCCGACGCCGGCTTGCCGGCGGCCTCAGTGAGCGAGCGCAGAGA
GGGAGTGGCCAATCCATCACTAGGGTCCCTAGATCTAAGTATATTAGAGCGAGTC
TTTCTGCACACAGATCACCTTCCTATCAACCCCCTAGCCTCTGGCAAATGAAGT
GGTAACCTTCCTCCTCTCGTCTCGCTCTGCTTTCCAGGGGTGTGTT
CGCCGAGAAGCAGTAAGAGTTTATGTTTCTGCTCTGCTGTATTTCTAGTA
ATGGAAGCCTGGTATTTAAATAGTTAAATTTCTTCTGCTGCTGATTTCTAGATTA
TATTACTGTTGTTGTTATTATTGTCATTATTGCACTGAGAACCTTTCTTGA
TCATGAAAACGCCAACAAAATTCTGAATCGCCAAAGAGGTATAATTCAAGTAAAT
TGGAAAGAGTTGTTCAAGGGAACCTTGAGAGAGAATGTATGAAAGAAAAGTGTAGT
TTGAAGAACGAGAAGTGGAAACACTGAAACAACGTGAATTGGAA
GCAGTATGTTGATGGAGATCAGTGTGAGTCCAATCCATGTTAAATGGCGCAGTTG
CAAGGATGACATTAATTCTATGAATGTTGGTCCCTTGATTGAAAGGAAAGAA
CTGTGAATTAGATGTAACATGTAACATTAAGAATGGCAGATGGCAGCTTGTAA
AAATAGTGTGATAACAAGGTGGTCTGCTCTGACTGAGGGATATGACTTGCAGA
AAACCAAGTCTGTGAACCAGCAGGCCATTCCATGTGAAAGAGTTCTGTT
ACAAACTCTAACGTCACCCGTGCTGAGACTGTTTCTGATGTGGACTATGTA
TTCTACTGAAGCTGAAACCATTGGATAACATCACTCAAAGCACCAATCATTAA
TGACTTCACTCGGGTTGGTGGAGAAGATGCCAACCCAGGTCAATTCCCTGGCA
GGTTGTTTGAATGGAAAGTTGATGCATTCTGTTGGAGGCTATGTTAATGAAAA
ATGGATTGTAACTGCTGCCACTGTGTTGAAACTGGTTAAATTACAGTTGTCGC
AGGTGAACATAATTGAGGAGACAGAACATACAGAGCAAAGCGAAATGTGATT
GAATTATTCCCTACCCACAACATGAGCTATTAAAGTACAACCATGACATTG
CCCTCTGGAACGGACGCCAACCTTAGTGCTAAACAGCTACGTTACACCTATTGCA
TTGCTGACAAGGAATACACGAACATCTCCCTAAATTGGATCTGGTATGTAAGTG
GCTGGGGAAAGAGTCTTCCACAAAGGGAGATCAGCTTAGTTCTCAGTACCTAGAG
TTCCACTGTTGACCGACGCCACATGCTTCTATCTACAAAGTCACCATCTATAACAA
CATGTTCTGCTGGCTTCCATGAAGGAGGTAGAGATTGTCAGGAGATAGTGG
GGGCCACCATGTTACTGAAGTGGAGGGACAGTTCTTAACGAAATTAGCTG
GGGTGAAGAGTGTGCAATGAAAGGCAAATATGGAATATACCAAGGTATCCCGT
ATGTCACACTGGATTAAGGAAAAACAAAGCTACTTAACCTCGACTGTGCCTCTAG
TTGCCAGCCATCTGTTGCCCCCTCCCCGTGCCCTCCTGACCCCTGGAAGGTGCC
ACTCCCACGTGCTTTCTAATAAAATGAGGAAATTGCATGCACTGAGTAGG

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TGTCATTCTATTCTGGGGGTGGGTGGGCAGGACAGCAAGGGGGAGGATGGGA
AGACAATAGCAGGCATGCTGGGATGCGGTGGCTCATGGCTCTGAGGCGGAAA
GAACCAGCTGGGCTCTAGGGGTATCCCCCTAGGTGGTTATATTATGATATATT
TTTGGTATCTTGATGACAATAATGGGGATTTGAAAGCTTAGCTTAAATTCTTT
TAATTAAAAAAAATGCTAGGCAGAATGACTCAAATTACGTTGGATACAGTTGAAT
TTATTACGGTCTCATAGGCCTGCCTGCGACCATGCTATACTAAAAATTAAAGT
GTGTGTTACTAATTATAAATGGAGTTCCATTATATTACCTTATTCTTATTAA
CATTGTCTTAGATATTACAAACATGACAGAACACTAAATCTTGAGTTGAA
TGCAAGATATAAACACTTAACGGGTTTAAAGATAATAATGTTGGTAAAAAATAT
AACTTTGAGTGTAGCAGAGAGGAACCATTGCCACCTCAGATTTCTGTAACGATC
GGGAACCTGCATCTCAGGGAGTAGCTTAGGTAGTCAGTGAAGAGAAGAACAAAAAGCA
GCATATTACAGTTAGTTGTCTTCATCAATCTTAAATATGTTGTGGTTTCTCTCC
CTGTTTACAGACAAGAGTGAGATGCCCATCGGTATAATGATTGGAGAACAA
CATTCAAAGGCCTGTAAGTTATAATGCTGAAAGCCCCTTAATATTCTGGTAGTA
TTAGTTAAAGTTTAAACACCTTTCCACCTTGAGTGTGAGAATTGAGAGCAGT
GCTGTCCAGTAGAAATGTGTGCATTGACAGAAAGACTGTGGATCTGTGCTGAGCAAT
GTGGCAGCCAGAGATCACAGGCTATCAAGCCTTGCACATGGCAAGTGTAACTG
AGAACACACATTCAAATAATAGTTAATTGAATGTATCTAGCCATGTGTGG
CTAGTAGCTCCTTCCTGGAGAGAGAATCTGGAGCCACATCTAATTGTTAAGTCT
GGAATCTATTCTGAAAGGTCTATGAACTATAGTTGGGGCAGCTCA
CTTACTAATTGCAATAAGATCCATGGTATCTTGAGAACATTATTGTCTCT
TTGTAGTACTGAAACCTTACATGTGAAGTAAGGGCTATACTTAAGTCACATCT
CCAACCTTAGTAATGTTAATGTAGTAAAAAAATGAGTAATTAAATTATTAGA
AGGTCAATAGTATCATGTATTCAAATAACAGAGGTATATGGTTAGAAAAGAAC
ATTCAAAGGACTTATATAATATCTAGCCTTGACAATGAATAAATTAGAGAGTAGTT
TGCCTGTTGCCTCATGTTCATAAATCTATTGACACATATGTGCATCTGCACCTCAGC
ATGGTAGAAGTCCATATCCTTGCTGGAAAGGCAGGTGTTCCATTACGCCCTCAG
AGAATAGCTGACGGGAAGAGGCTTCTAGATAGTTGTATGAAAGATATAACAAATC
TCGCAGGTACACAGGCATGATTGCTGGTGGAGAGGCCACTGCCTCATACTGA
GGTTTTGTGCTGCTTTCAGAGTCCTGATTGCCTTCCAGTATCTCCAGAAATG
CTCATACGATGAGCATGCCAAATTAGTCAGGAAGTAACAGACTTGCAGAACAGT
GTGTTGCCGATGAGTCTGCCGCAACTGTGACAATCCCTGTGAGTACCTCTGAT
TTTGTGGATCTACTTCCTGCTTCTGAACTCTGTTCAAAGCCAATCATGACTCCA
TCACCTAAGGCCCGGGAACACTGTGGCAGAGGGCAGCAGAGAGATTGATAAAGCC
AGGGTGATGGGAATTCTGTGGACTCCATTCTAGTAATTGCAGAACGCTACAAT

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ACACTCAAAAAGTCTACCACATGACTGCCAAATGGGAGCTTGACAGTGACAGTG
ACAGTAGATATGCCAAAGTGGATGAGGGAAAGACCACAAGAGCTAAACCTGTAAA
AAGAACTGTAGGCAACTAAGGAATGCAGAGAGAAAGATCTAGGAACCCCTAGTGAT
GGAGTTGGCCACTCCCTCTCGCGCTCGCTCGCTCACTGAGGCCGCCGGCAGA
GCCCGGGCGTCGGCGACCTTGGTCGCCCGCCTCAGTGAGCGAGCGAGCGCA
GAGAGGGAGTGCCAA
P00362: The 300/1500 bp HA F9 construct (for G551) (SEQ ID NO: 287)
TTGGCCACTCCCTCTCGCGCTCGCTCGCTCACTGAGGCCGCCGGCAGA
GCCCGACGCCCGGCTTGCCGGCGCCCTCAGTGAGCGAGCGAGA
GGGAGTGGCCAECTCATCACTAGGGTTCTAGATCTAAGTATATTAGCGAGTC
TTTCTGCACACAGATCACCTTCCTATCAACCCACTAGCCTGGCAAATGAAGT
GGTAACCTTCTCCTCCTCGTCTCCGGCTTGCTTTCCAGGGTGTGTT
CGCCGAGAACGAGCTAAGAGTTTATGTTTCTGCTGTATTTCTAGTA
ATGGAAGCTGGTATTTAAAAATAGTTAAATTTCCTTAGTGCTGATTTCTAGATTA
TTATTACTGTTGTTGTTATTATTCATTATTCATCTGAGAACCTTTCTTGA
TCATGAAAACGCCAACAAATCTGAATCGGCAAAGAGGTATAATTCAAGTAAAT
TGGAGAGTTGTTCAAGGGAACCTTGAGAGAGAATGTATGGAAGAAAAGTGTAGT
TTGAGAACGAGAGTTGAAAACACTGAAAGAACAACTGAATTGGAA
GCAGTATGTTGAGATCAGTGTGAGTCAATCCATGTTAAATGGCGCAGTTG
CAAGGATGACATTAATTCTATGAATGTTGGTGTCCCTTGATTGAGAAC
CTGTGAATTAGATGTAACATGTAACATTAAGAATGGCAGATGGCAGCAGTTGTA
AAATAGTGTGATAACAAGTGGTTGCTCCTGACTGAGGGATACTGACTTGCAGA
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Factor IX R338L polypeptide encoded in P00147

