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 gRNA and/or the Cas enzyme are delivered *in trans*; wherein the ceDNA vector comprises at least one nucleotide sequence between flanking inverted terminal repeats (ITRs), wherein the at least one 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.

[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

CLAIMS

1. 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 gRNA and/or the Cas enzyme are delivered *in trans*;

wherein the ceDNA vector comprises at least one nucleotide sequence between flanking inverted terminal repeats (ITRs), wherein the at least one 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.

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 the group consisting of: 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, wherein the dCas further comprises 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 250 bp to 2000 bp long.