

CLAIMS

1. A capsid-free closed-ended DNA (ceDNA) vector comprising:
at least one nucleic acid sequence between flanking inverted terminal repeats (ITRs), wherein the at least one nucleic acid sequence encodes at least one FIX protein.
2. The ceDNA vector of claim 1, wherein the least one nucleic acid sequence that encodes at least one FIX protein is selected from any of the sequences set forth in Table 1 or any open reading frame (ORF) sequence included in any ceDNA sequence listed in Table 12.
3. The ceDNA vector of claims 1 or 2, wherein the ceDNA vector comprises a promoter sequence selected from any of those in Table 7 operatively linked to the least one nucleic acid sequence that encodes at least one FIX protein.
4. The ceDNA vector of any of claims 1 to 3, wherein the ceDNA vector comprises an enhancer sequence selected from any of those in Table 8.
5. The ceDNA vector of any of claims 1 to 4, wherein the ceDNA vector comprises a 5' UTR and/or intron sequence selected from any of those in Table 9A.
6. The ceDNA vector of any of claims 1 to 5, wherein the ceDNA vector comprises a 3' UTR sequence selected from any of those in Table 9B.
7. The ceDNA vector of any of claims 1 to 6, wherein the ceDNA vector comprises at least one poly A sequence selected from any of those in Table 10.
8. The ceDNA vector of any one of claims 1-7, wherein the ceDNA vector comprises at least one promoter sequence operably linked to at least one nucleic acid sequence.
9. The ceDNA vector of any one of claims 1-8, wherein the at least one nucleic acid sequence is cDNA.
10. The ceDNA vector of any one of claims 1-9, wherein at least one ITR comprises a functional terminal resolution site and a Rep binding site.
11. The ceDNA vector of any one of claims 1-10, wherein one or both of the ITRs are from a virus selected from a *Parvovirus*, a *Dependovirus*, and an adeno-associated virus (AAV).
12. The ceDNA vector of any one of claims 1-11, wherein the flanking ITRs are symmetric or asymmetric with respect to one another.
13. The ceDNA vector of claim 12, wherein the flanking ITRs are symmetrical or substantially symmetrical.
14. The ceDNA vector of claim 12, wherein the flanking ITRs are asymmetric.
15. The ceDNA vector of any one of claims 1-14, wherein one or both of the ITRs are wild type, or wherein both of the ITRs are wild-type ITRs.
16. The ceDNA vector of any one of claims 1-15, wherein the flanking ITRs are from different viral serotypes.

17. The ceDNA vector of any one of claims 1-16, wherein the flanking ITRs are selected from any pair of viral serotypes shown in Table 2.
18. The ceDNA vector of any one of claims 1-17, wherein one or both of the ITRs comprises a sequence selected from one or more of the sequences in Table 3.
19. The ceDNA vector of any one of claims 1-18, wherein at least one of the ITRs is altered from a wild-type AAV ITR sequence by a deletion, addition, or substitution that affects the overall three-dimensional conformation of the ITR.
20. The ceDNA vector of any one of claims 1-19, wherein one or both of the ITRs are derived from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.
21. The ceDNA vector of any one of claims 1-20, wherein one or both of the ITRs are synthetic.
22. The ceDNA vector of any one of claims 1-21, wherein one or both of the ITRs are not a wild type ITR, or wherein both of the ITRs are not wild-type ITRs.
23. The ceDNA vector of any one of claims 1-22, wherein one or both of the ITRs are 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'.
24. The ceDNA vector of claim 23, 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.
25. The ceDNA vector of any one of claims 1-24, wherein one or both of the ITRs are 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.
26. The ceDNA vector of any one of claims 1-24, wherein one or both of the ITRs are 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.
27. The ceDNA vector of any one of claims 1-24, wherein one or both of the ITRs are 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.
28. The ceDNA vector of any one of claims 1-27, wherein one or both of the ITRs comprise 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.
29. The ceDNA vector of any one of claims 1-28, wherein one or both of the ITRs comprise 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.
30. The ceDNA vector of any one of claims 1-29, wherein one or both of the ITRs comprise 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.

