SUMMARY

[0012] The present disclosure provides, generally, non-viral capsid-free DNA vectors with covalently-closed ends (referred to herein as a "closed-ended DNA vector" or a "ceDNA vector"). 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 that are the same, but to the reverse complement of each other, *i.e.* the sequences are symmetrical or substantially symmetrical. According to some embodiments, the ceDNA vector comprises at least one heterologous nucleotide sequence operably positioned between two flanking inverted terminal repeat sequences (ITRs), wherein all or part of the heterologous nucleotide sequence is under the control of at least one regulatory switch. In other embodiments, the technology described herein relates to a ceDNA vector containing two modified AAV inverted terminal repeat sequences (ITRs), where the modified ITRs are symmetrical relative to each other and flank an expressible transgene. The ceDNA vectors disclosed herein can be produced in eukaryotic cells, thus devoid of prokaryotic DNA modifications and bacterial endotoxin contamination in insect cells.

[0012a] The present disclosure provides 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 two flanking symmetric inverted terminal repeats (symmetric ITRs), wherein the symmetric ITRs are not wild-type ITRs, and wherein each of the flanking symmetric ITRs is modified by a deletion, an insertion, and/or a substitution in the A' and/or A region, wherein the one of the flanking symmetric ITRs comprises a sequence having at least 95% identity to SEQ ID NO:497 (ITR-46 left), wherein the other flanking symmetric ITR comprises a sequence having 95% identity to SEQ ID NO: 481 (ITR-30, right), and wherein each flanking ITR has the same symmetrical modification.

[0013] In one aspect, 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 symmetrical modified inverted terminal repeat sequences (*e.g.*, mod-ITRs) (*e.g.* AAV ITRs). That is, both ITRs have the same sequence, but are reverse complements (inverted) of each other. In alternative embodiments, the 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. For example, one modified ITR can be from one serotype and the other modified ITR be from a different serotype, but they have the same mutation (*e.g.*, nucleotide insertion, deletion or substitution) in the same region. Stated differently, for illustrative purposes only, a 5' mod-ITR can be from AAV2 and have a deletion in the C region, and the 3' mod-ITR can be from AAV5 and have the corresponding deletion in the C'

region, and provided the 5'mod-ITR and the 3' mod-ITR have the same or symmetrical three-dimensional spatial organization, they are encompassed for use herein as a modified ITR pair.

[0014] In some embodiments, a modified ITR pair comprises at least one or any combination of a deletion, insertion, or substitution relative to wild type ITR sequence from the same AAV serotype. The additions, deletions, or substitutions in the symmetrical ITR are the same but the reverse complement of each other. For example, an insertion of 3 nucleotides in the C region of the 5' ITR would be reflected in the insertion of 3 reverse complement nucleotides in the corresponding section in the C' region of the 3' ITR. Solely for illustration purposes only, if the addition is AACG in the 5' ITR, the addition is CGTT in the 3' ITR at the corresponding site. For example, if the 5' ITR sense strand is ATC**GA**TCG with an addition of *AACG* between the G and A to result in the sequence

According to some embodiments, the ITRs are based on sequences from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12. According to some embodiments, the vector is in a nanocarrier. According to some embodiments, the nanocarrier comprises a lipid nanoparticle (LNP).

[0036] According to some embodiments of the aspects and embodiments herein, the ceDNA vector is obtained from a process comprising the steps of: (a) incubating a population of insect cells harboring a ceDNA expression construct in the presence of at least one Rep protein, wherein the ceDNA expression construct encodes the ceDNA vector, under conditions effective and for a time sufficient to induce production of the ceDNA vector within the insect cells; and (b) isolating the ceDNA vector from the insect cells. According to some embodiments, the ceDNA expression construct is selected from a ceDNA plasmid, a ceDNA bacmid, and a ceDNA baculovirus. According to some embodiments, the insect cell expresses at least one Rep protein**.** According to some embodiments, the at least one Rep protein is from a virus selected from a parvovirus, a dependovirus, and an adeno-associated virus (AAV). According to some embodiments, the at least one Rep protein is from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.

[0037] According to some embodiments, the disclosure provides a ceDNA expression construct that encodes the ceDNA vector of any of the aspects and embodiments herein. According to some embodiments, the construct is a ceDNA plasmid, ceDNA bacmid, or ceDNA baculovirus.

