gene editing therapy. The inventors have observed other limitations of current gene editing approaches relating to the various components such as nuclease(s), promoter(s) guide RNA(s) (if Cas9 is the nuclease), the 'corrected gene' donor template(s) (*e.g.,* a homology-directed recombination (HDR) repair template) and the separate delivery of homology regions. The current delivery of components is also problematic as components cannot be packaged in a single delivery particle and the use of multiple particles can raise immunogenicity issues. Since gene editing requires all the components are present within a single cell which is to be edited, the efficiency of gene editing is low as many cells do not get all of the delivered components.

[0012] Recombinant capsid-free AAV vectors can be obtained as an isolated linear nucleic acid molecule comprising an expressible transgene and promoter regions flanked by two wild-type AAV inverted terminal repeat sequences (ITRs) including the Rep binding and terminal resolution sites. These recombinant AAV vectors are devoid of AAV capsid protein encoding sequences, and can be single-stranded, double-stranded or duplex with one or both ends covalently linked through the two wild-type ITR palindrome sequences (e.g., WO2012/123430, U.S. Patent 9,598,703). They avoid many of the problems of AAV-mediated gene therapy in that the transgene capacity is much higher, transgene expression onset is rapid, and the patient immune system does recognize the DNA molecules as a virus to be cleared. However, constant expression of a transgene may not be desirable in all instances, and AAV canonical wild type ITRs may not be optimized for ceDNA function.

[0013] There is need in the field for a technology that allows precise targeting of nuclease activity (or other protein activities) to distinct locations within a target DNA in a manner that does not require the design of a new protein for each new target sequence. In addition, there is a need in the art for methods of controlling gene expression with minimal off-target effects, and there remains an important unmet need for controllable recombinant DNA vectors with improved production and/or expression properties.

~~BRIEF DESCRIPTION OF THE INVENTION~~SUMMARY

[0014] The ~~invention~~disclosure described herein is a non-viral capsid-free DNA vector with covalently-closed ends (referred to herein as a "closed-ended DNA vector" or a "ceDNA vector") for gene editing. The ceDNA vectors described herein are capsid-free, linear duplex DNA molecules formed from a continuous strand of complementary DNA with covalently-closed ends (linear, continuous and non-encapsidated structure), which comprise a 5' inverted terminal repeat (ITR) sequence and a 3' ITR sequence, where the 5' ITR and the 3' ITR can have the same symmetrical three-dimensional organization with respect to each other, (i.e., symmetrical or substantially symmetrical), or alternatively, the 5' ITR and the 3' ITR can have different three-dimensional organization with respect to each other (i.e., asymmetrical ITRs). In addition, the ITRs can be from the same or different serotypes. In some ~~embodiment~~examples, a ceDNA vector for gene editing can comprise ITR sequences that

have a symmetrical three-dimensional spatial organization such that their structure is the same shape in geometrical space, or have the same A, C-C' and B-B' loops in 3D space (i.e., they are the same or are mirror images with respect to each other). In such an ~~embodiment~~example, a symmetrical ITR pair, or substantially symmetrical ITR pair can both be modified ITRs (e.g., mod-ITRs) in the same manner and do not both have to be wild-type ITRs. A mod-ITR pair can have the same sequence which has one or more modifications from wild-type ITR and are reverse complements (inverted) of each other. In alternative ~~embodiment~~examples, a modified ITR pair are substantially symmetrical as defined herein, that is, the modified ITR pair can have a different sequence but have corresponding or the same symmetrical three-dimensional shape. In some ~~embodiment~~examples, one ITR can be from one AAV serotype, and the other ITR can be from a different AAV serotype.

[0015] Accordingly, some ~~aspect~~examples of the technology described herein relate to a ceDNA vector for gene editing that comprise ITR sequences selected from any of: (i) at least one WT ITR and at least one modified AAV inverted terminal repeat (ITR) (e.g., asymmetric modified ITRs); (ii) two modified ITRs where the mod-ITR pair have a different three-dimensional spatial organization with respect to each other (e.g., asymmetric modified ITRs), or (iii) symmetrical or substantially symmetrical WT-WT ITR pair, where each WT-ITR has the same three-dimensional spatial organization, or (iv) symmetrical or substantially symmetrical modified ITR pair, where each mod-ITR has the same three-dimensional spatial organization. The ceDNA vectors disclosed herein can be produced in eukaryotic cells, thus devoid of prokaryotic DNA modifications and bacterial endotoxin contamination in insect cells.

[0016] More particularly, ~~embodiment~~examples of the ~~invention~~disclosure are based on methods and compositions comprising a gene editing ceDNA vector that can express a transgene which is a gene editing molecule in a host cell (*e.g.,* a transgene is a nuclease such as ZFN, TALEN, Cas; one or more guide RNA; CRISPR; a ribonucleoprotein (RNP), or any combination thereof) and result in efficient genome editing. The ceDNA vectors described herein are not limited by size, thereby permitting, for example, expression of all of the components necessary for a gene editing system from a single vector (*e.g.,* a CRISPR/Cas gene editing system (*e.g.,* a Cas9 or modified Cas9 enzyme, a guide RNA and/or a homology directed repair template), or for a TALEN or Zinc Finger system). However, it is also contemplated that one or two of such components encoded on a single ceDNA vector, while the remaining component(s) can be expressed on a separate ceDNA vector or a traditional plasmid.

[0017] The technology described herein relates to a ceDNA vector containing two AAV inverted terminal repeat sequences (ITR) flanking a transgene or heterologous nucleic acid, where the heterologous nucleic acid is a gene editing nucleic acid sequence. In all ~~aspect~~examples provided herein, the gene editing nucleic acid sequence encodes a gene editing molecule selected from the group consisting of: a sequence specific nuclease, one or more guide RNA, CRISPR/Cas, a

ribonucleoprotein (RNP), or deactivated CAS for CRISPRi or CRISPRa systems, or any combination thereof.

[0018] In some ~~embodiment~~examples, the ceDNA vector comprises: (1) an expression cassette comprising a cis-regulatory element, a promoter and at least one transgene (e.g., a gene editing molecule); or (2) a promoter operably linked to at least one transgene (e.g., a gene editing molecule), and (3) two self-complementary sequences, e.g., asymmetrical or symmetrical or substantially symmetrical ITRs as defined herein, flanking said expression cassette, wherein the ceDNA vector is not associated with a capsid protein. In some ~~embodiment~~examples, the ceDNA vector comprises two self-complementary sequences found in an AAV genome, where at least one ITR comprises an operative Rep-binding element (RBE) (also sometimes referred to herein as "RBS") and a terminal resolution site (trs) of AAV or a functional variant of the RBE, and one or more cis-regulatory elements operatively linked to a transgene. In some ~~embodiment~~examples, the ceDNA vector comprises additional components to regulate expression of the transgene (e.g., a gene editing molecule), for example, regulatory switches, which are described herein in the section entitled "Regulatory Switches" for controlling and regulating the expression of the transgene, and can include a regulatory switch, e.g., a kill switch to enable controlled cell death of a cell comprising a ceDNA vector.

[0019] In some ~~embodiment~~examples, a ceDNA vector for gene editing described herein can be used for knock-in of desired nucleic acid sequence. In particular, the methods and compositions described herein can be used to introduce a new nucleic acid sequence, correct a mutation of a genomic sequence or introduce a mutation into a target gene sequence in a host cell. Such methods can be referred to as "DNA knock-in systems."