(SEQ ID NO: 702)

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SEQUENCE LISTING

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aaaaaaaaaa aaggtcagaa ttgttagtg actgtat tcttttcgcg actaaggaaa 240
gtgcagaatgactttagatc actgaaactt cacagaatag ggttgaatg tgaatttcata 300
actatccccaa agacatccatc attgcactt gcttatttta aaaaccacaa aacctgtgt 360
gttgatctca taaatagaac ttgttattt atttattttc atttttgtct gtcttcttgg 420
ttgctgttga tagacactaa aagagtatc gatattatct aagtttgaat ataaggctat 480
aaatatttaa taatttttaa aatgtatc ttggtatcg aattttctt ctgtttaaag 540
gcagaagaaa taattgaaca tcatcttgat ttttctgtat ggaatcagag cccaaatattt 600
tgaacaaat gcataatcta agtcaaatgg aaagaaatataaaaatgttacatttactt 660
cttggtttct tcagttttaa acaatccccc tttttcttcc ctggcccg 709

SEQ ID NO: 2 moltype = RNA length = 20
FEATURE Location/Qualifiers
source 1..20
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 2
gagcaacctc actctttgtct 20

SEQ ID NO: 3 moltype = RNA length = 20
FEATURE Location/Qualifiers
source 1..20
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 3
atgcattttt ttc当地at 20

SEQ ID NO: 4 moltype = RNA length = 20
FEATURE Location/Qualifiers
source 1..20
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 4
tgcattttgtt tcaaaaatatt 20

SEQ ID NO: 5 moltype = RNA length = 20
FEATURE Location/Qualifiers
source 1..20
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 5
atttatgaga tcaacagcac 20

SEQ ID NO: 6 moltype = RNA length = 20

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FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 6	
gatcaacagc acagggtttg	20
SEQ ID NO: 7	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 7	
ttaaataaaag catagtgcaa	20
SEQ ID NO: 8	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 8	
taaagcatag tgcaatggat	20
SEQ ID NO: 9	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 9	
tagtgcaatg gataggtctt	20
SEQ ID NO: 10	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 10	
tactaaaact ttattttact	20
SEQ ID NO: 11	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 11	
aaaggttgaac aatagaaaaaa	20
SEQ ID NO: 12	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 12	
aatgcataat ctaagtcaaa	20
SEQ ID NO: 13	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 13	
taataaaaatt caaacatcct	20
SEQ ID NO: 14	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 14	
gcatcttaa agaattttt	20
SEQ ID NO: 15	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct

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SEQUENCE: 15		
tttggcattt atttctaaaa		20
SEQ ID NO: 16	moltype = RNA length = 20	
FEATURE	Location/Qualifiers	
source	1..20	
	mol_type = other RNA	
	organism = synthetic construct	
SEQUENCE: 16		
tgtattttgtg aagtcttaca		20
SEQ ID NO: 17	moltype = RNA length = 20	
FEATURE	Location/Qualifiers	
source	1..20	
	mol_type = other RNA	
	organism = synthetic construct	
SEQUENCE: 17		
tccttagtaa aaaaaaaaaaa		20
SEQ ID NO: 18	moltype = RNA length = 20	
FEATURE	Location/Qualifiers	
source	1..20	
	mol_type = other RNA	
	organism = synthetic construct	
SEQUENCE: 18		
taattttctt ttgcgcacta		20
SEQ ID NO: 19	moltype = RNA length = 20	
FEATURE	Location/Qualifiers	
source	1..20	
	mol_type = other RNA	
	organism = synthetic construct	
SEQUENCE: 19		
tgactgaaac ttcacagaat		20
SEQ ID NO: 20	moltype = RNA length = 20	
FEATURE	Location/Qualifiers	
source	1..20	
	mol_type = other RNA	
	organism = synthetic construct	
SEQUENCE: 20		
gactgaaact tcacagaata		20
SEQ ID NO: 21	moltype = RNA length = 20	
FEATURE	Location/Qualifiers	
source	1..20	
	mol_type = other RNA	
	organism = synthetic construct	
SEQUENCE: 21		
ttcatttttag tctgtttct		20
SEQ ID NO: 22	moltype = RNA length = 20	
FEATURE	Location/Qualifiers	
source	1..20	
	mol_type = other RNA	
	organism = synthetic construct	
SEQUENCE: 22		
attatctaaat tttgaatata		20
SEQ ID NO: 23	moltype = RNA length = 20	
FEATURE	Location/Qualifiers	
source	1..20	
	mol_type = other RNA	
	organism = synthetic construct	
SEQUENCE: 23		
aatttttaaa atagttattct		20
SEQ ID NO: 24	moltype = RNA length = 20	
FEATURE	Location/Qualifiers	
source	1..20	
	mol_type = other RNA	
	organism = synthetic construct	
SEQUENCE: 24		
tgaatttttc ttctgtttaa		20
SEQ ID NO: 25	moltype = RNA length = 20	

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FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 25	
atcatcctga gtttttctgt	20
SEQ ID NO: 26	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 26	
ttaactaaac ttttatTTTAC	20
SEQ ID NO: 27	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 27	
acctttttt ttTTTTACCT	20
SEQ ID NO: 28	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 28	
agtgcataatgg ataggTCTTT	20
SEQ ID NO: 29	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 29	
tgattccTAC agaaaaaACTC	20
SEQ ID NO: 30	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 30	
tgggcaagggg aagaaaaaaaaa	20
SEQ ID NO: 31	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 31	
cctcaCTT GTCTGGGCAA	20
SEQ ID NO: 32	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 32	
acctcaCTCT TGTCTGGGCA	20
SEQ ID NO: 33	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 33	
tGAGCACCT CACTTTGTC	20
SEQ ID NO: 34	moltype = RNA length = 100
FEATURE	Location/Qualifiers
source	1..100
	mol_type = other RNA
	organism = synthetic construct

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SEQUENCE: 34
gagcaaccc actcttgtct gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 35      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 35
atgcatttgtt tccaaaatat gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 36      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 36
tgcatattttt tccaaaatatt gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 37      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 37
atttatgaga tcaacagcac gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 38      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 38
gatcaaacagc acagggtttt gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 39      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 39
ttaaataaag catagtgc当地 gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 40      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 40
taaaggcatag tgcaatggat gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 41      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 41
tagtgcaatg gataggctt gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 42      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 42
tactaaaact ttatttact gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

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SEQ ID NO: 43      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 43
aaagttgaac aatagaaaaaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 44      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 44
aatgcataat ctaagtcaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 45      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 45
taataaaaatt caaacatcct gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 46      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 46
gcatctttaa agaattttt gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 47      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 47
tttgcattt atttctaaaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 48      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 48
tgtatttttg aagtcttaca gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 49      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 49
tccttagttaa aaaaaaaaaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 50      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 50
taattttctt ttgcgcacta gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 51      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA

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organism = synthetic construct
 SEQUENCE: 51
 tgactgaaac ttccacagaat gttttagagc tagaaatagc aagttaaat aaggctagtc 60
 cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100
 SEQ ID NO: 52 moltype = RNA length = 100
 FEATURE Location/Qualifiers
 source 1..100
 mol_type = other RNA
 organism = synthetic construct
 SEQUENCE: 52
 gactgaaaat tcacagaata gttttagagc tagaaatagc aagttaaat aaggctagtc 60
 cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100
 SEQ ID NO: 53 moltype = RNA length = 100
 FEATURE Location/Qualifiers
 source 1..100
 mol_type = other RNA
 organism = synthetic construct
 SEQUENCE: 53
 ttatatttag tctgttctt gttttagagc tagaaatagc aagttaaat aaggctagtc 60
 cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100
 SEQ ID NO: 54 moltype = RNA length = 100
 FEATURE Location/Qualifiers
 source 1..100
 mol_type = other RNA
 organism = synthetic construct
 SEQUENCE: 54
 attatctaag ttgtatataa gttttagagc tagaaatagc aagttaaat aaggctagtc 60
 cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100
 SEQ ID NO: 55 moltype = RNA length = 100
 FEATURE Location/Qualifiers
 source 1..100
 mol_type = other RNA
 organism = synthetic construct
 SEQUENCE: 55
 aatttttaaa atagtattctt gttttagagc tagaaatagc aagttaaat aaggctagtc 60
 cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100
 SEQ ID NO: 56 moltype = RNA length = 100
 FEATURE Location/Qualifiers
 source 1..100
 mol_type = other RNA
 organism = synthetic construct
 SEQUENCE: 56
 tgaatttttc ttctgtttaa gttttagagc tagaaatagc aagttaaat aaggctagtc 60
 cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100
 SEQ ID NO: 57 moltype = RNA length = 100
 FEATURE Location/Qualifiers
 source 1..100
 mol_type = other RNA
 organism = synthetic construct
 SEQUENCE: 57
 atcatcctga gtttttctgt gttttagagc tagaaatagc aagttaaat aaggctagtc 60
 cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100
 SEQ ID NO: 58 moltype = RNA length = 100
 FEATURE Location/Qualifiers
 source 1..100
 mol_type = other RNA
 organism = synthetic construct
 SEQUENCE: 58
 ttactaaaac ttatattttac gttttagagc tagaaatagc aagttaaat aaggctagtc 60
 cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100
 SEQ ID NO: 59 moltype = RNA length = 100
 FEATURE Location/Qualifiers
 source 1..100
 mol_type = other RNA
 organism = synthetic construct
 SEQUENCE: 59
 accttttttt tttttttacctt gttttagagc tagaaatagc aagttaaat aaggctagtc 60
 cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