[0038] According to some embodiments, the disclosure provides a host cell comprising the ceDNA expression construct of any of the aspects or embodiments therein. According to some embodiments, the host cell expresses at least one Rep protein. According to some embodiments, the at least one Rep protein is from a virus selected from a parvovirus, a dependovirus, and an adeno-associated virus (AAV). According to some embodiments, the at least one Rep protein is from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12. According to some embodiments, the host cell is an insect cell. According to some embodiments, the insect cell is an Sf9 cell.

[0039] According to some embodiments, the disclosure provides a method of producing a ceDNA vector, comprising (a) incubating the host cell of any of the aspects and embodiments herein, under conditions effective and for time sufficient to induce production of the ceDNA vector; and (b) isolating the ceDNA from the host cells.

[0039a] According to some embodiments, the disclosure provides a use of the ceDNA vector as described herein in the manufacture of a medicament for treating, preventing, ameliorating, monitoring, or diagnosing a disease or disorder in a subject.

[0039b] According to some embodiments, the disclosure provides a use of the ceDNA vector as described herein in the manufacture of a medicament for delivering a therapeutic protein to a subject, wherein the at least one heterologous nucleic acid sequence encodes a therapeutic protein.

[0040] According to some embodiments, the disclosure provides a method for treating, preventing, ameliorating, monitoring, or diagnosing a disease or disorder in a subject, the method comprising: administering to a subject in need thereof, a composition comprising the ceDNA vector of any of the aspects and embodiments herein, wherein the at least one heterologous nucleotide sequence is selected to treat, prevent, ameliorate, diagnose, or monitor the disease or disorder.

and vascular or "wet" macular degeneration, glaucoma, uveitis, retinitis pigmentosa, Stargardt, Leber Congenital Amaurosis (LCA), Usher syndrome, pseudoxanthoma elasticum (PXE), x-linked retinitis pigmentosa (XLRP), x-linked retinoschisis (XLRS), Choroideremia, Leber hereditary optic neuropathy (LHON), Archomatopsia, cone-rod dystrophy, Fuchs endothelial corneal dystrophy, diabetic macular edema and ocular cancer and tumors. According to some embodiments, the blood disease or disorder is selected from the group consisting of hemophilia A, hemophilia B, thalassemia, anemia, and blood cancers. According to some embodiments, the liver disease or disorder is selected from the group consisting of progressive familial intrahepatic cholestasis (PFIC) and liver cancer, and tumors. According to some embodiments, the disease or disorder is cystic fibrosis. According to some embodiments, the ceDNA vector is administered in combination with a pharmaceutically acceptable carrier.

[0041] According to some embodiments, the disclosure provides a method for delivering a therapeutic protein to a subject, the method comprising administering to the subject a composition comprising the ceDNA vector of any of the aspects and embodiments herein, wherein the at least one heterologous nucleotide sequence encodes a therapeutic protein. According to some embodiments, the therapeutic protein is a therapeutic antibody. According to some embodiments, the therapeutic protein is selected from the group consisting of an enzyme, erythropoietin, angiostatin, endostatin, superoxide dismutase, globin, leptin, catalase, tyrosine hydroxylase, a cytokine, cystic fibrosis transmembrane conductance regulator (CFTR), a peptide growth factor, and a hormone.

[0042] According to some embodiments, the disclosure provides a kit comprising a ceDNA vector of any of the aspects and embodiments herein, and a nanocarrier, packaged in a container with a packet insert.

[0042a] According to some embodiments, the disclosure provides a kit comprising a ceDNA vector as described herein, and a nanocarrier, packaged in a container with instructions for use.

[0042b] According to some embodiments, the disclosure provides the ceDNA vector as described herein for use in therapy.

[0043] According to some aspects, the disclosure provides a kit for producing a ceDNA vector, the kit comprising an expression construct comprising at least one restriction site for insertion of at least one heterologous nucleotide sequence, or regulatory switch, or both, the at least one restriction site operatively positioned between either (i) symmetric inverted terminal repeat sequences (symmetrical ITRs), wherein the symmetrical ITRs are not wild-type ITRs or (ii) two wild-type inverted terminal repeat sequences (WT-ITRs). According to some embodiments, the kit is suitable for producing the ceDNA vector of any one of the aspects and embodiments herein. According to some embodiments, the kit further comprises a population of insect cells which is devoid of viral capsid coding sequences, that in the presence of Rep protein can induce production of the ceDNA vector. According to some embodiments, the kit further comprises a vector comprising a

polynucleotide sequence that encodes at least one Rep protein, wherein the vector is suitable for expressing the at least one Rep protein in an insect cell.

[0044] These and other aspects of the disclosure are described in further detail below.