[0020] In some ~~embodiment~~examples, a gene editing ceDNA vector disclosed herein comprises homology arms, e.g., at increase specificity of targeting to a target gene. Homology-directed repair (HDR) is a process of homologous recombination where a DNA template is used to provide the homology necessary for precise repair of a double-strand break (DSB) of insertion of the donor sequence of interest. For example, in one nonlimiting example, a ceDNA vector for gene editing can comprise a 5' and 3' homology arm to a specific gene, or target intergration site. In some ~~embodiment~~examples, a specific restriction site may be engineered 5' to the 5' homology arm, 3' to the 3' homology arm, or both. When the ceDNA vector is cleaved with the one or more restriction endonucleases specific for the engineered restriction site(s), the resulting cassette comprises the 5' homology arm-donor sequence-3' homology arm, and can be more readily recombined with the desired genomic locus. In some ~~embodiment~~examples, in the genomic DNA sequence to be targeted, located 5' of, and near to where the 5' end of the 3' homology arm homologous, and/or located 3' of, and near to where the 3' end of the 5' homology arm is homologous, there is a sgRNA target sequence (e.g., see **FIG. 17 and 18A**). It will be appreciated by one of ordinary skill in the art that this cleaved cassette may additionally comprise

other elements such as, but not limited to, one or more of the following: a regulatory region, a nuclease, and an additional donor sequence. In certain ~~aspect~~examples, the ceDNA vector itself may encode the restriction endonuclease such that upon delivery of the ceDNA vector to the nucleus, the restriction endonuclease is expressed and able to cleave the vector. In certain ~~aspect~~examples, the restriction endonuclease is encoded on a second ceDNA vector which is separately delivered. In certain ~~aspect~~examples, the restriction endonuclease is introduced to the nucleus by a non-ceDNA-based means of delivery. In certain ~~embodiment~~examples, the restriction endonuclease is introduced after the ceDNA vector is delivered to the nucleus. In certain ~~embodiment~~examples, the restriction endonuclease and the ceDNA vector are transported to the nucleus simultaneously. In certain ~~embodiment~~examples, the restriction endonuclease is already present upon introduction of the ceDNA vector.

[0021] Accordingly, in some ~~embodiment~~examples, the technology described herein enables more than one gene editing ceDNA being delivered to a subject. As discussed herein, in one ~~embodiment~~example, a ceDNA can have the homology arms flanking a donor sequence that targets a specific target gene or locus, and can in some ~~embodiment~~examples, also include one or more guide RNAs (e.g., sgRNA) for targeting the cutting of the genomic DNA, as described herein, and another ceDNA can comprise a nuclease enzyme and activator RNA, as described herein for the actual gene editing steps.

[0022] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the sequence-specific nuclease comprises: a TAL-nuclease, a zinc-finger nuclease (ZFN), a meganuclease, a megaTAL, or an RNA guided endonuclease (*e.g.,* CAS9, cpf1, dCAS9, nCAS9).

[0023] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the gene editing nucleic acid sequence is a homology-directed repair template.

[0024] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the homology-directed repair template comprises a 5' homology arm, a donor sequence, and a 3' homology arm.

[0025] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the composition further comprises a nucleic acid sequence that encodes an endonuclease, wherein the endonuclease cleaves or nicks at a specific endonuclease site on DNA of a target gene or a target site on the ceDNA vector.

[0026] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the 5' homology arm is homologous to a nucleotide sequence upstream of the DNA endonuclease cutting or nicking site on a chromosome.

[0027] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the 3' homology arm is homologous to a nucleotide sequence downstream of the DNA endonuclease cutting or nicking site.

[0028] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the homology arms are each about 250 to 2000bp.

[0029] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the DNA endonuclease comprises: a TAL-nuclease, a zinc-finger nuclease (ZFN), or an RNA guided endonuclease (*e.g.,* Cas9 or Cpf1).

[0030] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the RNA guided endonuclease comprises a Cas enzyme.

[0031] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the Cas enzyme is Cas9.

[0032] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the Cas enzyme is nicking Cas9 (nCas9).

[0033] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the nCas9 comprises a mutation in the HNH or RuVc domain (e.g. D10A) of Cas.

[0034] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the Cas enzyme is deactivated Cas nuclease (dCas) that complexes with a gRNA that targets a promoter region of a target gene.

[0035] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the composition further comprises a KRAB effector domain.

[0036] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the dCas is fused to a heterologous transcriptional activation domain that can be directed to a promoter region.

[0037] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the dCas fusion is directed to a promoter region of a target gene by a guide RNA that recruits additional transactivation domains to upregulate expression of the target gene.

[0038] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the dCas is *S. pyogenes* dCas9.

[0039] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the guide RNA sequence targets the proximity of the promoter of a target gene and CRISPR silences the target gene (CRISPRi system). As used herein, the phrase "proximity of the promoter of a target gene" refers to a region that is physically on, adjacent or near the promoter sequence of the target gene and a catalytically inactive DNA endonuclease can function to inhibit expression of the target gene. In some ~~embodiment~~examples, "proximity to the promoter" refers to a sequence within the promoter sequence itself, directly adjacent to the promoter sequence (either end) or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 nucleotides or more from a terminal end of the promoter sequence.

[0040] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the guide RNA sequence targets the transcriptional start site of a target gene and activates, or modulates, the target gene (CRISPRa system). As used herein, the term "transcriptional start site of a target gene" refers to a region that is physically on, adjacent or near the transcriptional start sequence ("ATG"; initiating

methionine) of the target gene and a catalytically inactive DNA endonuclease can function to recruit transcriptional machinery, such as RNA polymerase, to increase expression of the target gene, for example, by at least 10%. In some ~~embodiment~~examples, the guide RNA may comprise a sequence that includes the "ATG" transcriptional start site. In other ~~embodiment~~examples, the guide RNA may comprise a sequence directly upstream of the transcriptional start site. In additional ~~embodiment~~examples, the guide RNA can comprise a sequence 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 nucleotides or more upstream of the transcriptional start site, provided that the distance is not so large that the recruited translational machinery does not function to enhance expression of the target gene.

[0041] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the guide RNA sequence targets the proximity of a promoter of a target gene and activates, or modulates, the target gene (CRISPRa system), for example, to increase expression of the target gene.

[0042] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the composition further comprises a nucleic acid encoding at least one guide RNA (gRNA) for a RNA-guided DNA endonuclease.

[0043] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the guide RNA (gRNA) targets a splice acceptor or splice donor site of a defective gene to effect non-homologous end joining (NHEJ) and correction of the defective gene for expression of functional protein. The term "splice acceptor" as used herein refers to a nucleic acid sequence at the 3' end of an intron where it junctions with an exon. The consensus sequences for a splice acceptor include, but are not limited to: NTN(TC) (TC) (TC)TTT (TC) (TC)(TC) (TC) (TC) (TC)NCAGg (SEQ ID NO: 558). The intronic sequences are represented by upper case and the exonic sequence by lower case font. The term "splice donor" as used herein refers to a nucleic acid sequence at the 5' end of an intron where it junctions with an exon. The consensus sequence for a splice donor sequence includes, but is not limited to: naggt(ag)aGT (SEQ ID NO: 559). The intronic sequences are represented by upper case and the exonic sequence by lower case font. Theses sequences represent those of which are conserved from viral to primate genomes.

[0044] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the vector encodes multiple copies of one guide RNA sequence.

[0045] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the composition further comprises a regulatory sequence operably linked to the nucleic acid sequence encoding the gene editing sequence.