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SEQ ID NO: 60      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 60
atgtcaatgg ataggcttt gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 61      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 61
tgattccatc agaaaaactc gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 62      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 62
tgggcaagg aaaaaaaaaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 63      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 63
cctcaactt gtctggcaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 64      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 64
acctcaactt tgtctggca gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 65      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 65
tgagcaacct cactttgtc gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 66      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 66
gagcaacctc actcttgtc gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 67      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 67
atgcatttgtt ttcaaaatat gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 68      moltype = RNA  length = 100
FEATURE
source
1..100

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mol_type = other RNA
organism = synthetic construct
SEQUENCE: 68
tgcattttgtt tcaaaaattt gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 69      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 69
atttatgaga tcaacagcac gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 70      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 70
gatcaacagc acagggtttt gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 71      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 71
ttaataaaag catagtgc当地 gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 72      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 72
taaagcatag tgcaatggat gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 73      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 73
tagtgcaatg gataggcttt gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 74      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 74
tactaaaact ttatttact gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 75      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 75
aaagttgaac aatagaaaaaa gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 76      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 76
aatgcataat ctaagtcaaa gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60

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cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100
SEQ ID NO: 77      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 77
taataaaaatt caaacatcct gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 78      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 78
gcacatcttaa agaatttattt gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 79      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 79
tttggcattt atttctaaaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 80      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 80
tgtatatttg aagtcttaca gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 81      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 81
tccttagttaa aaaaaaaaaaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 82      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 82
taattttctt ttgcgcacta gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 83      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 83
tgactgaaac ttcacagaat gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 84      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 84
gactgaaact tcacagaata gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 85      moltype = RNA  length = 100
FEATURE           Location/Qualifiers

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source          1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 85
ttcatttttag tctgttttct gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 86      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 86
attatctaag tttgaatata gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 87      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 87
aattttaaa atagttttct gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 88      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 88
tgaattttc ttctgtttaa gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 89      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 89
atcatctga gttttctgt gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 90      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 90
ttactaaaac ttattttac gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 91      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 91
acctttttt ttttttacct gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 92      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 92
agtgcatagg ataggcttt gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 93      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 93

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tgattcctac agaaaaactc gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 94      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 94
tgggcaagg aaaaaaaaaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 95      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 95
cctcacttct gtctggcaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 96      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 96
acctcactct tgcgtggca gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 97      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 97
ttagcaacct cactttgtc gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 98      moltype = RNA length = 20
FEATURE           Location/Qualifiers
source            1..20
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 98
atttgcatct gagaaccctt 20

SEQ ID NO: 99      moltype = RNA length = 20
FEATURE           Location/Qualifiers
source            1..20
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 99
atcgggaact ggcatttca 20

SEQ ID NO: 100     moltype = RNA length = 20
FEATURE           Location/Qualifiers
source            1..20
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 100
gttacagggaa aatctgaagg 20

SEQ ID NO: 101     moltype = RNA length = 20
FEATURE           Location/Qualifiers
source            1..20
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 101
gatcgggaac tggcatttc 20

SEQ ID NO: 102     moltype = RNA length = 20
FEATURE           Location/Qualifiers
source            1..20
                  mol_type = other RNA
                  organism = synthetic construct

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SEQUENCE: 102 tgcatctgag aacccttagg	20
SEQ ID NO: 103 FEATURE source moltype = RNA length = 20 Location/Qualifiers 1..20 mol_type = other RNA organism = synthetic construct	
SEQUENCE: 103 cactcttgtc tgtggaaaca	20
SEQ ID NO: 104 FEATURE source moltype = RNA length = 20 Location/Qualifiers 1..20 mol_type = other RNA organism = synthetic construct	
SEQUENCE: 104 atcggttacag gaaaatctga	20
SEQ ID NO: 105 FEATURE source moltype = RNA length = 20 Location/Qualifiers 1..20 mol_type = other RNA organism = synthetic construct	
SEQUENCE: 105 gcatcttcag ggagtagctt	20
SEQ ID NO: 106 FEATURE source moltype = RNA length = 20 Location/Qualifiers 1..20 mol_type = other RNA organism = synthetic construct	
SEQUENCE: 106 caatcttaa atatgttgta	20
SEQ ID NO: 107 FEATURE source moltype = RNA length = 20 Location/Qualifiers 1..20 mol_type = other RNA organism = synthetic construct	
SEQUENCE: 107 tcactcttgt ctgtggaaac	20
SEQ ID NO: 108 FEATURE source moltype = RNA length = 20 Location/Qualifiers 1..20 mol_type = other RNA organism = synthetic construct	
SEQUENCE: 108 tgcttgatt tttctagtaa	20
SEQ ID NO: 109 FEATURE source moltype = RNA length = 20 Location/Qualifiers 1..20 mol_type = other RNA organism = synthetic construct	
SEQUENCE: 109 gtaaatatct actaagacaa	20
SEQ ID NO: 110 FEATURE source moltype = RNA length = 20 Location/Qualifiers 1..20 mol_type = other RNA organism = synthetic construct	
SEQUENCE: 110 tttttctagt aatggaagcc	20
SEQ ID NO: 111 FEATURE source moltype = RNA length = 20 Location/Qualifiers 1..20 mol_type = other RNA organism = synthetic construct	
SEQUENCE: 111 tttatattttt gatataatttt	20
SEQ ID NO: 112 moltype = RNA length = 20	

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FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 112	
gcacagatat aaacacttaa	20
SEQ ID NO: 113	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 113	
cacagatata aacacttaac	20
SEQ ID NO: 114	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 114	
ggttttaaaa ataataatgt	20
SEQ ID NO: 115	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 115	
tcagatttcc ctgttaacgat	20
SEQ ID NO: 116	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 116	
cagattttcc tgtaaacgatc	20
SEQ ID NO: 117	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 117	
caatggtaaa taagaataaa	20
SEQ ID NO: 118	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 118	
ggaaaatctg aagggtggcaa	20
SEQ ID NO: 119	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 119	
ggcgatctca ctcttgtctg	20
SEQ ID NO: 120	moltype = RNA length = 100
FEATURE	Location/Qualifiers
source	1..100
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 120	
atttgcacatct gagaaccctt gtttttagago tagaaatagc aagttaaaat aaggctagtc	60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt	100
SEQ ID NO: 121	moltype = RNA length = 100
FEATURE	Location/Qualifiers
source	1..100
	mol_type = other RNA

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SEQUENCE: 121          organism = synthetic construct
atcgggaact ggcacattca gtttttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 122          moltype = RNA   length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 122
gttacaggaa aatctgagg gtttttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 123          moltype = RNA   length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 123
gatcggaaac tggcatcttc gtttttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 124          moltype = RNA   length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 124
tgcatctgag aacccttagg gtttttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 125          moltype = RNA   length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 125
cactcttgtc tgtggaaaca gtttttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 126          moltype = RNA   length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 126
atcgttacag gaaaatctga gtttttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 127          moltype = RNA   length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 127
gcatcttcag ggagtagctt gtttttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 128          moltype = RNA   length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 128
caatcttaa atatgttgt gtttttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 129          moltype = RNA   length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 129
tcactcttgt ctgtggaaac gtttttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

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SEQ ID NO: 130      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 130
tgcttgatt tttcttagaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 131      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 131
gtaaatatct actaagacaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 132      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 132
tttttctagt aatggaaagcc gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 133      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 133
tttatattttt gatatatatttt gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 134      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 134
gcacagatata aacacttaac gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 135      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 135
cacagatata aacacttaac gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 136      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 136
ggttttaaa ataataatgt gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 137      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 137
tcagatttc ctgtaacgt gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 138      moltype = RNA  length = 100
FEATURE
source
1..100

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mol_type = other RNA
organism = synthetic construct
SEQUENCE: 138
cagattttcc tgttaacgatc gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 139      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 139
caatggtaaa taagaataa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 140      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 140
ggaaaatctg aaggtgccaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 141      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 141
ggcogatctca ctcttgcctg gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 142      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 142
atttgcatct gagaaccctt gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 143      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 143
atcgggaact ggcatctca gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 144      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 144
tttacaggaa aatctgaagg gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 145      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 145
gatcgggaac tggcatcttc gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 146      moltype = RNA  length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 146
tgcatctgag aacccttagg gtttagago tagaaatagc aagtaaaaat aaggctagtc 60

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cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100
SEQ ID NO: 147      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 147
cactcttgc tgtggaaaca gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 148      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 148
atcggttacag gaaaatctga gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 149      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 149
gcacatcttag ggagtagctt gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 150      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 150
caaatcttaa atatgttgt gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 151      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 151
tcactcttgt ctgtggaaac gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 152      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 152
tgcttgatt tttctagtaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 153      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 153
gttaatatct actaagacaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 154      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 154
tttttctagt aatggaaagcc gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt          100

SEQ ID NO: 155      moltype = RNA  length = 100
FEATURE           Location/Qualifiers

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source          1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 155
ttatattatt gatatatttt gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 156      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 156
gcacagatat aaacacttaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 157      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 157
cacagatata aacacttaac gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 158      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 158
ggttttaaa ataataatgt gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 159      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 159
tcagatttc ctgttaacgat gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 160      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 160
cagattttcc tgtaacgatc gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 161      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 161
caatggtaaa taagaataa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 162      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 162
ggaaaatctg aagggtggcaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                         100

SEQ ID NO: 163      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
               mol_type = other RNA
               organism = synthetic construct
SEQUENCE: 163

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ggcgatctca ctcttgtctg gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaagt ggcaccgagt cggtgccttt 100

SEQ ID NO: 164      moltype = length =
SEQUENCE: 164
000

SEQ ID NO: 165      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 165
agcaacctca ctcttgtctg 20

SEQ ID NO: 166      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 166
acctcaactc tgtctgggaa 20

