~~CLAIMS~~

~~What is claimed is:~~

1. ~~A lipid particle comprising an ionizable lipid and 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.~~
2. ~~The lipid nanoparticle of claim 1, wherein the ceDNA vector when digested with a restriction enzyme having a single recognition site on the ceDNA vector and analyzed by both native and denaturing gel electrophoresis displays characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls.~~
3. ~~The lipid nanoparticle of claim 1or 2, wherein one or more of the asymmetric ITR sequences are from a virus selected from a parvovirus, a dependovirus, and an adeno-associated virus (AAV).~~
4. ~~The lipid nanoparticle of claim 3, wherein the asymmetric ITRs are from different viral serotypes.~~
5. ~~The lipid nanoparticle of of claim 4, wherein the one or more asymmetric ITRs are from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.~~
6. ~~The lipid nanoparticle of any one of claims 1-3, wherein one or more of the asymmetric ITR sequences are synthetic.~~
7. ~~The lipid nanoparticle of any one of claims 1-6, wherein one or more of the ITRs is not a wild type ITR.~~
8. ~~The lipid nanoparticle of any one of claims 1-7, wherein one or more both of the asymmetric 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'.~~
9. ~~The lipid nanoparticle of any one of claims 1-8, wherein the ceDNA vector comprises at least two asymmetric ITRs selected from:~~
    a. ~~SEQ ID NO: 1 and SEQ ID NO:52; and~~
    a. ~~SEQ ID NO: 2 and SEQ ID NO: 51.~~
10. ~~The lipid nanoparticle of any one of claims 1-9, wherein 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.

11. The lipid nanoparticle of claim 10, wherein the ceDNA expression construct is selected from a ceDNA plasmid, a ceDNA bacmid, and a ceDNA baculovirus.

12. The lipid nanoparticle of claim 10 or claim 11, wherein the insect cell expresses at least one Rep protein.

13. The lipid nanoparticle of claim 10, wherein at least one Rep protein is from a virus selected from a parvovirus, a dependovirus, and an adeno-associated virus (AAV)

14. The lipid nanoparticle of claim 13, wherein 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.

15. The lipid particle of any one of claims 1-15, wherein the DNA vector is obtained from a vector polynucleotide, wherein the vector polynucleotide encodes a heterologous nucleic acid operatively positioned between two inverted terminal repeat sequences (ITRs), wherein the two ITS are different from each other (asymmetric), and at least one of the ITRs is a functional ITR comprising a functional terminal resolution site and a Rep binding site, and one of the ITRs comprises a deletion, insertion, and/or substitution relative to the functional ITR; the presence of Rep protein inducing replication of the vector polynucleotide and production of the DNA vector in an insect cell, the DNA vector being obtainable from a process comprising the steps of:

a. incubating a population of insect cells harboring the vector polynucleotide, which is devoid of viral capsid coding sequences, in the presence of Rep protein under conditions effective and for time sufficient to induce production of the capsid-free, non-viral DNA vector within the insect cells, wherein the insect cells do not comprise production of capsid-free, non-viral DNA within the insect cells in the absence of the vector; and

b. harvesting and isolating the capsid-free, non-viral DNA from the insect cells.

16. The lipid particle of any one of claims 10-15, wherein the presence of the capsid-free, non-viral DNA isolated from the insect cells can be confirmed.

17. The lipid particle of claim 16, wherein the presence of the capsid-free, non-viral DNA isolated from the insect cells can be confirmed by digesting DNA isolated from the insect cells with a restriction enzyme having a single recognition site on the DNA vector and analyzing the digested DNA material on a non-denaturing gel to confirm the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA.