[0046] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the regulatory sequence comprises an enhancer and/or a promoter. In certain ~~embodiment~~examples the promoter is an inducible promoter.

[0047] In another ~~embodiment~~example of this aspect and all other aspects provided herein, a promoter is operably linked to the nucleic acid sequence encoding the DNA endonuclease, wherein the nucleic

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 ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~example of this aspect and all other aspects provided herein, the nucleotide sequence encoding a nuclease is cDNA.

[0052] In another ~~embodiment~~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 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 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. ~~Another aspect~~ For example, provided herein ~~relates to~~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 ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~example of this aspect and all other aspects provided herein, a single ceDNA vector comprises all components of the gene editing system.

[0057] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the Cas protein is codon optimized for expression in the eukaryotic cell.

[0058] Also provided herein, in another ~~aspect~~ example is a method of genome editing comprising administering to a cell an effective amount of a ceDNA composition as described herein, under conditions suitable and for a time sufficient to edit a target gene.

[0059] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the target gene is targeted using one or more guide RNA sequences and edited by homology directed repair (HDR) in the presence of a HDR donor template.

[0060] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the target gene is targeted using one guide RNA sequence and the target gene is edited by non-homologous end joining (NHEJ). In one ~~embodiment~~example, the guide RNA targets a splice donor or acceptor to promote exon skipping and expression of functional protein, e.g. dystrophin protein.

[0061] In another ~~embodiment~~example of this aspect and all other aspects provided herein, the method is performed *in vivo* to correct a single nucleotide polymorphism (SNP), or deletion or insertion, associated with a disease.

[0062] In another ~~embodiment~~example of this aspect and all other aspects provided herein, a disease suitable for gene editing using the ceDNA vectors 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.

[0063] In another ~~embodiment~~example of this aspect and all other aspects provided herein, at least 2 different Cas proteins are present in the ceDNA vector, wherein one of the Cas proteins is catalytically inactive (Cas-i), and wherein the guide RNA associated with the Cas-I targets the promoter of the target cell, and wherein the DNA coding for the Cas-I is under the control of an inducible promoter so that it can turn-off the expression of the target gene at a desired time. As used herein, the term "catalytically inactive" refers to a molecule (e.*g.,* an enzyme or a kinase) with a catalytic site that has been altered from an active state to an inactive state, thereby hindering its activity. A molecule can be rendered catalytically inactive for example, from denaturation, inhibitory binding, mutations to the catalytic site, or secondary processing (*e.g.,* phosphorylation or other post-translational modifications). For example, a catalytically inactive, or deactivated Cas9 (dCas9), does not possess endonuclease activity and can be generated, for example, by introducing point mutations in the two catalytic residues, D10A

and H840A, of the gene encoding Cas9. In one ~~embodiment~~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 an *in vitro* 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 non-viral capsid-free close-ended DNA (ceDNA) vector composition, and wherein the Cas protein expressed from the ceDNA 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. In one example, the present disclosure provides 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 ~~embodiment~~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 ~~embodiment~~example of this aspect and all other aspects provided herein, the catalytically inactive Cas protein expressed from the vector is dCas9.

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

[0068] In another ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~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 an *in vitro* method of modulating expression of two or more target genes in a cell comprising: introducing into the cell: (i) a first composition comprising a vector that comprises: flanking terminal repeat (TR) sequences, and a nucleic acid sequence encoding at least two guide RNAs complementary to two or more target genes, wherein the vector is a non-viral capsid free closed ended DNA (ceDNA) vector, (ii) a second composition comprising a vector that comprises: flanking terminal repeat (TR) sequences and a nucleic acid sequence encoding at least two catalytically inactive DNA endonucleases that each associate with a guide RNA and bind to the two or more target genes, wherein the vector is a non-viral capsid free closed ended DNA (ceDNA) vector, and (iii) a third composition comprising a vector that comprises: flanking terminal repeat (TR) sequences, and a nucleic acid sequence encoding at least two transcriptional regulator proteins or domains, wherein the vector is a non-viral capsid free closed ended DNA (ceDNA) vector and wherein the at least two guide RNAs, the at least two catalytically inactive RNA-guided endonucleases and the at least two transcriptional regulator proteins or domains are expressed in the cell, wherein two or more co-localization complexes form between a guide RNA, a catalytically inactive RNA-guided endonuclease, a transcriptional regulator protein or domain and a target gene, and wherein the transcriptional regulator protein or domain regulates expression of the at least two target genes. In one example, the present disclosure provides 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

substantially symmetrical relative to each other as defined herein, and a nucleic acid sequence encoding at least two catalytically inactive DNA endonucleases that each associate with a guide RNA and bind to the two or more target genes, wherein the vector is a linear close-ended duplex DNA, and (iii) a third 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 transcriptional regulator proteins or domains, wherein the vector is a linear close-ended duplex DNA, and wherein the at least two guide RNAs, the at least two catalytically inactive RNA-guided endonucleases and the at least two transcriptional regulator proteins or domains are expressed in the cell, wherein two or more co-localization complexes form between a guide RNA, a catalytically inactive RNA-guided endonuclease, a transcriptional regulator protein or domain and a target gene, and wherein the transcriptional regulator protein or domain regulates expression of the at least two target genes.

[0072] In one ~~aspect~~example, non-viral capsid-free DNA vectors with covalently-closed ends are preferably linear duplex molecules, and are obtainable from a vector polynucleotide that encodes a heterologous nucleic acid operatively positioned between two inverted terminal repeat sequences (ITRs) (e.g. AAV ITRs), wherein at least one of the ITRs comprises a terminal resolution site and a replication protein binding site (RPS) (sometimes referred to as a replicative protein binding site), e.g. a Rep binding site. The 5' ITR and 3' ITR can be symmetrical or substantially symmetrical relative to each other where the 5' and 3' ITR have the same three-dimensional spatial organization (i.e., a symmetrical mod-ITR pair or a symmetrical or substantially symmetrical WT-ITR pair), or asymmetrical relative to each other such that the 5' ITR and the 3' ITR have different three-dimensional organization with respect to each other (i.e., asymmetrical ITRs) with respect to each other (e.g., a WT-ITR and a mod-ITR or a mod-ITR pair that , as these terms are defined herein.

[0073] In some ~~embodiment~~examples, the two self-complementary sequences can be ITR sequences from any known parvovirus, for example a dependovirus such as AAV (e.g., AAV1-AAV12). Any AAV serotype can be used, including but not limited to a modified AAV2 ITR sequence, that retains a Rep-binding site (RBS) such as 5´-GCGCGCTCGCTCGCTC-3´ (SEQ ID NO: 531) and a terminal resolution site (trs) in addition to a variable palindromic sequence allowing for hairpin secondary structure formation. In some ~~embodiment~~examples, the ITR is a synthetic ITR sequence that retains a functional Rep-binding site (RBS) such as 5'-GCGCGCTCGCTCGCTC-3' (SEQ ID NO: 531) and a terminal resolution site (TRS) in addition to a variable palindromic sequence allowing for hairpin secondary structure formation. In some examples, an ITR sequence retains the sequence of the RBS, trs and the structure and position of a Rep binding element forming the terminal loop portion of one of the ITR hairpin secondary structure from the corresponding sequence of the wild-type AAV2 ITR.