SEQ ID NO: 167      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 167
cctcaactt gtctgggaa 20

SEQ ID NO: 168      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 168
ctcaacttg tctgggaaag 20

SEQ ID NO: 169      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 169
gggaagggg agaaaaaaaaa 20

SEQ ID NO: 170      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 170
gggaaggggg gaaaaaaaaa 20

SEQ ID NO: 171      moltype = length =
SEQUENCE: 171
000

SEQ ID NO: 172      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 172
tgcatttgtt tcaaaatatt 20

SEQ ID NO: 173      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 173
tgattcctac agaaaaagtc 20

SEQ ID NO: 174      moltype = RNA length = 20

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FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 174	
tacagaaaaa gtcaggataa	20
SEQ ID NO: 175	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 175	
tttcttgc cttaaacag	20
SEQ ID NO: 176	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 176	
ttagatttt atattcaaac	20
SEQ ID NO: 177	moltype = length =
SEQUENCE: 177	
000	
SEQ ID NO: 178	moltype = length =
SEQUENCE: 178	
000	
SEQ ID NO: 179	moltype = length =
SEQUENCE: 179	
000	
SEQ ID NO: 180	moltype = length =
SEQUENCE: 180	
000	
SEQ ID NO: 181	moltype = length =
SEQUENCE: 181	
000	
SEQ ID NO: 182	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 182	
agtgcataatgg ataggcctta	20
SEQ ID NO: 183	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 183	
ttactttgca ctttccttag	20
SEQ ID NO: 184	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 184	
tactttgcac tttccttagt	20
SEQ ID NO: 185	moltype = RNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = other RNA
	organism = synthetic construct
SEQUENCE: 185	
tctgacccctt tattttacct	20
SEQ ID NO: 186	moltype = length =

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SEQUENCE: 186
000

SEQ ID NO: 187      moltype = length =
SEQUENCE: 187
000

SEQ ID NO: 188      moltype = length =
SEQUENCE: 188
000

SEQ ID NO: 189      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 189
attatcctga ctttttctgt                                20

SEQ ID NO: 190      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 190
tgaatttttc ctctgtttaa                                20

SEQ ID NO: 191      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 191
taattttctt ttgccccacta                                20

SEQ ID NO: 192      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 192
aaaaggtagt aattgtttag                                20

SEQ ID NO: 193      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 193
aacatccctag gtaaaaataaa                                20

SEQ ID NO: 194      moltype = length =
SEQUENCE: 194
000

SEQ ID NO: 195      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 195
tttgtcatgt tttctaaaaat                                20

SEQ ID NO: 196      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 196
tttgtcatgt atttctaaaaa                                20

SEQ ID NO: 197      moltype = length =
SEQUENCE: 197
000

SEQ ID NO: 198      moltype = RNA length = 100

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FEATURE          Location/Qualifiers
source           1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 198
agaaacctca ctcttgtctg gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                100

SEQ ID NO: 199      moltype = RNA length = 100
FEATURE          Location/Qualifiers
source           1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 199
acactcactct tgcgtgggaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                100

SEQ ID NO: 200      moltype = RNA length = 100
FEATURE          Location/Qualifiers
source           1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 200
cctcactctt gtctggggaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                100

SEQ ID NO: 201      moltype = RNA length = 100
FEATURE          Location/Qualifiers
source           1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 201
ctcactcttgcgtggggaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                100

SEQ ID NO: 202      moltype = RNA length = 100
FEATURE          Location/Qualifiers
source           1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 202
gggaagggg agaaaaaaaaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                100

SEQ ID NO: 203      moltype = RNA length = 100
FEATURE          Location/Qualifiers
source           1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 203
gggaagggg agaaaaaaaaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                100

SEQ ID NO: 204      moltype = length =
SEQUENCE: 204
000

SEQ ID NO: 205      moltype = length =
SEQUENCE: 205
000

SEQ ID NO: 206      moltype = RNA length = 100
FEATURE          Location/Qualifiers
source           1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 206
tgattccatc agaaaaaagt gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt                100

SEQ ID NO: 207      moltype = RNA length = 100
FEATURE          Location/Qualifiers
source           1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 207

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tacagaaaaa gtcaggataa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 208      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 208
ttcttcgtc cttaaacag gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 209      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 209
ttatagttt atattcaaac gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 210      moltype = length =
SEQUENCE: 210
000

SEQ ID NO: 211      moltype = length =
SEQUENCE: 211
000

SEQ ID NO: 212      moltype = length =
SEQUENCE: 212
000

SEQ ID NO: 213      moltype = length =
SEQUENCE: 213
000

SEQ ID NO: 214      moltype = length =
SEQUENCE: 214
000

SEQ ID NO: 215      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 215
atgtcaatgg ataggctta gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 216      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 216
ttacttgca ctttccttag gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 217      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 217
taccttgac tttccttagt gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 218      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 218
tctgacctt tatttacct gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

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SEQ ID NO: 219 moltype = length =
SEQUENCE: 219
000

SEQ ID NO: 220 moltype = length =
SEQUENCE: 220
000

SEQ ID NO: 221 moltype = length =
SEQUENCE: 221
000

SEQ ID NO: 222 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 222
attatcctga cttttctgt gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 223 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 223
tgaatttttc ctctgtttaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 224 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 224
taatttttctt ttgccacta gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 225 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 225
aaaagggttag aattgttttag gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 226 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 226
aacatccttag gtaaaaataaa gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 227 moltype = length =
SEQUENCE: 227
000

SEQ ID NO: 228 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 228
ttgtcatgtat tttctaaat gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 229 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct

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SEQUENCE: 229
tttgcatgt atttctaaaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 230      moltype = length =
SEQUENCE: 230
000

SEQ ID NO: 231      moltype = RNA length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 231
acgaacctca ctcttgctg gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 232      moltype = RNA length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 232
acctcactct tgcgtgggaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 233      moltype = RNA length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 233
ccctcactctt gtctggggaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 234      moltype = RNA length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 234
ctcaactcttgcgtggggaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 235      moltype = RNA length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 235
ggggaaaggaa agaaaaaaaaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 236      moltype = RNA length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 236
ggggaaaggaa agaaaaaaaaa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 237      moltype = length =
SEQUENCE: 237
000

SEQ ID NO: 238      moltype = length =
SEQUENCE: 238
000

SEQ ID NO: 239      moltype = RNA length = 100
FEATURE
source
1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 239

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tgattcctac agaaaaagtc gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 240      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 240
tacagaaaaa gtcaggataa gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 241      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 241
tttcttctgc cttaaacag gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 242      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 242
ttatagttt atattcaac gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 243      moltype = length =
SEQUENCE: 243
000

SEQ ID NO: 244      moltype = length =
SEQUENCE: 244
000

SEQ ID NO: 245      moltype = length =
SEQUENCE: 245
000

SEQ ID NO: 246      moltype = length =
SEQUENCE: 246
000

SEQ ID NO: 247      moltype = length =
SEQUENCE: 247
000

SEQ ID NO: 248      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 248
agtgcataatgg ataggctta gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 249      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 249
ttactttgca ctttccttag gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 250      moltype = RNA length = 100
FEATURE           Location/Qualifiers
source            1..100
                  mol_type = other RNA
                  organism = synthetic construct
SEQUENCE: 250
tactttgcac tttccttagt gtttagago tagaaatagc aagtaaaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

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SEQ ID NO: 251 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 251 tctgaccc ttatttaccc gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 252 moltype = length =
SEQUENCE: 252 000

SEQ ID NO: 253 moltype = length =
SEQUENCE: 253 000

SEQ ID NO: 254 moltype = length =
SEQUENCE: 254 000

SEQ ID NO: 255 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 255 attatccctga cttttctgt gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 256 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 256 tgaattttc ctctgtttaa gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 257 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 257 taattttctt ttgcccacta gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 258 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 258 aaaaggttcag aattgttttag gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 259 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 259 aacatccctag gtaaaaataaa gtttttagago tagaaatagc aagttaaaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 260 moltype = length =
SEQUENCE: 260 000

SEQ ID NO: 261 moltype = RNA length = 100
FEATURE Location/Qualifiers
source 1..100
mol_type = other RNA
organism = synthetic construct

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SEQUENCE: 261
ttgtcatgta tttctaaaat gtttagago tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 262      moltype = RNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other RNA
organism = synthetic construct

SEQUENCE: 262
ttgtcatgt atttctaaaa gtttagagc tagaaatagc aagttaaat aaggctagtc 60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgcttt 100

SEQ ID NO: 263      moltype = DNA  length = 145
FEATURE           Location/Qualifiers
source            1..145
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 263
ttggccactc ctctctgcg cgctcgctcg ctcactgagg ccgggggacc aaaggctgcc 60
cgacgccccg gtttgcggc ggcggccctca gtgagcgagc gagcgccgag agagggagtg 120
gccaactcca tcactagggg ttccct 145

SEQ ID NO: 264      moltype = DNA  length = 100
FEATURE           Location/Qualifiers
source            1..100
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 264
taggtcagtg aagagaagaa caaaaagcag catattacag ttagttgtct tcatcaatct 60
ttaaatatgt tgggtgttt ttctctccct gttccacag 100

