SUMMARY

[005] In an example, the present disclosure provides a construct comprising a promoter sequence operably linked to a first set of one or more copies of coding sequences each encoding a viral accessory protein or a recombinant viral vector genome, wherein the construct further comprises two recombinase recognition sites in opposite orientations.

[006] In an aspect, the present disclosure provides a construct comprising a promoter sequence flanked by two sets of oppositely oriented coding sequences each encoding a viral accessory protein or a recombinant viral vector genome, wherein the promoter is capable of being inverted upon a recombination event inducible by a recombinase, wherein the promoter is operably linked to a first set of the two sets of oppositely oriented coding sequences prior to recombination and becomes operably linked to a second set of the two sets of oppositely oriented coding sequences post recombination. In an example, the construct of the present disclosure may further comprise one or more endonuclease recognition motifs. In another aspect, the present disclosure provides a construct comprising a promoter sequence operably linked to one or more coding sequences each encoding a viral accessory protein, a recombinant viral vector genome or both, wherein at least one of said one or more coding sequences are flanked by two recombinase recognition sites, wherein said at least one of said one or more coding sequences are capable of being disrupted, deleted or inverted upon a recombination event inducible by a recombinase. In yet another aspect, the present disclosure provides a construct comprising (i) a promoter sequence operably linked to a first set of one or more coding sequences each encoding a viral accessory protein, a recombinant viral vector genome or both, and (ii) two recombinase recognition sites flanking said promoter sequence and said first set of one or more coding sequences, wherein, upon a recombination event inducible by a recombinase, said promoter sequence becomes operably linked to additional coding sequences besides said first set of one or more coding sequences. In a further aspect, the present disclosure provides a construct comprising a promoter sequence operably linked to two or more coding sequences each encoding a viral accessory protein, a recombinant viral vector genome or both, wherein at least one of said two or more coding sequences is codon optimized.

[006a] In one aspect, the present disclosure provides a cell comprising any one of the constructs as disclosed herein.

[007] In another example, the present disclosure provides a method of producing a viral vector producer cell with an optimized stoichiometric ratio of viral vector genome and viral accessory proteins, wherein the method comprises:

a. introducing a construct described here into a first clonal population of cells;
b. transiently providing a recombinase to the first clonal population; and
c. producing a second clonal population by inverting the promoter.

[007a] In one aspect, the present disclosure provides a method of producing a viral vector producer cell with an optimized stoichiometric ratio of viral vector genome and viral accessory proteins, wherein said method comprises:

a. introducing any one of the constructs as disclosed herein into a first clonal population of cells;
b. transiently providing a recombinase and/or a CRISPR-based complex to said first clonal population; and
c. producing a second clonal population by (i) inverting an invertible sequence flanked by recombinase recognition sites; and/or (ii) editing one or more copies of coding sequences each encoding a viral accessory protein, a recombinant viral vector genome or both.

[008] In another aspect, the present disclosure provides a method comprising:

a. obtaining a first stable viral vector producer cell line;
b. determining a viral titer from the first stable viral vector producer cell line; and
c. engineering one or more elements in the stable viral vector producer cell line to produce a second stable viral vector producer cell line with a stoichiometric ratio of viral vector genome and one or more viral accessory proteins different from the corresponding stoichiometric ratio in the first stable viral vector producer cell line.

BRIEF DESCRIPTION OF THE DRAWINGS

[009] **Figure 1** provides an illustration of the genome organization of the HIV-1 Virus. The HIV-1 genome contains 9,749 bp. In addition to the *gag*, *pol*, and *env* genes common to

CLAIMS

1. A construct comprising a promoter sequence flanked by two sets of oppositely oriented coding sequences each encoding a viral accessory protein or a recombinant viral vector genome, wherein said promoter is capable of being inverted upon a recombination event inducible by a recombinase, wherein said promoter is operably linked to a first set of said two sets of oppositely oriented coding sequences prior to recombination and becomes operably linked to a second set of said two sets of oppositely oriented coding sequences post recombination.

2. The construct of claim 1, wherein said promoter is flanked by two recombinase recognition sites in opposite orientations.

3. A construct comprising a promoter sequence operably linked to one or more coding sequences each encoding a viral accessory protein, a recombinant viral vector genome or both, wherein at least one of said one or more coding sequences are flanked by two recombinase recognition sites, wherein said at least one of said one or more coding sequences are capable of being disrupted, deleted or inverted upon a recombination event inducible by a recombinase.