[0074] In some ~~embodiment~~examples, a ceDNA vector comprising an asymmetric ITR pair can comprise a ITR with a modification in the ITR corresponding to any of the modifications in ITR sequences or ITR partial sequences shown in any one or more of Table 4A or 4B herein, or one or more of Tables 2, 3, 4, 5, 6, 7, 8, 9 and 10A-10B of PCT application PCT/US18/49996 which is incorporated herein in its entirety by reference. As an exemplary example, the present disclosure provides a closed-ended DNA vector for gene editing that comprises asymmetrical ITRs, the ceDNA vector comprising a promoter operably linked to a transgene, where the ceDNA is devoid of capsid proteins and is: (a) produced from a ceDNA-plasmid (e.g., see Examples 1-2 and/or **FIGS. 1A-1B**) that encodes a mutated right side AAV2 ITR having the same number of intramolecularly duplexed base pairs as SEQ ID NO:2 or a mutated left side AAV2 ITR having the same number of intramolecularly duplexed base pairs as SEQ ID NO:51 in its hairpin secondary configuration (preferably excluding deletion of any AAA or TTT terminal loop in this configuration compared to these reference sequences), and (b) is identified as ceDNA using the assay for the identification of ceDNA by agarose gel electrophoresis under native gel and denaturing conditions in Example 1. Examples of such 5' and 3' modified ITR sequences for ceDNA vector comprising asymmetric ITRs are provided in **Tables 4A or 4B** herein, or one or more of Tables 2, 3, 4, 5, 6, 7, 8, 9 and 10A-10B of PCT application PCT/US18/49996 which is incorporated herein in its entirety by reference.

[0075] Alternatively, in some ~~embodiment~~examples exemplary modified ITR sequences for use in a ceDNA vector that comprises symmetric modified ITRs, i.e., a ceDNA comprising a modified 5'ITR and a modified 3'ITR, where the modified 5'ITR and a modified 3'ITR are symmetrical or substantially symmetrical relative to each other are as shown in **Table 5**, which shows pairs of ITRs (modified 5' ITR and the symmetric modified 3' ITR). In some ~~embodiment~~examples, the symmetrical ITR-pair is a WT-WT ITR-pair which are shown in **Table** 2.

[0076] The technology described herein further relates to a ceDNA vector for gene editing, where the ceDNA vector comprises a heterologous nucleic acid expression cassette can comprise, e.g., more than 4000 nucleotides, 5000 nucleotides, 10,000 nucleotides or 20,000 nucleotides, or 30,000 nucleotides, or 40,000 nucleotides or 50,000 nucleotides, or any range between about 4000-10,000 nucleotides or 10,000-50,000 nucleotides, or more than 50,000 nucleotides. The ceDNA vectors do not have the size limitations of encapsidated AAV vectors, thus enable delivery of a large-size expression cassette to provide efficient expression of transgenes. In some ~~embodiment~~examples, the ceDNA vector is devoid of prokaryote-specific methylation.

[0077] The expression cassette can also comprise an internal ribosome entry site (IRES) and/or a 2A element. The cis-regulatory elements include, but are not limited to, a promoter, a riboswitch, an insulator, a mir-regulatable element, a post-transcriptional regulatory element, a tissue- and cell type-specific promoter and an enhancer. In some ~~embodiment~~examples the ITR can act as the promoter for the

transgene. In some ~~embodiment~~examples, the ceDNA vector comprises additional components to regulate expression of the transgene. For example, the additional regulatory component can be a regulator switch as disclosed herein, including but not limited to a kill switch, which can kill the ceDNA infected cell, if necessary, and other inducible and/or repressible elements.

[0078] The technology described herein further provides novel methods of gene editing using the ceDNA vectors. A ceDNA vector has the capacity to be taken up into host cells, as well as to be transported into the nucleus in the absence of the AAV capsid. In addition, the ceDNA vectors described herein lack a capsid and thus avoid the immune response that can arise in response to capsid-containing vectors.

[0079] ~~Aspects~~ Examples of the ~~invention~~disclosure relate to methods to produce the ceDNA vectors useful for gene editing as described herein. Other ~~embodiment~~examples relate to a ceDNA vector produced by the method provided herein. In one ~~embodiment~~example, the capsid free non-viral DNA vector (ceDNA vector) is obtained from a plasmid (referred to herein as a "ceDNA-plasmid") comprising a polynucleotide expression construct template comprising in this order: a first 5' inverted terminal repeat (e.g. AAV ITR); a heterologous nucleic acid sequence; and a 3' ITR (e.g. AAV ITR), where the 5' ITR and 3'ITR can be asymmetric relative to each other, or symmetric (e.g., WT-ITRs or modified symmetric ITRs) as defined herein.

[0080] The ceDNA vector disclosed herein is obtainable by a number of means that would be known to the ordinarily skilled artisan after reading this disclosure. For example, a polynucleotide expression construct template used for generating the ceDNA vectors of the present ~~invention~~disclosure can be a ceDNA-plasmid (e.g. see **Table 8** or **FIG. 7B),** a ceDNA-bacmid, and/or a ceDNA-baculovirus. In one ~~embodiment~~example, the ceDNA-plasmid comprises a restriction cloning site (e.g. SEQ ID NO: 7) operably positioned between the ITRs where an expression cassette comprising e.g., a promoter operatively linked to a transgene, e.g., a reporter gene and/or a therapeutic gene) can be inserted. In some ~~embodiment~~examples, ceDNA vectors are produced from a polynucleotide template (e.g., ceDNA-plasmid, ceDNA-bacmid, ceDNA-baculovirus) containing symmetric or asymmetric ITRs (modified or WT ITRs).

[0081] In a permissive host cell, in the presence of e.g., Rep, the polynucleotide template having at least two ITRs replicates to produce ceDNA vectors. ceDNA vector production undergoes two steps: first, excision ("rescue") of template from the template backbone (e.g. ceDNA-plasmid, ceDNA-bacmid, ceDNA-baculovirus genome etc.) via Rep proteins, and second, Rep mediated replication of the excised ceDNA vector. Rep proteins and Rep binding sites of the various AAV serotypes are well known to those of ordinary skill in the art. One of ordinary skill understands to choose a Rep protein from a serotype that binds to and replicates the nucleic acid sequence based upon at least one

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 ~~aspect~~example of the ~~invention~~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 an *in vitro* 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 non-viral capsid-free close-ended DNA (ceDNA) vector composition, wherein the ceDNA nucleic acid vector composition comprises 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, 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. In one example, the present disclosure provides 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 ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~example of this aspect and all other aspects provided herein, the genomic safe harbor gene is the AAVS1 locus.

[0088] In another ~~embodiment~~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 ~~embodiment~~example of this aspect and all other aspects provided herein, the protein of interest is a receptor. In another ~~embodiment~~example of this aspect and all other aspects provided herein, the protein of interest is a protease.

[0089] In another ~~embodiment~~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 ~~embodiment~~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.

In one aspect, the present disclosure provides a kit for inserting a donor sequence at an insertion site on a chromosome in a cell, comprising: a) a first non-viral capsid-free close-ended DNA (ceDNA) vector comprising: two AAV inverted terminal repeat (ITR); and a first nucleotide sequence comprising a 5' homology arm, a donor sequence, and a 3' homology arm, wherein the donor sequence has gene editing functionality; and b) a second ceDNA vector comprising: at least one AAV ITR; and a nucleotide sequence encoding at least one gene editing molecule, wherein in the first ceDNA vector, the 5' homology arm is homologous to a sequence upstream of a cleavage site for gene editing molecule on the chromosome and wherein the 3' homology arm is homologous to a sequence downstream of the gene editing molecule cleavage site on the chromosome; and wherein the 5' homology arm or the 3' homology arm are proximal to the ITR. In one further aspect, the present disclosure provides an *in vitro* method of inserting a donor sequence at a predetermined insertion site on a chromosome in a host cell, comprising: a) introducing into the host cell a first non-viral capsid-free close-ended DNA (ceDNA) vector having at least one inverted terminal repeat (ITR), wherein the ceDNA vector comprises a first linear nucleic acid comprising a 5' homology arm, a donor sequence, and a 3' homology arm; and b) introducing into the host cell a second ceDNA vector comprising 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 that cleaves the chromosome at or adjacent to the insertion site, wherein the donor sequence is inserted into the chromosome at or adjacent to the insertion site through homologous recombination.