SEQ ID NO: 265      moltype = DNA  length = 1296
FEATURE           Location/Qualifiers
source            1..1296
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 265
tttcttgatc atgaaaacgc caacaaaatt ctgaatcgcc caaagaggta taattcagg 60
aaatttggaa agtttgtca agggAACCTT gagagagaat gtatggaga aaagtgttagt 120
tttgaagaag cacgagaagt ttttggaaac actgaaagaa caactgaatt ttggaaaggc 180
tatgtttagt gagatcagt tgagtccat ccatgtttaa atggcggcag ttgcaaggat 240
gacattaaatt ctatgtatg ttgggtgtcc ttggattttt aaggaaaggaa ctgtgtat 300
gatgttaacat gtaacattaa gaatggcaga tgccgacgtt ttgtttaaaa tagtgtctgt 360
acaagggtgg tttgtctctt tactggggg ttttgcattt cagaaaaacca gaagtcctgt 420
gaaccagcag tgccattttcc atgttggaaa gtttctgttt cacaacttc taagtcacc 480
cgtgtctgaga ctgtttttcc tgatgtggac tatgttaaat ctactgaagc tgaaaccatt 540
ttggataaca tcactcaaag caccataca ttaatgtact tcactcggtt tggtgggtaa 600
gaagatgoca aaccaggta atcccttggg cagggtgtt tgaatggtaa agttgtatc 660
ttctgtggg gtttgcattgt taatggaaa tggattgtta ctgtgtccca ctgtgttgaa 720
actgtgttta aatttacatgt tgccgacgtt gaacataata ttgaggagac agaacataaca 780
gagcaaaaggc gaaatgtgtat tgaatattt cctcaccacca actacaatgc agctttaat 840
aagtacaacc atgacatgtc ctttttggaa ctggacgac ctttgcgtt aacacgtac 900
gttacaccta ttgcattgc tgacaaggaa tacacgaaaca ttttccatca atttggatct 960
ggctatgtaa gtgggtgggg aagagtcttc cacaaggaa gatcgttccat agtttttcgt 1020
taccttagag ttccacttgt tgccgacgtt acatgttccat ttttgcattt gttcaccatc 1080
tataacaaca tcgttctgtgt tgggttccat gaaggaggta gagatgtatc tcaaggagat 1140
agtgggggac cccatgttac tgaagtggaa gggaccaggat ttttactgg aattttagc 1200
tgggggttggaa agtgtgtcaat gaaaggcataat ttttgcattt atcccgat 1260
gtcaactggaa ttaaggaaa aacaaagtc acttaa 1296

SEQ ID NO: 266      moltype = DNA  length = 276
FEATURE           Location/Qualifiers
source            1..276
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 266
cctcgactgt gccttcttagt tgcccgccat ctgttggttt cccctcccccc gtgccttcc 60
tgaccctgaa aggtgcact cccactgtcc ttccactataa aatggaggaa attgcattcgc 120
attgtctgag taggtgtcat ttatgttggggt ggggtggggt gggcaggac agcaaggggg 180
aggattggga agacaatagc aggcatgtg gggatgcggt gggctctatg gtttctgagg 240
cgaaaaaaac cagctggggc tcttaggggt atcccc 276

SEQ ID NO: 267      moltype = DNA  length = 192
FEATURE           Location/Qualifiers
source            1..192

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mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 267
 aaaaaaaaaaccc tcacacacccccc cccttgcaccc gaaacataaa atgaatgcaat ttgttttgtt 60
 taacttggttt attgcaggctt ataattggta caaataaagc aatagcatca caaatttcac 120
 aaataaaagca ttttttcac tgcatcttag ttgttggtttt cccaaactca tcaatgtatc 180
 ttatcatgtc tg 192

SEQ ID NO: 268 moltype = DNA length = 1296
FEATURE Location/Qualifiers
source 1..1296
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 268
 ttaggtggcc tttagtctttt cttttatcca attcacgtcg cgagagaccc tcgtatagat 60
 gccccatccccc cccttcatcg cacattccccc ccccaactt attatcccg tcaagaact 120
 tggcccttcg acttcgtcg cgtgtggcc accttgcattca ccttggcatg agtcgcgacc 180
 gcccctgtga aaccaggcac aaaaactgtt attgtaaatc gtaaatttcg tggacagaag 240
 acagggtgcgt ctatcgacca acgggacccgca caaatatgc agaacgaggg ctgatcgacc 300
 tggatgtggaa accccggccccc acccaactac atatccgctc cccaaatitca agaaagatt 360
 tggatattctt ttatcgccca tacaatccgg ggttacatcg ggttgcattca cggatgttc 420
 gtccagtcggcc aggaggcgctt tattcatgtt ttacttgcgtt atagcggcat tataattttgt 480
 atggggatgtt atccgtatcaa catccctttt ctgttgcattca tgctcgtt cttcaatgtt 540
 gtgttgcgcacca gcccacggccgca taatcttaccc cccctgtcg acacagtgtg cggccgttac 600
 aatccacttcc ttatcgacta tggaggccccccca aaaaactacgca tcgtatcc cgttgacac 660
 cacttgcctat gggaaatttgcg cagggtttacq gtcctccggcc cccgacaaacc tagtaatgc 720
 attaaatgac tggatgtggattt gttttatattt atcaagaatc gtttgcgtt cagtagagtt 780
 aacgttgcgttcc acatcgggaa aaaaactgttcc ggccttgcgtt aacttgcgtt cttggacac 840
 acttaccccgaa cccgacccggaa agggccacccg cgggttgcacccg ctctttgtat ttcagcgac 900
 cccgttgcgttcc tcgttgcacca tacacaaatcc ttgttgcgtt cggggattttt tacagaatgg 960
 ctgcgtatgtt ccattttttaa ttgttgcagggtt gacgttccac tcgcgttcc ttcttcacaa 1020
 accaaaaaggcc caccacaaactt cgttggaaattt tatatcgctt ttacaactcc ccccatccag 1080
 acatcggttacccatc gatccgttcc ggttccatcc gatccatgc ttccacactt cgttggcc 1140
 ttcttgcgttcc tcaaaacactt cccgcgttcc ttccaaacttcc ctttttctt ccataactt 1200
 tcgttgcgttcc ttcccttgcgtt ccgttccatcc aagtttccctt gaggatatacc tttagggcc 1260
 gttaaatgttcc ttatcgccgtt tttcggttcc cagaaaa

SEQ ID NO: 269 moltype = DNA length = 100
FEATURE Location/Qualifiers
source 1..100
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 269
 ctgtggaaac agggagagaaa aaaccacaca acatatttaa agattgtatga agacaactaa 60
 ctgtatatgtt ccgttttttctt cactgaccta 100

SEQ ID NO: 270 moltype = DNA length = 145
FEATURE Location/Qualifiers
source 1..145
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 270
 aggaacccctt agtgatggag ttggccactc cctctctgcg cgctcgctcg ctcaactgggg 60
 cccggccggcc aaagccgggg cgttggggca cctttggatcg cccggccctca gtggaggcggc 120
 gagccgcgcgag agaggggatgttcc gccaa

SEQ ID NO: 271 moltype = DNA length = 100
FEATURE Location/Qualifiers
source 1..100
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 271
 gattatttgg attaaaaacaa aagactttctt taagagatgtt aaaattttca tttttttttt 60
 ttttttgcataaaactaaaga attattttttt tacatttcag 100

SEQ ID NO: 272 moltype = DNA length = 1329
FEATURE Location/Qualifiers
source 1..1329
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 272
 ttcttgcattt atgaaaacgc caaaaaattt ctgaatccgc cccaaaggatca taatttgcgtt 60
 aaatttggaaatc agttttttcc agggaaacctt gagagatgtt gatggaaatc aaaaatgtgtt 120
 tttttggaaatc cccggccggcc cccggccggcc cccggccggcc cccggccggcc 180
 ttgttgcgttcc gatccgttcc ttttttttttcc cccggccggcc cccggccggcc 240
 gacattaaattt cccatgttcc ttgttgcgttcc ttttttttttcc cccggccggcc cccggccggcc 300

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gatgtacat	gtaacattaa	gaatggcaga	tgcgagcagt	ttttaaaaaa	tagtgctgat	360
aacaaggctgg	tttgcctctg	tactggggaa	tatcgacttg	cagaaaaacc	gaagtcctgt	420
gaaccacag	tgccattttcc	atgtggaaa	gttctgttt	cacaaacttc	taagtcacc	480
cgtgctgaga	ctgttttcc	tgatgtggac	tatgtaaat	ctactga	tgaaaccatt	540
ttggataaca	tcaactcaaag	caccaatca	ttaatgact	tcactcggtt	tggtgggtaa	600
gaagatgoca	aaccaggta	atccccttg	caggtgttt	tgaatggtaa	agtgtatgca	660
ttctgtggag	gtctatcgt	taatggaaaa	tggattgtta	ctgtgc	cccaatgttt	720
actctgttta	aaattacagt	tgtcgaggt	gaacataata	tgtggagac	agaacataaca	780
gagccaaagc	gaaatgtat	togaattt	cctcaccaca	actacaatgc	agctattaat	840
aagtacaacc	atgacattgc	ccttctggaa	ctgacgacaa	ccttagtgc	aaacagctac	900
gttacaccta	tttgcattgc	tgcacaaaggaa	tacacgaa	tcttcctaa	atttggatct	960
ggctatgtaa	gtggctgggg	aagagtctc	cacaaaggaa	gatcagott	agtcttcag	1020
taccttagag	ttccacttgt	tgaccgac	acatgttctt	tatctacaaa	gttcaccatc	1080
tataacaaca	tggttctgtt	tggttccat	gaaggaggaa	gatgtatgc	tcaaggagat	1140
agtgggggac	cccatgttac	tgaatggaa	gggaccagg	tcttaactgg	aattatttagc	1200
tggggtaa	agtgtcaat	gaaaaggaa	tatggatat	ataccaagtc	ctcccgat	1260
gtcaactgga	ttaaggaaaa	aacaaagctc	actgtcagcg	gatggagact	gttcaagaag	1320
atcagctaa						1329

