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 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 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 examples, one ITR can be from one AAV serotype, and the other ITR can be from a different AAV serotype.

[0014a] In one aspect, the present disclosure refers to an *in vitro* method of inserting a donor sequence at a predetermined insertion site on a chromosome in a host cell, comprising:
introducing into the host cell an mRNA encoding a Cas enzyme, a guide RNA (gRNA) and a composition comprising a lipid nanoparticle (LNP) comprising a capsid-free closed-ended DNA (ceDNA) vector;
wherein the ceDNA vector comprises at least one heterologous nucleotide sequence between flanking inverted terminal repeats (ITRs), wherein at least one heterologous nucleotide sequence is a gene editing nucleic acid sequence, wherein the gene editing nucleic acid sequence comprises a 5' homology arm, a donor sequence, and a 3' homology arm, wherein the donor sequence is inserted into the chromosome at or adjacent to the insertion site through homologous recombination. ~~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.~~

[0015] Accordingly, some 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, examples of the 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 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

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 example and all other examples provided herein, a poly-A-site is upstream and proximate to the 5' homology arm.
[0049] In another example of this example and all other examples provided herein, the donor sequence is foreign to the 5' homology arm or the 3' homology arm.
[0050] In another example of this example and all other examples 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 example and all other examples 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 example provided herein relates to 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.

[0053a] In another ~~aspect~~example, the present disclosure refers 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.
[0054] In another example of this example and all other examples 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 example and all other examples 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 example and all other examples provided herein, a single ceDNA vector comprises all components of the gene editing system.

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 example, 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.

[0084a] In another ~~aspect~~example, the present disclosure refers to 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.

[0089a] In another ~~aspect~~example, the present disclosure refers to 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.

[0089b] In another ~~aspect~~example, the present disclosure refers to use of a first ceDNA vector and a second ceDNA in the manufacture of a medicament for treating a genetic disease, wherein: a) the first non-viral capsid-free close-ended DNA (ceDNA) vector has 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) the second ceDNA vector comprises at least one heterologous nucleotide sequence between flanking inverted terminal repeats (ITRs), wherein the at least one heterologous nucleotide sequence encodes at least one gene editing molecule that cleaves a chromosome at or adjacent to a predetermined insertion site on the chromosome in a host cell, wherein a donor sequence is inserted into the chromosome at or adjacent to the insertion site through homologous recombination.

[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. 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.~~1. An *in vitro* method of inserting a donor sequence at a predetermined insertion site on a chromosome in a host cell, ~~for genome editing~~ comprising:

introducing into the host cell an mRNA encoding a Cas enzyme, a guide RNA (gRNA) and a composition comprising a lipid nanoparticle (LNP) comprising a capsid-free closed-ended DNA (ceDNA) vector; wherein ~~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~~the ~~(~~ceDNA~~)~~ vector comprises~~ing~~ at least one heterologous nucleotide sequence between flanking inverted terminal repeats (ITRs), wherein at least one heterologous nucleotide sequence is a ~~encodes at least one~~ gene editing nucleic acid sequence~~molecule~~, wherein the gene editing nucleic acid sequence comprises a 5' homology arm, a donor sequence, and a 3' homology arm, wherein the donor sequence is inserted into the chromosome at or adjacent to the insertion site through homologous recombination.

~~8. The method of claim 7, wherein at least one gene editing molecule is selected from a nuclease, a guide RNA (gRNA), a guide DNA (gDNA), and an activator RNA.~~

~~9. The method of claim 8, wherein at least one gene editing molecule is a nuclease.~~

~~10. The method of claim 9, wherein the nuclease is a sequence specific nuclease.~~

~~11. The method of claim 10, 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.~~

12. The method of claim 10, 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.

13. 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.

14. The method of claim 13, 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.

15. The method of claim 13, wherein the genomic safe harbor gene comprises a site in or near any one of: the albumin gene, CCR5 gene, AAVS1 locus.

16. 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.

17. The kit of claim 16, wherein the gene editing molecule is a nuclease.

18. The kit of claim 17, wherein the nuclease is a sequence specific nuclease.

19. Use of a first ceDNA vector and a second ceDNA in the manufacture of a medicament for treating a genetic disease, wherein:

a) the first non-viral capsid-free close-ended DNA (ceDNA) vector has 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) the second ceDNA vector comprises at least one heterologous nucleotide sequence between flanking inverted terminal repeats (ITRs), wherein the at least one heterologous nucleotide sequence encodes at least one gene editing molecule that cleaves a chromosome at or adjacent to a predetermined insertion site on the chromosome in a host cell, wherein a donor sequence is inserted into the chromosome at or adjacent to the insertion site through homologous recombination.~~

~~20. The use of claim 19, wherein the gene editing molecule is a nuclease.~~

~~21. The use of claim 20, wherein the nuclease is a sequence specific nuclease.~~

2. The method of claim 1, wherein the donor sequence is promoterless.

3. The method of claim 1, wherein the donor sequence is a repair template.

4. The method of claim 1, wherein the donor sequence is a polynucleotide for insertion into a host cell genome.

5. The method of claim 4, wherein the donor sequence is a polynucleotide that is to be inserted into a host cell genome to manufacture a therapeutic protein.

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

7. The method of claim 6, wherein the nCas9 contains a mutation in the HNH or RuVc domain of Cas.

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

9. The method of claim 8, further comprising a KRAB effector domain.

10. The method of claim 1, wherein the guide RNA sequence targets the transcriptional start site of a target gene and activates a target gene.

11. The method of claim 1, wherein the gRNA comprises a first guide RNA and a second guide RNA.

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

13. The method of claim 12, wherein targeting the splice acceptor or splice donor site effects non-homologous end joining (NHEJ) and correction of a defective gene.

14. The method of any one of the previous claims, wherein the homology arms are each about 250 bp to 2000 bp.

15. The method of claim 1, wherein the gRNA and/or the Cas enzyme are delivered *in trans*.