acid sequence encoding the DNA endonuclease further comprises an intron sequence upstream of the endonuclease sequence, and wherein the intron comprises a nuclease cleavage site.

[0048] In another example of this aspect and all other aspects provided herein, a poly-A-site is upstream and proximate to the 5' homology arm.

[0049] In another example of this aspect and all other aspects provided herein, the donor sequence is foreign to the 5' homology arm or the 3' homology arm.

[0050] In another example of this aspect and all other aspects provided herein, the 5' homology arm or the 3' homology arm are proximal to the at least one ITR as defined herein.

[0051] In another example of this aspect and all other aspects provided herein, the nucleotide sequence encoding a nuclease is cDNA.

[0052] In another example, the editing is directed at RNA instead of DNA. For example, using Cas13, such as Cas13 from *Prevotella* spp. bacteria. This enzyme is combined with another molecule that corrects the RNA. For example, the ADAR2 protein changes individual RNA's from adenosine to inosines. See *e.g.,* Science, Cox, D.B.T. *et al.* 25 Oct 2017 "RNA editing with CRISPR-Cas13. RNA editing and/or tracking using ceDNA vector(s) encoding a gene editing system as described herein can be performed with methods known in the art, for example, Abudayyeh et al. *Science* 353:1-9 (2016); O'Connell et al. *Nature* 516:263-266 (2014); Nelles et al. *Cell* 165:488-496 (2016); the contents of each of which are incorporated by reference herein in their entirety.

[0053] Another aspect provided herein relates to an *in vitro* method for genome editing of a chromosome in a host cell comprising: contacting a cell with a) capsid-free closed ended DNA (ceDNA) vector comprising at least one nucleotide sequence between flanking inverted terminal repeats (ITRs), wherein the at least one nucleotide sequence encodes a Cas enzyme; and a guide RNA (gRNA); or b) a ceDNA vector comprising at least one nucleotide sequence between flanking ITRs, wherein the at least one nucleotide sequence encodes a gRNA; and a Cas enzyme, wherein the chromosome is repaired via non-homologous end joining (NHEJ). For example, provided herein is a method for genome editing comprising: contacting a cell with a gene editing system, wherein one or more components of the gene editing system are delivered to the cell by contacting the cell with a composition comprising the ceDNA vector as disclosed herein, wherein the ceDNA nucleic acid vector composition comprises flanking inverted terminal repeat (ITR) sequences where the ITR sequences are asymmetrical, symmetrical or substantially symmetrical relative to each other as defined herein, and at least one gene editing nucleic acid sequence.

[0054] In another example of this aspect and all other aspects provided herein, the gene editing system is selected from the group consisting of: a TALEN system, a zinc-finger endonuclease (ZFN) system, a CRISPR/Cas system, A CRISPRi system, a CRISPRa system, and a meganuclease system.

[0055] In another example of this aspect and all other aspects provided herein, the at least one gene editing nucleic acid sequence encodes a gene editing molecule selected from the group consisting of: an RNA guided nuclease, a guide RNA, guide DNA, ZFN, TALEN, a Cas, CRISPR/Cas molecule or orthologue thereof, a ribonucleoprotein (RNP), or deactivated CAS for CRISPRi or CRISPRa systems.

[0056] In another example of this aspect and all other aspects provided herein, a single ceDNA vector comprises all components of the gene editing system.

and H840A, of the gene encoding Cas9. In one example, a catalytically inactive state of a molecule refers to a molecule with less than 0.1% catalytic activity compared to its catalytically active state and further encompasses a molecule having any activity discernable by standard laboratory methods.

[0064] Also provided herein, in another aspect, is a method for editing a single nucleotide base pair in a target gene of a cell, the method comprising contacting a cell with a CRISPR/Cas gene editing system, wherein one or more components of the CRISPR/Cas gene editing system are delivered to the cell by contacting the cell with a close-ended DNA (ceDNA) nucleic acid vector composition, wherein the ceDNA nucleic acid vector composition is a linear close-ended duplex DNA comprising flanking terminal repeat (TR) sequences and at least one gene editing nucleic acid sequence for targeting a target gene or a regulatory sequence for the target gene, wherein the Cas protein expressed from the vector is catalytically inactive and is fused to a base editing moiety, wherein the method is performed under conditions and for a time sufficient to modulate expression of the target gene.

[0065] In another example of this aspect and all other aspects provided herein, the base editing moiety comprises a single-strand-specific cytidine deaminase, a uracil glycosylase inhibitor, or a tRNA adenosine deaminase.

[0066] In another example of this aspect and all other aspects provided herein, the catalytically inactive Cas protein expressed from the vector is dCas9.

[0067] In another example of this aspect and all other aspects provided herein, the cell contacted is a T cell, or a $CD34^+$ cell.