SEQ ID NO: 273	moltype = DNA	length = 1329				
FEATURE	Location/Qualifiers					
source	1..1329					
	mol_type = other DNA					
	organism = synthetic construct					
SEQUENCE: 273						
tttagaaatc	ttcttaaaca	ggcccgcc	gctcacggtg	agcttagtct	tttcttttat	60
ccaaatccac	tgacgagaga	ccttcgtata	gtgcacat	ttccccctca	tcgcacat	120
ctccccccaa	cttattatcc	ccgtcaagaa	acttgc	tgcacttc	tgacgtgtgg	180
tccacactgaa	tcaccttggc	atgatcg	ccgc	ccctcg	tgaacccag	240
gttattgtaa	atcgtaaaat	tcgtggacag	aagacagg	tc	ccaacggac	300
gcccacat	atgc	gggtgatcg	ac	tttgc	gggaa	360
cacatatcgc	ttcccaaaat	tcaaga	at	tttgc	tttgc	420
cggggtaaca	taggagttaa	gtacggatgg	ctcg	tccaggagg	ctat	480
gttgtacttg	ttttagcgg	cattata	gtatgggt	atgatc	tgc	540
tttctgttca	gtatgttca	gttgc	ccgc	ccgt	aatctt	600
aaccccccgc	tgcacacat	gtgcggccgt	tacaatcc	tttgc	tgc	660
ccccacaaac	gogtcgactt	ttccgttgc	caccac	tgc	catggaa	720
agcgtcc	cccccgacaa	ccctg	at	tttgc	ggcagg	780
attatcaaga	atcg	tttgc	tgc	cc	aaaaactgt	840
ctcgcc	tttgc	atgttggg	ca	atc	ccgc	900
cgc	tttgc	tc	g	cc	ggggcac	960
aacttgc	tcggcggat	tttgc	tttgc	at	tttgc	1020
gtgtgac	acttcg	tttgc	aaa	cc	actcgtagg	1080
atttatc	tcttacaa	ccccccat	caga	at	tttgc	1140
atcgacat	tgcttc	caga	act	tc	tttgc	1200
tttctcaaa	ctgc	at	tc	tc	tc	1260
ttcaagctt	ctcg	at	tc	tc	tc	1320
gtcc	agaaa					1329

SEQ ID NO: 274	moltype = DNA	length = 100				
FEATURE	Location/Qualifiers					
source	1..100					
	mol_type = other DNA					
	organism = synthetic construct					
SEQUENCE: 274						
ctgaaatgt	aaagaataat	tctttat	tagaaaaaa	gaaaacatca	tgaaaat	60
acatcttta	agaaagtctt	tgttttaat	ccaaataatc			100

SEQ ID NO: 275	moltype = DNA	length = 1446				
FEATURE	Location/Qualifiers					
source	1..1446					
	mol_type = other DNA					
	organism = synthetic construct					
SEQUENCE: 275						
tttcttgc	atgaaaacgc	caacaaatt	ctgaatcg	ccaaagg	taattc	60
aaattggaa	agtttgtca	agggaa	ctt	gag	aaatgt	120
tttgaaga	ag	tttggagg	tttgc	gg	actggagg	180
tataatctcg	acca	ggaa	gggg	gg	aaat	240
gtaa	ccat	gggt	cc	cc	gggt	300
atccatgtt	tttgc	at	tc	cc	gg	360
atttcaagg	tttgc	at	tc	cc	actat	420
acacttgc	tcgacgg	aact	tc	tc	at	480
ggcatcg	tttgc	at	tc	tc	tc	540
aagataatc	acg	at	tc	tc	tc	600
aacggcgt	tttgc	at	tc	tc	tc	660
aaggcagg	tttgc	at	tc	tc	tc	720

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accgggggtgg	tgcctatcct	ggtcgagctg	gacggcgacg	taaaggccca	caagttagc	780
gtgtccggc	aggcggaggg	cgtggccaco	tacggcaac	tgaccctgaa	gttcatctgc	840
accacccggca	agetcggcgt	gcccggccc	accctcgta	ccaccctgac	ctacggcg	900
cagtgcgttca	gocgttaccc	cgaccatcg	aaggcagc	acttcttcaa	gtccggcat	960
ccccaaaggct	acgttccagga	gcccacccat	ttcttcaagg	acgacggca	ctacaagacc	1020
cggcgccgagg	tgaagttcg	ggggacac	cttgtgaac	gcatcgac	gaagggcac	1080
gacttcaagg	aggacggca	cattctggg	cacaagctgg	agtacaacta	caacagccac	1140
aacgtctata	tcatggccg	caacagaag	aacggcat	aggtaac	caagatccgc	1200
cacaacatcg	aggacggcag	cgtgcagtc	gcccggact	accagcaga	cacccatc	1260
ggcgacggcc	ccgtgtgtct	gcccggacaa	cactacttgc	gacacccatc	cgccctgac	1320
aaagacccca	acgagaagcg	cgatcacat	gtctgtgt	agttcgtac	cgccggccgg	1380
atcaactctcg	gcatggacg	gctgtacaag	ggaggaggaa	gcccgaaga	gaagagaaag	1440
gtctaa						1446

SEQ ID NO: 276 moltype = DNA length = 1446
 FEATURE Location/Qualifiers
 source 1..1446
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 276

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ggtgatcg	gggggggtc	cgaactcc	cggcaccat	tggtccct	tctcggttgg	120
gtcttgc	aggggcgtct	gggtgtcg	gtatgtgtt	tggggcaga	gcacggggcc	180
gtcgccgt	gggggtgtt	gtgtgtgt	gtggcggac	tgcacgtgc	cgtctcgat	240
gttgtgc	atcttga	tcacottgt	gcccgttcc	tgttgttgc	ccatgtatgt	300
cacgttgc	ctgtgtgt	tgtatcc	cttggggccc	aggatgtgc	cgtctctt	360
gaagtgc	cccttgc	cgatgtgt	caccagggt	tgcgttcc	acttacactc	420
ggccctgg	tgttagtgc	cgtcgtt	gaagaatgt	gttccctt	gcacgtagcc	480
ctcggttgc	ggtgttgc	agaagtgc	ctgtttat	tgggtgggt	acctgtgtaa	540
gcaactc	cgttaggtc	gggtgttac	cagggtggc	cagggtcagg	gcagcttgc	600
gtgtgtgc	acgggttgc	gggtgttgc	gcccgttgc	ggtgttgc	gcgttgcct	660
gttcacgt	aacttgc	cggttacgt	gcccgttgc	tccaccagg	tgggcacac	720
gcccgttgc	acttgc	ggggccggg	tttcccttca	cgtcgccggc	780	
ctgtttgc	agggtgt	tgggtggc	gatcccttc	cacagcc	agccggc	840
gcccgttgc	gttccatc	acagcagg	gcccgttgc	tttgcgttgc	tctcgat	900
gatcttgc	ccgttccaca	gggtgttgc	cacgggttgc	tttgcgttgc	cgaacacggc	960
gatccctc	tagggctgc	cgaatgtt	gatcatgtt	gggggtc	cgtcgat	1020
cagggtgc	tagtgc	tcacccgtt	gtgtgttgc	tccacgggt	acaccac	1080
aaaaatctc	tccatc	ccatcgttgc	gcccgttgc	ccctgttgc	ggatgtatc	1140
gtggatgt	atcttgc	cggttgc	gctcgttgc	atcttgc	tgggggtc	1200
gttcacgc	agggttgc	acaggctgt	cacccgc	tgctccag	cctgggtc	1260
gttgtagc	gggggttgc	tccagtc	cacgaatgc	tccagggt	acacggc	1320
ctcgaagct	cacttctt	ccatgc	cctcttcc	tggccctgc	cgaactc	1380
cagttgc	ctgtgttac	tettgggc	gttcaggat	tgttgttgc	tctcgat	1440
caggaa						1446

SEQ ID NO: 277 moltype = DNA length = 3570
 FEATURE Location/Qualifiers
 source 1..3570
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 277

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cgtacggcc	gttttgc	ggccggct	ca	gtggcgac	gagcgcgc	agggggat	120
gccaactc	tca	tacttgc	ttcc	tgttgc	gtggatggaa	gaacaaa	180
cagcatat	cagttagt	tctt	catca	tctttaaa	tgttgttgc	ttttcttc	240
cctgttca	cagt	tttct	tgtatca	aacggca	aaatcttgc	tccggca	300
aggtataatt	caggtaatt	ggaagat	tttgc	ggatgggg	accttgc	agaatgtat	360
gaagaaaaat	gtatgttgc	agaacac	ggatgttt	aaaacat	gaaacaact	420	
gaattttgc	aggcgtat	tgatgttgc	cgtgtgt	ccatccat	ttttaatggc	480	
ggcgttgc	aggatgtat	catccat	aatccat	gttgcgttgc	atttgttgc	540	
aagaactgt	attagatgt	aacatgt	aatagaat	gcatgtgc	gcagatgt	600	
aaaaatatgt	ctgataacaa	ggtgggttgc	tccgtact	agggtat	acttgc	660	
aaccacaaat	cctgttgc	acgtgttgc	tttccat	gttgc	tttttcac	720	
acttctaa	tcacccgt	tgatgttgc	tttccat	gttgc	atgttact	780	
gaagcttgc	aaatgttgc	taatcact	caaaaccc	aatc	tgc	840	
cgggttgc	gtggaga	tgccaa	ggtcaatt	cttgc	gggttgc	900	
ggtaaagtgc	atgcattct	tggaggct	atcgtt	aaaatgtt	tgtactgt	960	
gcccactgt	ttgaaaact	acagtgt	tgc	cagggt	taatatttgc	1020	
gagacacaa	atacagac	aaagc	aaatgt	gttgc	ttatctca	1080	
atgcgtca	ttaatgttgc	caaccat	gttgc	acttgc	ccacaact	1140	
gtgttgc	actacgttac	acattttgc	attgt	gttgc	atgttact	1200	
ctcaat	gtatgttgc	tgtatgttgc	ttggg	aaag	tccacaa	1260	
gttttagt	tgcgttac	taggttca	cttgc	ggaccat	tcttctat	1320	
acaatgttca	ccatctata	caacatgtt	tgtgttgc	tccat	gttgc	1380	
tcatgtca	gagatgttgc	ggggcc	tttact	gttgc	aggatgtat	1440	