4. A construct comprising (i) a promoter sequence operably linked to a first set of one or more coding sequences each encoding a viral accessory protein, a recombinant viral vector genome or both, and (ii) two recombinase recognition sites flanking said promoter sequence and said first set of one or more coding sequences, wherein, upon a recombination event inducible by a recombinase, said promoter sequence becomes operably linked to additional coding sequences besides said first set of one or more coding sequences.

5. The construct of claim 1, wherein said viral accessory protein or recombinant viral vector genome is from a virus selected from the group consisting of lentivirus, retrovirus, herpesvirus, adenovirus, and adeno-associated virus.

6. The construct of claim 1, wherein said first and second sets of one or more copies of coding sequences are capable of providing different levels of expression of said coding sequences depending on an orientation of said promoter.

7. The construct of claim 1, wherein said first and second sets of one or more copies of coding sequences comprise different copy numbers.

8. The construct of claim 1, wherein said promoter comprises an inducible, recombined, or edited promoter.

9. The construct of claim 1, wherein said recombinase recognition sites comprise two palindromic recognition regions flanking a spacer region.

10. The construct of claim 1, wherein each of said recombinase recognition sites comprises a lox site.

11. The construct of claim 10, wherein said lox site comprises a wildtype lox site or a mutant lox site.

12. The construct of claim 9, wherein said spacer region is selected from the group consisting of loxP, lox511, lox2272, lox5171, m2, m3, and m7.

13. The construct of any one of claims 1 to 12, wherein said recombinase is a Cre-recombinase.

14. The construct of claim 1, wherein each of said recombinase recognition sites are a Flippase recombinase target sequence.

15. The construct of claim 14, wherein said recombinase is a Flippase recombinase.

16. The construct of claim 1, wherein said first set of said one or more copies of coding sequences comprises two or more copies of said coding sequences arranged as a polycistronic expression cassette.

17. The construct of claim 16, wherein said polycistronic expression cassette is in the sense orientation downstream of said promoter prior to promoter inversion.

18. The construct of claim 16, wherein said polycistronic expression cassette further comprises one or more viral skip sequences, internal ribosome entry site elements, or both.

19. The construct of claim 18, wherein said viral skip sequences are selected from the group consisting of P2A, T2A, E2A, and F2A.

20. The construct of claim 1, wherein said second set of said one or more copies of coding sequences comprises a monocistronic expression cassette.

21. The construct of claim 20, wherein said monocistronic expression cassette is in the antisense orientation upstream of said promoter prior to promoter inversion.

22. The construct of any of claims 1 to 21, wherein said viral accessory protein is a fusion protein.

23. The construct of any of claims 1 to 21, wherein said viral accessory protein comprises a sequence encoding a structural viral protein, a regulatory viral protein, or both.

24. The construct of claim 23, wherein said structural and regulatory proteins are selected from the group consisting of Gag, Pol, Rev, Env, Tat, Nef, Vpr, Vif, Vpu, and Vpx.

25. The construct of any of claims 1 to 21, wherein said viral accessory protein comprises a sequence encoding one or more viral accessory protein domains.

26. The construct of claim 25, wherein said one or more viral accessory protein domains are selected from the group consisting of CA, MA, NC, p6, SP1, RT, IN, PR, and DU.

27. The construct of any one of claims 1 to 26, wherein a sequence encoding said viral accessory protein or one or more viral accessory protein domains comprise a wild-type sequence, a codon optimized sequence, or both

28. A construct comprising a promoter sequence operably linked to two or more coding sequences each encoding a viral accessory protein, a recombinant viral vector genome or both, wherein at least one of said two or more coding sequences is codon optimized.

