CLAIMS

1. 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 ceDNA vector 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 ceDNA vector of claims 1 or 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 ceDNA vector of claim 3, wherein the asymmetric ITRs are from different viral serotypes.

5. The ceDNA vector 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 ceDNA vector of any one of claims 1-3, wherein one or more of the asymmetric ITR sequences are synthetic.

7. The ceDNA vector of any one of claims 1-3 and 6, wherein one or more of the ITRs is not a wild type ITR.

8. The ceDNA vector 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 ceDNA vector of claim 8, 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.

10. The ceDNA vector of claim 8 or claim 9, wherein one or both of the asymmetric ITRs is modified by a deletion, insertion, and/or substitution that results in the deletion of all or part of a stem-loop structure normally formed by the B and B' regions.

11. The ceDNA vector of any one of claims 8-10, wherein one or both of the asymmetric ITRs is modified by a deletion, insertion, and/or substitution that results in the deletion of all or part of a stem-loop structure normally formed by the C and C' regions.

12. The ceDNA vector of claim 10 or claim 11, wherein one or both of the asymmetric ITRs is modified by a deletion, insertion, and/or substitution that results in the deletion of part of a stem-loop structure normally formed by the B and B' regions and/or part of a stem-loop structure normally formed by the C and C' regions.

13. The ceDNA vector of any one of claims 1-12, wherein one or both of the asymmetric ITRs comprises a single stem-loop structure in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.

14. The ceDNA vector of claim 13, wherein one or both of the asymmetric ITRs comprises a single stem and two loops in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.

15. The ceDNA vector of claim 13 or claim 14, wherein one or both of the asymmetric ITRs comprises a single stem and a single loop in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.

16. The ceDNA vector of any one of claims 1-15, wherein at least one asymmetric ITR is a modified AAV2 ITR comprising a nucleotide sequence selected from: the ITRs in FIGS. 26A or 26B, SEQ ID NOS: 101-499 or 545-547, an ITR having at least 95% sequence identity to an ITR in FIGS 26A or 26B, and an ITR having at least 95% sequence identity to SEQ ID NOS: 101-499 and 545-547.

17. The ceDNA vector of any one of claims 1-16, wherein at least one asymmetric ITR is a modified AAV2 ITR comprising a nucleotide sequence of SEQ ID NOS. 2, 52, 63, or 64, or a nucleotide sequence having at least 95% sequence identity to SEQ ID NOS. 2, 52, 63, or 64.

18. The ceDNA vector of any one of claims 1-16, wherein the 5' ITR is a wild type AAV ITR and the 3' ITR comprises a sequence selected from SEQ ID NO: 2, 64, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128,130, 132, 134, 469-483 and 546, and ITR sequences shown in FIG. 26A, and sequences having at least 95% sequence identity to any of the foregoing sequences.

19. The ceDNA vector of any one of claims 1-16, wherein the 3' ITR is a wild type AAV ITR and the 5' ITR comprises a sequence selected from SEQ ID NO: 52, 63, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 484-499, 545 and 547, and ITR sequences shown in FIG. 26B, and sequences having at least 95% sequence identity to any of the foregoing sequences.

20. The ceDNA vector of any one of claims 1-16, wherein the 5' ITR comprises a sequence selected from SEQ ID NO: 52, 63, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 484-499, 545 and 547, and ITR sequences shown in FIG. 26B, and sequences having at least 95% sequence identity to any of the foregoing sequences; and the 3' ITR comprises a sequence selected from SEQ ID NO: 2, 64, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128,130, 132, 134, 469-483 and 546, and ITR sequences shown in FIG. 26A, and sequences having at least 95% sequence identity to any of the foregoing sequences.

21. The ceDNA vector of claim 1, comprising at least two asymmetric ITRs selected from:

a. SEQ ID NO: 1 and SEQ ID NO:52; and

b. SEQ ID NO: 2 and SEQ ID NO: 51.

22. The ceDNA vector of claim 1, comprising a pair of asymmetric ITRs selected from:

a. SEQ ID NO:1 and SEQ ID NO:52; and

b. SEQ ID NO:2 and SEQ ID NO:51.