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actggaaatta	ttagctgggg	tgaagagtgt	gcaatgaaag	gcaaatatgg	aatatatacc	1500
aaggatcc	ggtatgtcaa	ctggattaa	aaaaaaacaa	agtcactta	acctcgactg	1560
tgcccttgc	ttgcgcggca	tctgtttt	ccccccccc	cgtgcctcc	ttgaccctgg	1620
aagggtccac	tcccaactgtc	ctttcttaat	aaaaatggag	aattgcatcg	cattgtctgg	1680
qtaggtgtca	ttctatttcg	gggggtgggg	tggggcaggag	cgaacagggg	qaaggatggg	1740
aagacaataq	caggcatgt	ggggatgggg	tgggctctat	ggcttcttag	gcccggaaagaa	1800
ccagctgggg	ctcttaggggg	tatccccaaa	aaacccccc	caccctcccc	tgaacactgaa	1860
acataaaaat	aatgcaatgt	tttgtttaa	cttggtttt	gcagcttata	atggttacaa	1920
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tggtttgtcc	aaactcatca	atgtatctta	tcatgtctgt	taggtgaget	tagtctttt	2040
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aaacatgtta	ttgttaaatcg	taaatttgc	ggcagacaga	caggtcgttc	tatcgaccaa	2280
cgggacgcgc	aaatattgc	gaacggggc	tgatcgaccc	ttgtggaaga	ccggccccc	2340
cccaactcaca	tatccgctcc	caaatttca	gaagatattt	gtatatttt	tatccggtat	2400
acaatccggg	gtacatcgat	taatggat	gagtggctcg	tccagtc	ggaggggctat	2460
atcatgggtt	tacttggttt	tageggccat	ataatgttga	tggggatgt	tccgttataac	2520
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aatcttaacc	cccgcttcga	cacagtgtc	ggccgttaca	atccacttt	cattgtactat	2640
ggggccccca	caaaacgggt	cgactttcc	gttgacccac	acccgttgc	gaatttggcc	2700
aggttttagcg	tcctcgcccc	cgacaaaccc	agtaaagtca	ttaaatgact	gtgtggattt	2760
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aactgtctcg	gcccttgc	actttgtat	ctgggacaca	tttaccgc	cgccacggaa	2880
ggggcacccgg	gggttccacgc	tctttgtatt	ctcggcggc	cgtgtaccc	cagtgcata	2940
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SEQ ID NO: 278 moltype = DNA length = 3636
FEATURE Location/Qualifiers
source 1..3636
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 278 Organism - Synthetic Construct

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cgacgccccg	gctttcccg	ggggccgtca	gtgaaggcagc	gaggcgcag	agaggggatgy	120
gccaactcc	tcactagggg	ttctcatgatc	ttctgtatatt	ttggataaaa	acaagaactt	180
tcttaaagaa	tgtaaaaaatt	tcatgtatgt	ttcttttttg	ctaaacaa	aaagattttc	240
ttttacattt	cagttttct	tgatcatgaa	aacgocaa	aaattctgaa	tcggccaaag	300
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cggggttgtt	gtggagaaga	tgccaaacc	ggtaatccc	cttggcagg	tgttttgaat	900
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gcccactgt	tttggaaactt	tggtttaaat	acagtgtcg	cagggtacaa	taatattgt	1020
gagacagaaac	atacagagca	aaagcgaat	gtgattcgaa	tttattctca	ccacaactac	1080
aatgcagcta	ttaataaagta	caaccatgc	attgccttc	tggacttgg	cgaaccctta	1140
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ctcaaaatttg	gatctggcta	tgtaaatgtc	ttggggaaag	ttttccacaa	aggggatata	1260
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gggtggggca	ggacagcga	ggggaggatt	gggaagacaa	tagcaggat	gtctggggat	1800
cgggtgggtt	tatgtgtt	ggggggggaa	gaaccacgt	gggtctttag	gggtatcccc	1860
aaaaaaatcc	ccacatcc	ccctgtacat	gaaacataaa	atgtatgc	ttgtgttgt	1920
taacttgtt	attgcacgtt	ataatgttta	caataaaagc	ataagatca	caatatttcac	1980

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cttttcttt	atccaattca	cgtagcgaga	gaccttctga	tagatgcac	atttcccctt	2160
catccgacat	tctcccccc	aacttattat	cccggtcaag	aaacttgttc	cttcgacttc	2220
agtgacgtgt	ggtccacctg	aatcacctt	gcatgagtgc	cgaccgcct	cgtgaaaccc	2280
agcacaaaaac	atgttattgt	aaatcgtaaa	tttcgtggac	agaagacagg	tcgctctatc	2340
gaccaacggg	acgcgcacat	atgcgagaad	gagggtctgat	gcaccttgc	ggaagacccg	2400
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 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 279

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SEQ ID NO: 280 moltype = DNA length = 3636
 FEATURE Location/Qualifiers
 source 1..3636
 mol_type = other DNA
 organism = synthetic construct
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SEQ ID NO: 281 moltype = DNA length = 1954
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 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 281

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SEQ ID NO: 282 moltype = DNA length = 2359
 FEATURE Location/Qualifiers
 source 1..2359
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 282

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 catgttgc ttttttttttcccaataa ataacaggaa ttttttttttcccaataa ataacaggaa ttttttttttcccaataa ataacaggaa tttttttttt 1620

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aaatatggaa	tataaccaa	ggatatcccg	tatgtcaact	ggattaaggaa	aaaaacaag	1740
ctcaactaac	ctcgactgt	ccttctatgt	gccagccatc	tgtgtttgc	ccctcccccg	1800
tgcccttc	gaccctggaa	ggtgcactc	ccactgtct	ttcctaataa	aataggaaaa	1860
ttgcatcgca	ttgtctgagt	aggtgtcatt	ctattctggg	gggtgggtg	ggcgaggaca	1920
gcaaggggaa	ggatttggaa	gacaatagca	ggcatgtgg	ggatgegggt	ggcttatgg	1980
cttctgagcc	ggaaaagaacc	agctgggtc	ctagggggta	cccccttag	gtggttatat	2040
tattgtat	tttttgtat	ctttgtatc	aataatgggg	gattttgaaa	gttttagctt	2100
aaattttt	taattaaaaa	aaaatgttag	gcagaatgac	tcaaaattacg	ttggatacacag	2160
ttgaattttt	tacggctca	tagggcctgc	ctgctcgacc	atgtataact	aaaaattaaa	2220
agtgtgttt	taattttta	taatttggagt	ttccatttat	atttacattt	attttttattt	2280
taccatgtc	tttagtagata	tttacaaaaca	tgacagaaaac	actaaatctt	gagtttgaat	2340
gcacagat	aaacacttaa	cgggtttaa	aaataataat	gttggtaaaa	aaataataact	2400
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acaagagtga	gateccccat	cggtataatg	atttgggaga	acaacattt	aaaggctgt	2640
aagttaataat	gtgaaagcc	cacttaat	ttctgttagt	atttggtaaa	gttttaaaaac	2700
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tcaagcactt	tgcacatggc	aagtgtact	gagaaggcaca	cattcaaaa	atagtttaatt	2880
tttaattgtat	gtatctagcc	atgtgtggc	atgtgtccct	ttccctggaga	gagaatctgg	2940
agccccatc	taacttggta	agtctggaa	tttattttt	atttctggaa	aggcttatgt	3000
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tataacttaag	tcacatctcc	aaccttagta	atgttttaat	gttagaaaaa	aatgagtaat	3180
taattttttt	tttagaaggc	aatagttca	tgtatttcca	ataacagagg	tatatggta	3240
gaaaagaaac	aattcaaaagg	attttatataa	tatttagcct	tgacaatgaa	taaattttaga	3300
gagtagttt	cctgtttgcc	tcatgttcat	aaatctattt	acacatatgt	gcacatctgcac	3360
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cagagaatag	ctgacgggaa	gagggtttct	atagatgtgt	atgaaagata	tacaaaatct	3480
cggcggatata	cacaggcatg	atttgcgtt	tgggagagc	acttagatct	aggaacccct	3540
agtgtatgg	ttggccactc	cctctctgcg	cgctcgctcg	ctcaactgggg	ccgccccggc	3600
aaagcccccgg	cgtcggccga	ccttgggtcg	cccgccctca	gtgagcgcagc	gagcgcgcag	3660
agaggggatg	gcacaa					3675

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SEQ ID NO: 299 moltype = length =
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SEQ ID NO: 300 moltype = RNA length = 100
FEATURE Location/Qualifiers
misc_difference 1..20
note = n=a, c, u, g, unknown or other
source 1..100
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 300
nnnnnnnnnn nnnnnnnnnn gtttagago tagaaatagg aagttaaat aaggctagtc 60
cgtttatcaac ttgaaaaagt ggcaccgagt cggtgccttt 100

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SEQ_ID NO: 400 moltype = RNA length = 22
FEATURE
source
1..22
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 400
gttttagagc tatgctgttt tg 22

SEQ_ID NO: 401 moltype = RNA length = 80
FEATURE
source
1..80
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 401
gttttagagc tagaaatagc aagttaaaat aaggctagtc cgtttatcaac ttgaaaaagt 60
ggcaccgagt cggtgcttt 80

SEQ_ID NO: 402 moltype = RNA length = 76
FEATURE
source
1..76
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 402
gttttagagc tagaaatagc aagttaaaat aaggctagtc cgtttatcaac ttgaaaaagt 60
ggcaccgagt cggtgc 76