[0090] In some ~~embodiment~~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.

176. A method of inserting a donor sequence at a predetermined insertion site on a chromosome in a host cell, comprising:

a) introducing into the host cell a first non-viral capsid-free close-ended DNA (ceDNA) vector having at least one inverted terminal repeat (ITR), wherein the ceDNA vector comprises a first linear nucleic acid comprising a 5' homology arm, a donor sequence, and a 3' homology arm; and

b) introducing into the host cell a second ceDNA vector comprising 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 that cleaves the chromosome at or adjacent to the insertion site, wherein the donor sequence is inserted into the chromosome at or adjacent to the insertion site through homologous recombination.

177. The method of paragraph 176, wherein the gene editing molecule is a nuclease.

178. The method of paragraph 177, wherein the nuclease is a sequence specific nuclease.

179. The method of any of paragraphs 176-178, wherein the first ceDNA vector is a ceDNA vector of any of paragraphs 1, 40-56, 57-69.

180. The method of any of paragraphs 176-179, wherein the second ceDNA vector is a ceDNA vector of any of paragraphs 1-39 or paragraphs 57-69.

181. The method of any of paragraphs 179-180, wherein the second ceDNA vector further comprises a third nucleotide sequence encoding a guide sequence recognizing the insertion site.

182. A cell containing a ceDNA vector of any of paragraphs 1-69.

183. A composition comprising a vector of any of paragraphs 1-69 and a lipid.

184. The composition of paragraph 184, wherein the lipid is a lipid nanoparticle (LNP).

185. A kit comprising a composition of paragraph 183 or 184 or a cell of paragraph 182.

[0091] In some ~~embodiment~~examples, one ~~aspect~~ example of the technology described herein relates to a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises at least one heterologous nucleotide sequence, operably positioned between asymmetric inverted terminal repeat sequences (asymmetric ITRs), wherein at least one of the asymmetric ITRs comprises a functional terminal resolution site and a Rep binding site, and optionally the heterologous nucleic acid sequence encodes a transgene, and wherein the vector is not in a viral capsid.

[0092] These and other aspects of the ~~invention~~disclosure are described in further detail below.

CLAIMS

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 claim 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 claim 2, wherein at least one gene editing molecule is a nuclease.

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

5. The ceDNA vector of claim 4, wherein the sequence specific nuclease is selected from a nucleic acid-guided nuclease, zinc finger nuclease (ZFN), a meganuclease, a transcription activator-like effector nuclease (TALEN), or a megaTAL.

6. The ceDNA vector of claim 5, wherein the sequence specific nuclease is a nucleic acid-guided nuclease selected from a single-base editor, an RNA-guided nuclease, and a DNA-guided nuclease.

7. The ceDNA vector of claim 2 or claim 6, wherein at least one gene editing molecule is a gRNA or a gDNA.

8. The ceDNA vector of claim 2, 6 or 7, wherein at least one gene editing molecule is an activator RNA.

9. The ceDNA of any one of claims 6-8, wherein the nucleic acid-guided nuclease is a CRISPR nuclease.

10. The ceDNA vector of claim 9, wherein the CRISPR nuclease is a Cas nuclease.

11. The ceDNA vector of claim 10, wherein the Cas nuclease is selected from Cas9, nicking Cas9 (nCas9), and deactivated Cas (dCas).

12. The ceDNA vector of claim 11, wherein the nCas9 contains a mutation in the HNH or RuVc domain of Cas.

13. The ceDNA vector of claim 11, wherein the Cas nuclease is a deactivated Cas nuclease (dCas) that complexes with a gRNA that targets a promoter region of a target gene.

14. The ceDNA vector of claim 13, further comprising a KRAB effector domain.

15. The ceDNA vector of claim 13 or claim 14, wherein the dCas is fused to a heterologous transcriptional activation domain that can be directed to a promoter region.

16. The ceDNA vector of claim 15, wherein the dCas fusion is directed to a promoter region of a target gene by a guide RNA that recruits additional transactivation domains to upregulate expression of the target gene.

17. The ceDNA vector of any one of claims 13-16, wherein the dCas is *S. pyogenes* dCas9.

18. The ceDNA vector of any one of claims 7-17, wherein the guide RNA sequence targets the promoter of a target gene and CRISPR silences the target gene (CRISPRi system).

19. The ceDNA vector of any one of claims 7-17, wherein the guide RNA sequence targets the transcriptional start site of a target gene and activates the target gene (CRISPRa system).

20. The ceDNA vector of any one of claims 6-19, wherein the at least one gene editing molecule comprises a first guide RNA and a second guide RNA.

21. The ceDNA vector of any one of claims 7-20, wherein the gRNA targets a splice acceptor or splice donor site.

22. The ceDNA vector of claim 21, wherein targeting the splice acceptor or splice donor site effects non-homologous end joining (NHEJ) and correction of a defective gene.

23. The ceDNA vector of any one of claims 7-22, wherein the vector encodes multiple copies of one guide RNA sequence.

24. The ceDNA vector of any one of claims 1-23, wherein a first heterologous nucleotide sequence comprises a first regulatory sequence operably linked to a nucleotide sequence that encodes a nuclease.

25. The ceDNA vector of claim 24, wherein the first regulatory sequence comprises a promoter.

26. The ceDNA vector of claim 25, wherein the promoter is CAG, Pol III, U6, or H1.

27. The ceDNA vector of any one of claims 24-26, wherein the first regulatory sequence comprises a modulator.

28. The ceDNA vector of claim 27, wherein the modulator is selected from an enhancer and a repressor.

29. The ceDNA vector of any one of claims 24-28, wherein the first heterologous nucleotide sequence comprises an intron sequence upstream of the nucleotide sequence that encodes the nuclease, wherein the intron sequence comprises a nuclease cleavage site.

30. The ceDNA vector of any one of claims 1-29, wherein a second heterologous nucleotide sequence comprises a second regulatory sequence operably linked to a nucleotide sequence that encodes a guide RNA.