31. The ceDNA vector of any one of claims 1-30, wherein both ITRs are altered in a manner that results in an overall three-dimensional symmetry when the ITRs are inverted relative to each other.

32. The ceDNA vector of any one of claims 1-31, wherein one or both of the ITRs comprises a nucleic acid sequence selected from the sequences set forth in Tables 3, 5A, 5B, and 6.

33. The ceDNA vector of any one of claims 1-32, wherein at least one nucleic acid sequence is under the control of at least one regulatory switch.

34. The ceDNA vector of claim 33, wherein the at least one regulatory switch is selected from the group consisting of: a binary regulatory switch, a small molecule regulatory switch, a passcode regulatory switch, a nucleic acid-based regulatory switch, a post-transcriptional regulatory switch, a radiation-controlled or ultrasound controlled regulatory switch, a hypoxia-mediated regulatory switch, an inflammatory response regulatory switch, a shear-activated regulatory switch, and a kill switch.

35. A capsid-free close-ended DNA (ceDNA) vector comprising a nucleic acid sequence selected from Table 12.

36. A capsid-free close-ended DNA (ceDNA) vector comprising a nucleic acid sequence at least 85% identical to SEQ ID NO: 404, SEQ ID NO: 405 or SEQ ID NO: 406.

37. A capsid-free close-ended DNA (ceDNA) vector consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 404, SEQ ID NO: 405 and SEQ ID NO: 406.

38. A method of expressing an FIX protein in a cell comprising contacting the cell with the ceDNA vector of any one of claims 1-37.

39. The method of claim 38, wherein the cell is a hepatocyte.

40. The method of claim 38 or 39, wherein the cell is *in vitro* or *in vivo*.

41. The method of any one of claims 38-40, wherein the at least one nucleic acid sequence is codon optimized for expression in the eukaryotic cell.

42. The method of any one of claims 38-41, wherein the at least one nucleic acid sequence that is codon optimized is selected from any one of the sequences set forth in Table 1 or any open reading frame (ORF) sequence included in any ceDNA sequence listed in Table 12.

43. A method of treating a subject with hemophilia B, comprising administering to the subject a ceDNA vector of any one of claims 1-37, wherein at least one nucleic acid sequence encodes at least one FIX protein.

44. The method of claim 43, wherein the least one nucleic acid sequence that encodes the at least one FIX protein is selected from any one of the sequences set forth in Table 1.

45. The method of claim 43 or 44, wherein the ceDNA vector is administered to a hepatocyte.

46. The method of any of claims 44 to 45, wherein the ceDNA vector expresses the FIX protein in a hepatocyte.

47. The method of any of claims 44-46, wherein the ceDNA vector is administered by intravenous or intramuscular injection.

48. The method of any one of claims 44-47, further comprising administering to the subject an immune modulating agent.
49. The method of claim 48, wherein the immune modulating agent is an immunosuppressant.
50. The method of claim 49, wherein the immunosuppressant is a tyrosine kinase inhibitor (TKI).
51. The method of claim 50, wherein the TKI is administered to the subject at a dosage of about 0.5 mg/kg to about 700 mg/kg.
52. A pharmaceutical composition comprising the ceDNA vector of any one of claims 1-37.
53. The pharmaceutical composition of claim 52, further comprising an additional compound.
54. The pharmaceutical composition of claim 52, wherein the additional compound is an immune modulating agent.
55. The pharmaceutical composition of claim 54, wherein the immune modulating agent is an immunosuppressant.
56. The pharmaceutical composition of claim 55, wherein the immunosuppressant is a tyrosine kinase inhibitor (TKI).
57. The pharmaceutical composition of claim 56, wherein the composition further comprises an excipient or carrier.
58. A cell containing a ceDNA vector of any of claims 1-37.
59. The cell of claim 58, wherein the cell is a hepatocyte.
60. A composition comprising a ceDNA vector of any of claims 1-37 and a lipid.
61. The composition of claim 60, wherein the lipid is a lipid nanoparticle (LNP).
62. A kit comprising the ceDNA vector of any one of claims 1-37 or the composition of any one of claims 52-57, or the cell of claim 58.