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SEQ ID NO: 573      moltype = length =
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SEQ ID NO: 576      moltype = length =
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SEQ ID NO: 577      moltype = length =
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SEQ ID NO: 582      moltype = length =
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SEQ ID NO: 585      moltype = length =
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SEQ ID NO: 587      moltype = length =
SEQUENCE: 587
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SEQ ID NO: 588      moltype = length =
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SEQ ID NO: 592      moltype = length =
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SEQUENCE: 593
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SEQ ID NO: 594      moltype = length =
SEQUENCE: 594
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SEQ ID NO: 595      moltype = length =
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SEQ ID NO: 596      moltype = length =
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SEQ ID NO: 598 moltype = length =
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SEQ ID NO: 599 moltype = length =
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FEATURE Location/Qualifiers
source 1..7
mol_type = protein
organism = synthetic construct
REGION 1..7
note = Simian virus 40 NLS
SEQUENCE: 600
PKKKRKV 7

SEQ ID NO: 601 moltype = AA length = 7
FEATURE Location/Qualifiers
source 1..7
mol_type = protein
organism = synthetic construct
REGION 1..7
note = Simian virus 40 NLS
SEQUENCE: 601
PKKKRRV 7

SEQ ID NO: 602 moltype = AA length = 16
FEATURE Location/Qualifiers
REGION 1..16
note = Nucleoplasmin bipartite NLS sequence
source 1..16
mol_type = protein
organism = synthetic construct
SEQUENCE: 602
KRPAATKKAG QAKKKK 16

SEQ ID NO: 603 moltype = length =
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SEQ ID NO: 608 moltype = length =
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SEQ ID NO: 609 moltype = length =
SEQUENCE: 609
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SEQ ID NO: 610 moltype = length =
SEQUENCE: 610
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SEQ ID NO: 611 moltype = length =

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SEQUENCE: 611
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SEQ_ID NO: 630 moltype = length =

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SEQUENCE: 630
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SEQ_ID NO: 667 moltype = length =
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SEQ_ID NO: 687 moltype = length =

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SEQUENCE: 687
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SEQ ID NO: 688      moltype = length =
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SEQ ID NO: 690      moltype = length =
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SEQ ID NO: 691      moltype = length =
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SEQ ID NO: 698      moltype = length =
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SEQ ID NO: 699      moltype = length =
SEQUENCE: 699
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SEQ ID NO: 700      moltype = AA length = 461
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source          Location/Qualifiers
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mol_type = protein
organism = Homo sapiens
SEQUENCE: 700
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ERECMEEKCS FEEAREVFN TERTTEFWKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP 120
FGFEGKNCEL VTDCNIKNGR CEQFCCKNSAD NKVVCSTEG YRLAENQKSC EPAPVFPCGR 180
VSVSQTSLT RAETVFPDVN YVNSTEATI LDNITQSTQS FNDFTRVGG EDAKPGQQFPW 240
QVVLNGKVA FCAGGSIVNEK WIVTAAHCV TGVKITTVVAG EHNLIEETHT EQKRNVIRII 300
PHINYNNAAIN KYNHDIALLE LDEPLVLNSY VTPICIADEK YTNIFLKFGS GYVSGWGRVF 360
HKGRSALVHQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE 420
GTSFLTGIIS WGEECAMKGK YGIYTKVSRV VNWIKEKTKL T 461

SEQ ID NO: 701      moltype = AA length = 415
FEATURE
source          Location/Qualifiers
1..415
mol_type = protein
organism = Homo sapiens
SEQUENCE: 701
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SCKDDINSYE CWCPFGFEGK NCEDVTCNI KNGRCEQFCK NSADNKVVCS CTEGYRLAEN 120
QKSCEPAVPF PCGRVSVSQT SKLTRAETVF PDVDYVNSTE AETILDNTQ STQSFNDFTF 180
VVGGEDAKPG QFPWQVVLNG KVDAFCGGSI VNEKWIVTAA HCVENTGVKIT VVAGEHNIEE 240

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TEHTEQKRVN	IRIIPHYN	AAINKYNHD	ALLELDEPLV	LNSYVTPI	ADKEYTNIFL	300
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CQGDSGGPHV	TEVEGTSFLT			GIISWGEECA	MKGKYGIYTK	415

SEQ ID NO: 702	moltype = AA	length = 415				
FEATURE	Location/Qualifiers					
source	1..415					
	mol_type = protein					
	organism = synthetic construct					
SEQUENCE: 702						
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SCKDDINSYE	CWCPFGFEGH	NCELDVTNCI	KNGRCEQFCK	NSADNKVVC	CTEGYRLAEN	120
QKSCEPAVPF	PCGRVSVSQT	SKLTRAETVF	PDVYVNSTE	AETILDNTQ	STQSFNDFT	180
VVAGGEDAKPG	QFPWQVVLNG	KVDAGCGGS	VNEKWIVITA	HCVETGVKIT	VVAGEHNIEE	240
TEHTEQKRVN	IRIIPHYN	AAINKYNHD	ALLELDEPLV	LNSYVTPI	ADKEYTNIFL	300
KFGSGYVSGW	GRVFHKGRSA	LVLQYLRVPL	VDRATCLLST	KFTIYNNMFC	AGFHBRGRDS	360
CQGDSGGPHV	TEVEGTSFLT			GIISWGEECA	MKGKYGIYTK	415
SEQ ID NO: 703	moltype = DNA	length = 4104				
FEATURE	Location/Qualifiers					
source	1..4104					
	mol_type = other DNA					
	organism = synthetic construct					
SEQUENCE: 703						
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cacagcatca	agaaaaaatct	catecgagcc	ctgctgttt	actccggcga	aaccgcagaa	180
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SEQ ID NO: 704 moltype = DNA length = 4140
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 source 1..4140
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 704

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 organism = Homo sapiens

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SEQUENCE: 1121
000

SEQ_ID NO: 1122 moltype = length =
SEQUENCE: 1122
000

SEQ_ID NO: 1123 moltype = length =
SEQUENCE: 1123
000

SEQ_ID NO: 1124 moltype = length =

-continued

```

SEQUENCE: 1124
000

SEQ_ID NO: 1125      moltype = length =
SEQUENCE: 1125
000

SEQ_ID NO: 1126      moltype = length =
SEQUENCE: 1126
000

SEQ_ID NO: 1127      moltype = length =
SEQUENCE: 1127
000

SEQ_ID NO: 1128      moltype = length =
SEQUENCE: 1128
000

SEQ_ID NO: 1129      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 1129
gagtccgagc agaagaagaa                                         20

SEQ_ID NO: 1130      moltype = RNA length = 20
FEATURE
source
1..20
mol_type = other RNA
organism = synthetic construct
SEQUENCE: 1130
gacccccctcc accccgcctc                                         20

```

1.-143. (canceled)

144. A method of expressing Factor IX in a liver cell or population of liver cells, comprising administering:

- (i) a nucleic acid construct comprising a Factor IX protein coding sequence;
 - (ii) an RNA-guided DNA binding agent or a nucleic acid encoding the RNA-guided DNA binding agent; and
 - (iii) a guide RNA (gRNA) comprising a sequence targeting intron 1 of an albumin locus,
- thereby expressing Factor IX in the liver cell or population of liver cells.

145. The method of claim **144**, wherein the RNA-guided DNA binding agent is Cas9.

146. The method of claim **145**, wherein the method comprises administering an mRNA encoding the Cas9.

147. The method of claim **146**, wherein the gRNA and the mRNA encoding the Cas9 are administered in a lipid nanoparticle.

148. The method of claim **144**, wherein the nucleic acid construct is administered in a viral vector.

149. The method of claim **148**, wherein the viral vector is an adeno-associated viral (AAV) vector.

150. The method of claim **144**, wherein the nucleic acid construct is a bidirectional nucleic acid construct comprising: (a) a first segment comprising a first coding sequence for Factor IX and (b) a second segment comprising a reverse complement of a second coding sequence for Factor IX.

151. The method of claim **150**, wherein the bidirectional nucleic acid construct does not comprise a homology arm, does not comprise a promoter that drives expression of the first coding sequence, and does not comprise a promoter that drives expression of the second coding sequence.

152. The method of claim **144**, wherein the nucleic acid construct is administered in an AAV vector, the RNA-guided DNA binding agent or the nucleic acid encoding the RNA-guided DNA binding agent and the gRNA are administered in a lipid nanoparticle, and the AAV vector and the lipid nanoparticle are administered simultaneously or sequentially, in any order.

153. The method of claim **144**, wherein the liver cell or population of liver cells is a human liver cell or population of human liver cells.

154. A method of treating a Factor IX deficiency, comprising administering to an individual with the Factor IX deficiency:

- (i) a nucleic acid construct comprising a Factor IX protein coding sequence;
 - (ii) an RNA-guided DNA binding agent or a nucleic acid encoding the RNA-guided DNA binding agent; and
 - (iii) a guide RNA (gRNA) comprising a sequence targeting intron 1 of an albumin locus,
- thereby expressing Factor IX in the individual.

155. The method of claim **154**, wherein the RNA-guided DNA binding agent is Cas9.

156. The method of claim **155**, wherein the method comprises administering an mRNA encoding the Cas9.

157. The method of claim **156**, wherein the gRNA and the mRNA encoding the Cas9 are administered in a lipid nanoparticle.

158. The method of claim **154**, wherein the nucleic acid construct is administered in a viral vector.

159. The method of claim **158**, wherein the viral vector is an adeno-associated viral (AAV) vector.

160. The method of claim **154**, wherein the nucleic acid construct is a bidirectional nucleic acid construct compris-

ing: (a) a first segment comprising a first coding sequence for Factor IX and (b) a second segment comprising a reverse complement of a second coding sequence for Factor IX.

161. The method of claim **160**, wherein the bidirectional nucleic acid construct does not comprise a homology arm, does not comprise a promoter that drives expression of the first coding sequence, and does not comprise a promoter that drives expression of the second coding sequence.

162. The method of claim **154**, wherein the nucleic acid construct is administered in an AAV vector, the RNA-guided DNA binding agent or the nucleic acid encoding the RNA-guided DNA binding agent and the gRNA are administered in a lipid nanoparticle, and the AAV vector and the lipid nanoparticle are administered simultaneously or sequentially, in any order.

163. The method of claim **154**, wherein the individual is a human.

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