23. The ceDNA vector of any one of claims 1-20, wherein one or both asymmetric ITRs comprises a sequence other than SEQ ID NO: 2, 52, 63-64, 113, 114, and 557.

24. The ceDNA vector of any one of claims 1-24, wherein all or part of the heterologous nucleotide sequence is under the control of at least one regulatory switch.

25. The ceDNA vector of claim 24, wherein at least one regulatory switch is selected from the regulatory switches in Table 11.

26. The ceDNA vector of any one of claims 1-25, wherein the vector is in a nanocarrier.

27. The ceDNA vector of claim 26, wherein the nanocarrier comprises a lipid nanoparticle (LNP).

28. A non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector) of any one of claims 1-25, the ceDNA vector being 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.

29. The ceDNA vector of claim 28, wherein the ceDNA expression construct is selected from a ceDNA plasmid, a ceDNA bacmid, and a ceDNA baculovirus.

30. The ceDNA vector of claim 28 or claim 29, wherein the insect cell expresses at least one Rep protein.

31. The ceDNA vector of claim 30, wherein at least one Rep protein is from a virus selected from a parvovirus, a dependovirus, and an adeno-associated virus (AAV).

32. The ceDNA vector of claim 31, 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.

33. A ceDNA expression construct that encodes the ceDNA vector of any one of claims 1-25.

34. The ceDNA expression construct of claim 33, which is a ceDNA plasmid, ceDNA bacmid, or ceDNA baculovirus.

35. A host cell comprising the ceDNA expression construct of claim 33 or claim 34.

36. The host cell of claim 35, which expresses at least one Rep protein.

37. The host cell of claim 36, wherein at least one Rep protein is from a virus selected from a parvovirus, a dependovirus, and an adeno-associated virus (AAV).

38. The host cell of claim 37, 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.

39. The host cell of any one of claims 35 to 38, which is an insect cell.

40. The host cell of claim 39, which is an Sf9 cell.

41. A method of producing a ceDNA vector, comprising:

a. incubating the host cell of any one of claims 35-40 under conditions effective and for time sufficient to induce production of the ceDNA vector; and

b. isolating the ceDNA from the host cells.

42. 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 one of claims 1-25, wherein the at least one heterologous nucleotide sequence is selected to treat, prevent, ameliorate, diagnose, or monitor the disease or disorder.

43. The method of claim 42, wherein the at least one heterologous nucleotide sequence, when transcribed or translated corrects for an abnormal amount of an endogenous protein in the subject.

44. The method of claim 42, wherein the at least one heterologous nucleotide sequence, when transcribed or translated corrects for an abnormal function or activity of an endogenous protein or pathway in the subject.

45. The method of any one of claims 42-44, wherein the at least one heterologous nucleotide sequence encodes or comprises a nucleotide molecule selected from an RNAi, an siRNA, an miRNA, an lncRNA, and an antisense oligo- or polynucleotide.

46. The method of any one of claims 42-44, wherein the at least one heterologous nucleotide sequence encodes a protein.

47. The method of claim 42, wherein the at least one heterologous nucleotide sequence encodes a marker protein.

48. The method of any one of claims 42-46, wherein the at least one heterologous nucleotide sequence encodes an agonist or an antagonist of an endogenous protein or pathway associated with the disease or disorder.

49. The method of any one of claims 42-46, wherein the at least one heterologous nucleotide sequence encodes an antibody.

50. The method of claims 42-49, wherein the ceDNA vector is administered in combination with a pharmaceutically acceptable carrier.

51. A method for delivering a therapeutic protein to a subject, the method comprising: administering to a subject a composition comprising the ceDNA vector of any of claims 1-25, wherein at least one heterologous nucleotide sequence encodes a therapeutic protein.

52. The method of claim 51, wherein the therapeutic protein is a therapeutic antibody.

53. A kit comprising a ceDNA vector of any of claims 1-25, and a nanocarrier, packaged in a container with a packet insert.

54. A kit for producing a ceDNA vector, the kit comprising:

a. 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 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.

55. The kit of claim 54, which is suitable for producing the ceDNA vector of any one of claims 1-25.

56. The kit of claim 54 or claim 55, further comprising 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.

57. The kit of any one of claims 54-56, further comprising 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.

Claims

1. An invention not claimed in any divisional application.