31. The ceDNA vector of claim 30, wherein the second regulatory sequence comprises a promoter.

32. The ceDNA vector of claim 31, wherein the promoter is CAG, Pol III, U6, or H1.

33. The ceDNA vector of any one of claims 30-32, wherein the second regulatory sequence comprises a modulator.

34. The ceDNA vector of claim 33, wherein the modulator is selected from an enhancer and a repressor.

~~35. The ceDNA vector of any one of claims 1-34, wherein a third heterologous nucleotide sequence comprises a third regulatory sequence operably linked to a nucleotide sequence that encodes an activator RNA.~~

~~36. The ceDNA vector of claim 35, wherein the third regulatory sequence comprises a promoter.~~

~~37. The ceDNA vector of claim 36, wherein the promoter is CAG, Pol III, U6, or H1.~~

~~38. The ceDNA vector of any one of claims 35-37, wherein the third regulatory sequence comprises a modulator.~~

~~39. The ceDNA vector of claim 38, wherein the modulator is selected from an enhancer and a repressor.~~

~~40. The ceDNA vector of any one of claims 1-39, wherein the ceDNA vector comprises a 5' homology arm and a 3' homology arm to a target nucleic acid sequence.~~

~~41. The ceDNA vector of claim 40, wherein the 5' homology arm and the 3' homology arm are each between about 250 to 2000 bp.~~

~~42. The ceDNA vector of claim 40 or claim 41, wherein the 5' homology arm and/or the 3' homology arm are proximal to an ITR.~~

~~43. The ceDNA vector of any one of claims 40-42, wherein at least one heterologous nucleotide sequence is between the 5' homology arm and the 3' homology arm.~~

~~44. The ceDNA vector of claim 43, wherein the at least one heterologous nucleotide sequence that is between the 5' homology arm and the 3' homology arm comprises a target gene.~~

~~45. The ceDNA vector of any one of claims 40-44, wherein the ceDNA vector at least one heterologous nucleotide sequence that encodes a gene editing molecule is not between the 5' homology arm and the 3' homology arm.~~

~~46. The ceDNA vector of claim 45, wherein none of the heterologous nucleotide sequences that encode gene editing molecules are between the 5' homology arm and the 3' homology arm.~~

~~47. The ceDNA vector of any one of claims 40-46, comprising a first endonuclease restriction site upstream of the 5' homology arm and/or a second endonuclease restriction site downstream of the 3' homology arm.~~

~~48. The ceDNA vector of claim 47, wherein the first endonuclease restriction site and the second endonuclease restriction site are the same restriction endonuclease sites.~~

~~49. The ceDNA vector of claim 47 or claim 48, wherein at least one endonuclease restriction site is cleaved by an endonuclease which is also encoded on the ceDNA vector.~~

~~50. The ceDNA vector of any one of claims 40-49, wherein further comprises one or more poly-A sites.~~

~~51. The ceDNA vector of any one of claims 40-50, comprising at least one of a transgene regulatory element and a poly-A site downstream and proximate to the 3' homology arm and/or upstream and proximate to the 5' homology arm.~~

~~52. The ceDNA vector of any one of claims 40-51, comprising a 2A and selection marker site upstream and proximate to the 3' homology arm.~~

~~53. The ceDNA vector of any one of claims 40-52, wherein the 5' homology arm is homologous to a nucleotide sequence upstream of a nuclease cleavage site on a chromosome.~~

~~54. The ceDNA vector of any one of claims 40-53, wherein the 3' homology arm is homologous to a nucleotide sequence downstream of a nuclease cleavage site on a chromosome.~~

~~55. The ceDNA vector of any one of claims 1-54, comprising a heterologous nucleotide sequence encoding an enhancer of homologous recombination.~~

~~56. The ceDNA vector of claim 55, wherein the enhancer of homologous recombination is selected from SV40 late polyA signal upstream enhancer sequence, the cytomegalovirus early enhancer element, an RSV enhancer, and a CMV enhancer.~~

~~57. The ceDNA vector of any one of claims 1-56, wherein at least one ITR comprises a functional terminal resolution site and a Rep binding site.~~

~~58. The ceDNA vector of any one of claims 1-57, wherein the flanking ITRs are symmetric or asymmetric.~~

~~59. The ceDNA vector of claim 58, 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, addition, or substitution that affects the overall three-dimensional conformation of the ITR.~~

~~60. The ceDNA vector of any one of claims 1-59, wherein at least one heterologous nucleotide sequence is cDNA.~~

~~61. The ceDNA vector of claims 1-60, wherein one or more of the flanking ITRs are derived from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.~~

~~62. The ceDNA vector of any one of claims 1-61, wherein one or more of the ITRs are synthetic.~~

~~63. The ceDNA vector of any one of claims 1-62, wherein one or more of the ITRs is not a wild type ITR.~~

~~64. The ceDNA vector of any one of claims 1-63, wherein one or more both of the ITRs is modified by a deletion, insertion, and/or substitution in at least one of the ITR regions selected from A, A', B, B', C, C', D, and D'.~~

~~65. The ceDNA vector of claim 64, 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, or C' regions.~~

~~66. The ceDNA vector of any one of claims 1-58 or 56-65, wherein the ITRs are symmetrical.~~

~~67. The ceDNA vector of any one of claims 1-58, 60, 61 and 66, wherein the ITRs are wild type.~~

~~68. The ceDNA vector of any one of claims 1-66, wherein both ITRs are altered in a manner that results in an overall three-dimensional symmetry when the ITRs are inverted relative to each other.~~

~~69. The ceDNA vector of claim 68, wherein the alteration is a deletion, insertion, and/or substitution in the ITR regions selected from A, A', B, B', C, C', D, and D'.~~

~~70.~~1. An *in vitro* 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 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.

~~71. The method of claim 70, wherein at least one gene editing molecule is selected from a nuclease, a guide RNA (gRNA), a guide DNA (gDNA), and an activator RNA.~~

~~72. The method of claim 71, wherein at least one gene editing molecule is a nuclease.~~

~~73. The method of claim 72, wherein the nuclease is a sequence specific nuclease.~~

~~74. The method of claim 73, wherein the sequence specific nuclease is selected from a nucleic acid-guided nuclease, zinc finger nuclease (ZFN), a meganuclease, a transcription activator-like effector nuclease (TALEN), or a megaTAL.~~

~~75. The method of claim 73, wherein the sequence specific nuclease is a nucleic acid-guided nuclease selected from a single-base editor, an RNA-guided nuclease, and a DNA-guided nuclease.~~

~~76. The method of claim 70 or 75, wherein at least one gene editing molecule is a gRNA or a gDNA.~~

~~77. The method of claim 70, 75 or 76, wherein at least one gene editing molecule is an activator RNA.~~

~~78. The method of any one of methods 74-77, wherein the nucleic acid-guided nuclease is a CRISPR nuclease.~~

~~79. The method of claim 78, wherein the CRISPR nuclease is a Cas nuclease.~~

~~80. The method of claim 79, wherein the Cas nuclease is selected from Cas9, nicking Cas9 (nCas9), and deactivated Cas (dCas).~~

81. The method of claim 80, wherein the nCas9 contains a mutation in the HNH or RuVc domain of Cas.

82. The method of claim 80, wherein the Cas nuclease is a deactivated Cas nuclease (dCas) that complexes with a gRNA that targets a promoter region of a target gene.

83. The method of claim 82, further comprising a KRAB effector domain.

84. The method of claim 82 or 83, wherein the dCas is fused to a heterologous transcriptional activation domain that can be directed to a promoter region.

85. The method of claim 84, wherein the dCas fusion is directed to a promoter region of a target gene by a guide RNA that recruits additional transactivation domains to upregulate expression of the target gene.

86. The method of any of claims 82-85, wherein the dCas is *S. pyogenes* dCas9.

87. The method of any of claims 78-86, wherein the guide RNA sequence targets the promoter of a target gene and CRISPR silences the target gene (CRISPRi system).

88. The method of any of claims 78-86, wherein the guide RNA sequence targets the transcriptional start site of a target gene and activates the target gene (CRISPRa system).

89. The method of any of claims 76-88, wherein the at least one gene editing molecule comprises a first guide RNA and a second guide RNA.

90. The method of any of claims 76-89, wherein the gRNA targets a splice acceptor or splice donor site.

91. The method of claim 22, wherein targeting the splice acceptor or splice donor site effects non-homologous end joining (NHEJ) and correction of a defective gene.