[0068] In another example of this aspect and all other aspects provided herein, the target gene encodes for a programmed death protein (PD1), cytotoxic T-lymphocyte-associated antigen 4(CTLA4), or tumor necrosis factor-α (TNF-α).

[0069] In another example of this aspect and all other aspects provided herein, further comprising administering the cells (e.g. T cells or CD34+ cells) produced by a method described herein to a subject in need thereof.

[0070] In another example of this aspect and all other aspects provided herein, the subject in need thereof has a viral infection, bacterial infection, cancer, or autoimmune disease.

[0071] Another aspect provided herein relates to a method of modulating expression of two or more target genes in a cell comprising: introducing into the cell: (i) a composition comprising a ceDNA vector that comprises: flanking ITR sequences, where the ITR sequences are asymmetrical, symmetrical or substantially symmetrical relative to each other as defined herein, and a nucleic acid sequence encoding at least two guide RNAs complementary to two or more target genes, wherein the vector is a linear close-ended duplex DNA, (ii) a second composition comprising a ceDNA vector that comprises: flanking ITR sequences, where the ITR sequences are asymmetrical, symmetrical or

functional ITR. For example, if the replication competent ITR is from AAV serotype 2, the corresponding Rep would be from an AAV serotype that works with that serotype such as AAV2 ITR with AAV2 or AAV4 Rep but not AAV5 Rep, which does not. Upon replication, the covalently-closed ended ceDNA vector continues to accumulate in permissive cells and ceDNA vector is preferably sufficiently stable over time in the presence of Rep protein under standard replication conditions, e.g. to accumulate in an amount that is at least 1 pg/cell, preferably at least 2 pg/cell, preferably at least 3 pg/cell, more preferably at least 4 pg/cell, even more preferably at least 5 pg/cell.

[0082] Accordingly, one example of the disclosure relates to a process of producing a ceDNA vector for gene editing comprising the steps of: a) incubating a population of host cells (e.g. insect cells) harboring the polynucleotide expression construct template (e.g., a ceDNA-plasmid, a ceDNA-bacmid, and/or a ceDNA-baculovirus), which is devoid of viral capsid coding sequences, in the presence of a Rep protein under conditions effective and for a time sufficient to induce production of the ceDNA vector within the host cells, and wherein the host cells do not comprise viral capsid coding sequences; and b) harvesting and isolating the ceDNA vector from the host cells. The presence of Rep protein induces replication of the vector polynucleotide with a modified ITR to produce the ceDNA vector in a host cell. However, no viral particles (e.g. AAV virions) are expressed. Thus, there is no virion-enforced size limitation.

[0083] The presence of the ceDNA vector useful for gene editing is isolated from the host cells can be confirmed by digesting DNA isolated from the host cell with a restriction enzyme having a single recognition site on the ceDNA vector and analyzing the digested DNA material on denaturing and non-denaturing gels to confirm the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA.

[0084] Also provided herein in another aspect, is a method for inserting a nucleic acid sequence into a genomic safe harbor gene, the method comprising: contacting a cell with (i) a gene editing system and (ii) a homology directed repair template having homology to a genomic safe harbor gene and comprising a nucleic acid sequence encoding a protein of interest, wherein one or more components of the gene editing system are delivered to the cell by contacting the cell with a ceDNA vector composition as disclosed herein, wherein the ceDNA vector composition is a linear close-ended duplex DNA comprising flanking ITR sequences, where the ITR sequences are asymmetrical, symmetrical or substantially symmetrical relative to each other as defined herein, and at least one gene editing nucleic acid sequence having a region complementary to a genomic safe harbor gene, and wherein the method is performed under conditions and for a time sufficient to insert the nucleic acid sequence encoding the protein of interest into the genomic safe harbor gene.

[0085] In another example of this aspect and all other aspects provided herein, the genomic safe harbor gene comprises an active intron close to at least one coding sequence known to express proteins at a high expression level.

[0086] In another example of this aspect and all other aspects provided herein, the genomic safe harbor gene comprises a site in or near the albumin gene.

[0087] In another example of this aspect and all other aspects provided herein, the genomic safe harbor gene is the AAVS1 locus.

[0088] In another example of this aspect and all other aspects provided herein, the protein of interest is a receptor, a toxin, a hormone, an enzyme, or a cell surface protein. In another example of this aspect and all other aspects provided herein, the protein of interest is a receptor. In another example of this aspect and all other aspects provided herein, the protein of interest is a protease.