18. The lipid particle of any one of claims 1-17, wherein the DNA vector is obtained from a vector polynucleotide, wherein the vector polynucleotide encodes a heterologous nucleic acid operatively positioned between a first and a second AAV2 inverted terminal repeat DNA polynucleotide sequence (ITRs), with at least one of the ITRs having at least one polynucleotide deletion, insertion, and/or substitution with respect to the corresponding AAV2 wild type ITR of SEQ ID NO:1 or SEQ ID NO:51 to induce replication of the DNA vector in an insect cell in the presence of Rep protein, the DNA vector being obtainable from a process comprising the steps of:

    a. incubating a population of insect cells harboring the vector polynucleotide, which is devoid of viral capsid coding sequences, in the presence of Rep protein, under conditions effective and for a time sufficient to induce production of the capsid-free, non-viral DNA within the insect cells, wherein the insect cells do not comprise viral capsid coding sequences; and

    b. harvesting and isolating the capsid-free, non-viral DNA from the insect cells.

19. The lipid particle of claim 18, wherein the presence of the capsid-free, non-viral DNA isolated from the insect cells can be confirmed.

20. The lipid particle of claim 19, wherein the presence of the capsid-free, non-viral DNA isolated from the insect cells can be confirmed by digesting DNA isolated from the insect cells with a restriction enzyme having a single recognition site on the DNA vector and analyzing the digested DNA material on a non-denaturing gel to confirm the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA.

21. The lipid particle of any one of claims 1-20, wherein the lipid particle further comprises one or more of a non-cationic lipid; a PEG conjugated lipid; and a sterol.

22. The lipid particle of any one of claims 1-21, wherein the ionizable lipid is a lipid described in Table 1.

23. The lipid particle of any one of claims 1-22, wherein the lipid particle further comprises a non-cationic lipid, wherein the non-ionic lipid is selected from the group

consisting of distearoyl-sn-glycero-phosphoethanolamine, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierucoylphosphatidylcholine (DEPC), palmitoyloleyolphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidicacid,cerebrosides, dicetylphosphate, lysophosphatidylcholine, and dilinoleoylphosphatidylcholine.

24. The lipid particle of any one of claims 1-23, wherein the lipid particle further comprises a conjugated lipid, wherein the conjugated lipid, wherein the conjugated-lipid is selected from the group consisting of PEG-diacylglycerol (DAG), PEG-dialkyloxypropyl (DAA), PEG-phospholipid, PEG-ceramide (Cer), a pegylated phosphatidylethanoloamine (PEG-PE), PEG-succinate-diacylglycerol (PEGS-DAG), PEG-dialkoxypropylcarbam, and N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt.

25. The lipid particle of any one of claims 1-24, wherein the lipid particle further comprises cholesterol or a cholesterol derivative.

26. The lipid particle of any one of claims 1-25, wherein the lipid particle comprises:

(i) an ionizable lipid;

(ii) a non-cationic lipid;

(iii) a conjugated lipid that inhibits aggregation of particles; and

(iv) a sterol.

27. The lipid particle of any one of claims 1-26, wherein the lipid particle comprises:

(a) an ionizable lipid in an amount from about 20 mol % to about 90 mol % of the total lipid present in the particle;

(b) a non-cationic lipid in an amount from about 5 mol % to about 30 mol % of the total lipid present in the particle;

(c) a conjugated lipid that inhibits aggregation of particles in an amount from about 0.5 mol % to about 20 mol % of the total lipid present in the particle; and

(d) a sterol in an amount from about 20 mol % to about 50 mol % of the total lipid present in the particle.

28. The lipid particle of any one of claims 1-27, wherein total lipid to DNA vector (mass or weight) ratio is from about 10:1 to about 30:1.

29. A composition comprising a first lipid nanoparticle and an additional compound, wherein the first lipid nanoparticle comprises a first capsid free, non-viral vector, and is a lipid nanoparticle of any one of claims 1-28.

30. The composition of claim 29, wherein said additional compound is encompassed in a second lipid nanoparticle, and wherein the first and second lipid nanoparticles are different.

31. The composition of claim 28 or 29, wherein said additional compound is encompassed in the first lipid nanoparticle.

32. The composition of any one of claims 28-30, wherein said additional compound is a therapeutic agent.

33. The composition of claim 28, where said additional compound is a second capsid free, non-viral vector, wherein the first and second capsid free, non-viral vectors are different.

Claims

1. An invention not claimed in any divisional application.