92. The method of claim 76-91, wherein the vector encodes multiple copies of one guide RNA sequence.

93. The method of any of claims 70-92, wherein a first heterologous nucleotide sequence comprises a first regulatory sequence operably linked to a nucleotide sequence that encodes a nuclease.

94. The method of claim 93, wherein the first regulatory sequence comprises a promoter.

95. The method of claim 94, wherein the promoter is CAG, Pol III, U6, or H1.

96. The method of any of claims 93-95, wherein the first regulatory sequence comprises a modulator.

97. The method of claim 96, wherein the modulator is selected from an enhancer and a repressor.

98. The method of any of claims 93-97, wherein the first heterologous nucleotide sequence comprises an intron sequence upstream of the nucleotide sequence that encodes the nuclease, wherein the intron sequence comprises a nuclease cleavage site.

99. The method of any of claims 70-98, wherein a second heterologous nucleotide sequence comprises a second regulatory sequence operably linked to a nucleotide sequence that encodes a guide RNA.

100. The method of claim 99, wherein the second regulatory sequence comprises a promoter.

101. The method of claim 100, wherein the promoter is CAG, Pol III, U6, or H1.

102. The method of any of claims 99-101, wherein the second regulatory sequence comprises a modulator.

103. The method of claim 102, wherein the modulator is selected from an enhancer and a repressor.

104. The method of any of claims 70-103, wherein a third heterologous nucleotide sequence comprises a third regulatory sequence operably linked to a nucleotide sequence that encodes an activator RNA.

105. The method of claim 104, wherein the third regulatory sequence comprises a promoter.

106. The method of claim 105, wherein the promoter is CAG, Pol III, U6, or H1.

107. The method of claim 104-106, wherein the third regulatory sequence comprises a modulator.

108. The method of claim 107, wherein the modulator is selected from an enhancer and a repressor.

109. The method of any of claims 70-108, wherein the ceDNA vector comprises a 5' homology arm and a 3' homology arm to a target nucleic acid sequence.

110. The method of claim 109, wherein the 5' homology arm and the 3' homology arm are each between about 250 to 2000 bp.

111. The method of claim 109 or 110wherein the 5' homology arm and/or the 3' homology arm are proximal to an ITR.

112. The method of any of claims 109-111, wherein at least one heterologous nucleotide sequence is between the 5' homology arm and the 3' homology arm.

113. The method of claim 112, wherein the at least one heterologous nucleotide sequence that is between the 5' homology arm and the 3' homology arm comprises a target gene.

114. The method of claim 109-113, wherein the ceDNA vector at least one heterologous nucleotide sequence that encodes a gene editing molecule is not between the 5' homology arm and the 3' homology arm.

115. The method of claim 114, wherein none of the heterologous nucleotide sequences that encode gene editing molecules are between the 5' homology arm and the 3' homology arm.

116. The method of claim 109-115, comprising a first endonuclease restriction site upstream of the 5' homology arm and/or a second endonuclease restriction site downstream of the 3' homology arm.

~~117. The method of claim 116, wherein the first endonuclease restriction site and the second endonuclease restriction site are the same restriction endonuclease sites.~~

~~118. The method of claim 116 or 117, wherein at least one endonuclease restriction site is cleaved by an endonuclease which is also encoded on the ceDNA vector.~~

~~119. The method of any of claims 109-118, wherein further comprises one or more poly-A sites.~~

~~120. The method of any of claims 109-119, comprising at least one of a transgene regulatory element and a poly-A site downstream and proximate to the 3' homology arm and/or upstream and proximate to the 5' homology arm.~~

~~121. The method of any of claims 109-120, comprising a 2A and selection marker site upstream and proximate to the 3' homology arm.~~

~~122. The method of any of claims 109-121, wherein the 5' homology arm is homologous to a nucleotide sequence upstream of a nuclease cleavage site on a chromosome.~~

~~123. The method of any of claims 109-122, wherein the 3' homology arm is homologous to a nucleotide sequence downstream of a nuclease cleavage site on a chromosome.~~

~~124. The method of any of claims 109-123, comprising a heterologous nucleotide sequence encoding an enhancer of homologous recombination.~~

~~125. The method of claim 124, wherein the enhancer of homologous recombination is selected from SV40 late polyA signal upstream enhancer sequence, the cytomegalovirus early enhancer element, an RSV enhancer, and a CMV enhancer.~~

~~126. The method of any of claims 70-125, wherein at least one ITR comprises a functional terminal resolution site and a Rep binding site.~~

~~127. The method of any of claims 70-126, wherein the flanking ITRs are symmetric or asymmetric.~~

~~128. The method of claim 127, 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, addition, or substitution that affects the overall three-dimensional conformation of the ITR.~~

~~129. The method of any of claims 70-128, wherein at least one heterologous nucleotide sequence is cDNA.~~

~~130. The method of any of claims 70-129, wherein one or more of the flanking ITRs are derived from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.~~

~~131. The method of any of claims 70-130, wherein one or more of the ITRs are synthetic.~~

~~132. The method of any of claims 70-131, wherein one or more of the ITRs is not a wild type ITR.~~

~~133. The method of any of claims 70-132, wherein one or more both of the ITRs is modified by a deletion, insertion, and/or substitution in at least one of the ITR regions selected from A, A', B, B', C, C', D, and D'.~~

~~134. The method of claim 133, 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, or C' regions.~~

~~135. The method of any of claims 70-127 or 129-134, wherein the ITRs are symmetrical.~~

~~136. The method of any one of claims 70-127 or 129-130, wherein the ITRs are wild type.~~

~~137. The method of any of claims 70-136, wherein both ITRs are altered in a manner that results in an overall three-dimensional symmetry when the ITRs are inverted relative to each other.~~

~~138. The method of claim 137, wherein the alteration is a deletion, insertion, and/or substitution in the ITR regions selected from A, A', B, B', C, C', D, and D'.~~

~~139. The method of any of claims 70-138, wherein the cell contacted is a eukaryotic cell.~~

~~140. The method of any of claims 84-139, wherein the CRISPR nuclease is codon optimized for expression in the eukaryotic cell.~~

~~141. The method of any of claims 84-140, wherein the Cas protein is codon optimized for expression in the eukaryotic cell.~~

~~142. A method of genome editing comprising administering to a cell an effective amount of a non-viral capsid-free closed-ended DNA (ceDNA vector) of any one of claims 1-69, under conditions suitable and for a time sufficient to edit a target gene.~~

~~143. The method of any of claims 113-142, wherein the target gene is gene targeted using one or more guide RNA sequences and edited by homology directed repair (HDR) in the presence of a HDR donor template.~~

~~144. The method of any of claims 142-143, wherein the target gene is targeted using one guide RNA sequence and the target gene is edited by non-homologous end joining (NHEJ).~~

~~145. The method of any of claims 70-144, wherein the method is performed *in vivo* to correct a single nucleotide polymorphism (SNP) associated with a disease.~~

~~146. The method of claim 145, wherein the disease comprises sickle cell anemia, hereditary hemochromatosis or cancer hereditary blindness.~~

~~147. The method of any of claims 70-146, wherein at least 2 different Cas proteins are present in the ceDNA vector, and wherein one of the Cas protein is catalytically inactive (Cas-i), and wherein the guide RNA associated with the Cas-I targets the promoter of the target cell, and wherein the DNA~~

~~coding for the Cas-I is under the control of an inducible promoter so that it can turn-off the expression of the target gene at a desired time.~~

~~148.~~2. An *in vitro* 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 non-viral capsid-free close-ended DNA (ceDNA) vector composition, and

wherein the Cas protein expressed from the ceDNA 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.