[0089] In another example of this aspect and all other aspects provided herein, exemplary nonlimiting genes to be targeted, or protein of interest can be, Factor VIII (FVIII) or Factor IX (FIX). In another example of this aspect and all other aspects provided herein, the method is performed *in vivo* for the treatment of hemophilia A, or hemophilia B. Uses of the gene editing ceDNA vectors as disclosed herein is discussed in the sections entitled "Exemplary diseases to be treated with a gene editing ceDNA" and "Additional diseases for gene editing" herein. Exemplary disease to be treated are, for example, but not limited to, Duchene Muscular Dystrophy (DMD gene), transthyretin amyloidosis (ATTR) (correct mutTTR gene), ornithine transcarbamylase deficiency (OTC deficiency), haemophilia, cystic fibrosis, sickle cell anemia, hereditary hemochromatosis, cancer, or hereditary blindness, and genes to be corrected, include but are not limited to; erythropoietin, angiostatin, endostatin, superoxide dismutase (SOD1), globin, leptin, catalase, tyrosine hydroxylase, a cytokine, cystic fibrosis transmembrane conductance regulator (CFTR), or a peptide growth factor, and the like.

[0090] In some examples, the present application may be defined in any of the following paragraphs:

1. A non-viral capsid-free close-ended DNA (ceDNA) vector comprising:

at least one heterologous nucleotide sequence between flanking inverted terminal repeats (ITRs), wherein at least one heterologous nucleotide sequence encodes at least one gene editing molecule.

2. The ceDNA vector of paragraph 1, wherein at least one gene editing molecule is selected from a nuclease, a guide RNA (gRNA), a guide DNA (gDNA), and an activator RNA.

3. The ceDNA vector of paragraph 2, wherein at least one gene editing molecule is a nuclease.

4. The ceDNA vector of paragraph 3, wherein the nuclease is a sequence specific nuclease.

CLAIMS

1. An *in vitro* method for genome editing of a chromosome in a host cell comprising:

contacting a cell with a) capsid-free closed ended DNA (ceDNA) vector comprising at least one nucleotide sequence between flanking inverted terminal repeats (ITRs), wherein the at least one nucleotide sequence encodes a Cas enzyme; and a guide RNA (gRNA); or

b) a ceDNA vector comprising at least one nucleotide sequence between flanking ITRs, wherein the at least one nucleotide sequence encodes a gRNA; and a Cas enzyme, wherein the chromosome is repaired via non-homologous end joining (NHEJ).

2. The method of claim 1, wherein the Cas enzyme is provided as an mRNA encoding the Cas enzyme.

3. The method of claim 1, wherein the Cas enzyme is provided in an expression vector encoding the Cas enzyme.

4. The method of claim 1, wherein the Cas enzyme is provided as a purified protein.

5. The method of claim 1, wherein the Cas enzyme is selected from the group consisting of: Cas9, nicking Cas9 (nCas9), and deactivated Cas (dCas).

6. The method of claim 1, wherein the Cas enzyme is a deactivated Cas nuclease (dCas) that complexes with the gRNA that targets a promoter region of a target gene.

7. The method of claim 1, wherein the gRNA targets a transcriptional start site of a target gene and activates the target gene (CRISPRa system).

8. The method of claim 1, wherein the gRNA comprises a first gRNA; or a first gRNA and a second gRNA.

9. The method of claim 1, wherein the gRNA is a single guide RNA (sgRNA).

10. The method of claim 1, wherein the gRNA targets a splice acceptor or splice donor site.

11. The method of claim 7, wherein the target gene encodes a therapeutic protein.

12. The method of claim 1, wherein:

at least one ITR comprises a functional terminal resolution site and a Rep binding site;
the flanking ITRs are symmetric or asymmetric;
one or more of the ITRs is not a wild type ITR;
at least one of the ITRs is modified by a deletion, insertion, and/or substitution in at least one of the ITR regions selected from the group consisting of A, A', B, B', C, C', D, and D', wherein the deletion, insertion, and/or substitution results in the deletion of all or part of a stem-loop structure normally formed by the A, A', B, B' C, and/or C' regions;
both ITRs are altered in a manner that results in an overall three-dimensional symmetry when the ITRs are inverted relative to each other, wherein the alteration is a deletion, insertion, and/or substitution in the ITR regions selected from the group consisting of A, A', B, B', C, C', D, and D'; and/or
one or more of the flanking ITRs are derived from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.

13. The method of claim 1, wherein one or more of the flanking ITRs are synthetic.

14. The method of claim 12, wherein the flanking ITRs are asymmetric, wherein at least one of the ITRs is altered from a wild-type AAV ITR sequence by a deletion, an addition, and/or a substitution that affects the overall three-dimensional conformation of the ITR.

15. The method of claim 1, wherein the host cell is a eukaryotic cell.

16. The method of claim 1, wherein the ceDNA is in a composition comprising a lipid.

17. The method of claim 16, wherein the lipid is a lipid nanoparticle.