~~149. The method of claim 148, wherein the ceDNA vector is a ceDNA vector of any of claims 1-69.~~

~~150. The method of claim 148, wherein the base editing moiety comprises a single-strand-specific cytidine deaminase, a uracil glycosylase inhibitor, or a tRNA-adenosine deaminase.~~

~~151. The method of claim 148, wherein the catalytically inactive Cas protein is dCas9.~~

~~152. The method of any of claims 70-151, wherein the cell is a T cell, or $CD34^+$.~~

~~153. The method of any of claims 70-152, wherein the target gene encodes for a programmed death protein (PD1), cytotoxic T-lymphocyte-associated antigen 4(CTLA4), or tumor necrosis factor-α (TNF-α).~~

~~154. The method of any of claims 70-153, further comprising administering the cells produced to a subject in need thereof.~~

~~155. The method of claim 154, wherein the subject in need thereof has a genetic disease, viral infection, bacterial infection, cancer, or autoimmune disease.~~

~~156.~~3. An *in vitro* method of modulating expression of two or more target genes in a cell comprising: introducing into the cell:

(i) a first composition comprising a vector that comprises: flanking terminal repeat (TR) sequences, and a nucleic acid sequence encoding at least two guide RNAs complementary to two or more target genes, wherein the vector is a non-viral capsid free closed ended DNA (ceDNA) vector,

(ii) a second composition comprising a vector that comprises: flanking terminal repeat (TR) sequences and a nucleic acid sequence encoding at least two catalytically inactive DNA

endonucleases that each associate with a guide RNA and bind to the two or more target genes, wherein the vector is a non-viral capsid free closed ended DNA (ceDNA) vector, and

(iii) a third composition comprising a vector that comprises: flanking terminal repeat (TR) sequences, and a nucleic acid sequence encoding at least two transcriptional regulator proteins or domains, wherein the vector is a non-viral capsid free closed ended DNA (ceDNA) vectorand

wherein the at least two guide RNAs, the at least two catalytically inactive RNA-guided endonucleases and the at least two transcriptional regulator proteins or domains are expressed in the cell,

wherein two or more co-localization complexes form between a guide RNA, a catalytically inactive RNA-guided endonuclease, a transcriptional regulator protein or domain and a target gene, and

wherein the transcriptional regulator protein or domain regulates expression of the at least two target genes.

~~157. The method of claim 156, wherein the ceDNA vector of the first composition is a ceDNA vector of any of claims 1-69, the ceDNA vector of the second composition is a ceDNA vector of any of claims 1-69, and the third composition is a ceDNA vector of any of claims 1-69.~~

~~158.~~4. An *in vitro* 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 non-viral capsid-free close-ended DNA (ceDNA) vector composition, wherein the ceDNA nucleic acid vector composition comprises 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, 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.

~~159. The method of claim 158, wherein the ceDNA vector is a ceDNA vector of any of claims 1-69.~~

~~160. The method of claim 158, wherein 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.~~

~~161. The method of claim 158, wherein the genomic safe harbor gene comprises a site in or near any one of: the albumin gene, CCR5 gene, AAVS1 locus.~~

~~162. The method of any of claims 158-161, wherein the protein of interest is a receptor, a toxin, a hormone, an enzyme, or a cell surface protein.~~

~~163. The method of any of claims 162, wherein, the protein of interest is a secreted protein.~~

~~164. The method of claim 163, wherein the protein of interest comprises Factor VIII (FVIII) or Factor IX (FIX).~~

~~165. The method of claim 164, wherein the method is performed *in vivo* for the treatment of hemophilia A, or hemophilia B.~~

~~166. A method of inserting a donor sequence at a predetermined insertion site on a chromosome in a host cell, comprising: introducing into the host cell the ceDNA vector of claims 1-69, wherein the donor sequence is inserted into the chromosome at or adjacent to the insertion site through homologous recombination.~~

~~167. A method of generating a genetically modified animal comprising a donor sequence inserted at a predetermined insertion site on the chromosome of the animal, comprising a) generating a cell with the donor sequence inserted at the predetermined insertion site on the chromosome according to claim 167; and b) introducing the cell generated by a) into a carrier animal to produce the genetically modified animal.~~

~~168. The method of claim 167, wherein the cell is a zygote or a pluripotent stem cell.~~

~~169. A genetically modified animal generated by the method of claim 168.~~

~~170. The genetically modified animal of claim 169, wherein the animal is a non-human animal.~~

~~171.~~5. A kit for inserting a donor sequence at an insertion site on a chromosome in a cell, comprising: a) a first non-viral capsid-free close-ended DNA (ceDNA) vector comprising:

two AAV inverted terminal repeat (ITR); and

a first nucleotide sequence comprising a 5' homology arm, a donor sequence, and a 3' homology arm, wherein the donor sequence has gene editing functionality; and

~~(b)~~ b) a second ceDNA vector comprising:

at least one AAV ITR; and

a nucleotide sequence encoding at least one gene editing molecule,

wherein in the first ceDNA vector, the 5' homology arm is homologous to a sequence upstream of a cleavage site for gene editing molecule on the chromosome and wherein the 3' homology arm is homologous to a sequence downstream of the gene editing molecule

cleavage site on the chromosome; and wherein the 5' homology arm or the 3' homology arm are proximal to the ITR.

~~172. The method of claim 171, wherein the gene editing molecule is a nuclease.~~

~~173. The method of claim 172, wherein the nuclease is a sequence specific nuclease.~~

~~174. The method of any of claims 171-173, wherein the first ceDNA vector is a ceDNA vector of any of claims 1, 40-56, 57-69.~~

~~175. The method of any of claims 171-173, wherein the second ceDNA vector is a ceDNA vector of any of claims 1-39 or claims 57-69.~~

~~176.~~6. An *in vitro* method of inserting a donor sequence at a predetermined insertion site on a chromosome in a host cell, comprising:

a) introducing into the host cell a first non-viral capsid-free close-ended DNA (ceDNA) vector having at least one inverted terminal repeat (ITR), wherein the ceDNA vector comprises a first linear nucleic acid comprising a 5' homology arm, a donor sequence, and a 3' homology arm; and

b) introducing into the host cell a second ceDNA vector comprising 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 that cleaves the chromosome at or adjacent to the insertion site, wherein the donor sequence is inserted into the chromosome at or adjacent to the insertion site through homologous recombination.

~~177. The method of claim 176, wherein the gene editing molecule is a nuclease.~~

~~178. The method of claim 177, wherein the nuclease is a sequence specific nuclease.~~

~~179. The method of any of claims 176-178, wherein the first ceDNA vector is a ceDNA vector of any of claims 1, 40-56, 57-69.~~

~~180. The method of any of claims 176-179, wherein the second ceDNA vector is a ceDNA vector of any of claims 1-39 or claims 57-69.~~

~~181. The method of any of claims 179-180, wherein the second ceDNA vector further comprises a third nucleotide sequence encoding a guide sequence recognizing the insertion site.~~

~~182. A cell containing a ceDNA vector of any of claims 1-69.~~

~~183. A composition comprising a vector of any of claims 1-69 and a lipid.~~

~~184. The composition of claim 184, wherein the lipid is a lipid nanoparticle (LNP).~~

~~185. A kit comprising a composition of claim 183 or 184 or a cell of claim 182.~~