29. A cell comprising the construct of any of claims 1 to 28.

30. The cell of claim 29, wherein said cell is a eukaryotic cell.

31. The cell of claim 29, wherein said cell is a mammalian cell.

32. The cell of claim 29, wherein said cell is a human cell.

33. The cell of claim 29, wherein said cell is a viral vector producer cell.

34. The cell of claim 29, wherein said construct is integrated in the genome of said cell.

35. The cell of claim 33, wherein said viral vector producer cell is adherent or in suspension.

36. The cell of claim 33, wherein said viral vector producer cell is cultured in serum-supplemented or serum-free media.

37. The cell of claim 33, wherein said viral vector producer cell is immortalized.

38. The cell of claim 33, wherein said viral vector producer cell is a HEK293 cell, or a derivative thereof.

39. The cell of claim 38, wherein said HEK293 cell is a HEK293T cell.

40. A method of producing a viral vector producer cell with an optimized stoichiometric ratio of viral vector genome and viral accessory proteins, wherein said method comprises:

a. introducing the construct of any of claims 1 to 28 into a first clonal population of cells;

b. transiently providing a recombinase and/or a CRISPR-based complex to said first clonal population; and

c. producing a second clonal population by (i) inverting an invertible sequence flanked by recombinase recognition sites; and/or (ii) editing one or more copies of coding sequences each encoding a viral accessory protein, a recombinant viral vector genome or both.

41. The method of claim 40, wherein said second clonal population comprises a viral vector genome construct and one or more accessory constructs encoding one or more viral accessory proteins.

42. The method of claim 40, wherein said method further comprises generating from said second clonal population a stable viral vector producer cell line.

43. The method of claim 40, wherein said invertible sequence comprises a promoter sequence, a viral accessory protein sequence, a recombinant viral vector genome sequence, or a combination thereof.

44. The method of claim 42, wherein said stable viral vector producer cell line exhibits a higher viral titer relative to said first clonal population.

45. The method of claim 44, wherein said viral titer is determined by physical titration, functional titration, or both.

46. The method of claim 44, wherein said viral titer is determined by assaying for viral nucleic acid via an assay selected from the group consisting of PCR, RT-PCR, and quantitative detection by blot hybridization, or by assaying for viral protein via an immunoassay.

47. The method of claim 40, wherein said method further comprises determining the stoichiometric ratio of viral vector genome RNA and said viral accessory proteins in said first clonal population, second clonal population, or both.

48. The method of claim 40, wherein said method further comprises quantifying the level of said viral vector genome and said viral accessory proteins in said first clonal population, second clonal population, or both.

49. The method of claim 42, wherein said method further comprises storing said stable viral vector producer cell line by cryopreservation.

50. The method of claim 42, wherein said method further comprises expanding cells from said cryopreserved cell line to produce viral vectors.

51. The method of claim 40, wherein said recombinase recognition sites comprise two palindromic recognition regions flanking a spacer region.

52. The method of claim 40, wherein each of said recognition sites comprises a lox site.

53. The method of claim 52, wherein said lox site comprises a wildtype lox site or a mutant lox site.

54. The method of claim 51, wherein said spacer region is selected from the group consisting of loxP, lox511, lox2272, lox5171, m2, m3, and m7.

55. The method of claim 40, wherein said recombinase is a Cre-recombinase.

56. The method of claim 40, wherein each of said recombinase recognition sites is a Flippase recombinase target sequence.

57. The method of claim 40, wherein said recombinase is a Flippase recombinase.

58. The method of claim 40, wherein said viral vector genome construct comprises one or more elements selected from the group consisting of a 5' long terminal repeat, a 3' long terminal repeat, a packaging signal, and a central polypurine tract.

59. The method of claim 40, wherein said viral vector genome construct does not comprise a 5' long terminal repeat, a 3' long terminal repeat, a packaging signal, or a central polypurine tract.

60. The method of claim 40, wherein said viral vector genome construct comprises a self-inactivating long terminal repeat.

61. The method of claim 40, wherein said viral vector genome construct comprises a promoter and a polyadenylation sequence.

62. The method of claim 61, wherein said promoter of said viral vector genome construct is constitutive or inducible.

63. The method of claim 40, wherein said viral vector genome construct comprises a concatemer.

64. The method of claim 63, wherein said concatemer comprises multiple copies of a viral vector genome construct.

65. The method of claim 63, wherein said concatemer comprises one or more selection genes.

66. The method of claim 63, wherein said concatemer comprises one or more transcription factors.

67. The method of claim 40, wherein said first clonal population or said second clonal population comprises viral vector producer cells.

68. The method of claim 40, wherein said introducing step may be chemical, biological or physical.

69. The method of claim 40, wherein said introducing step comprises optical, magnetic, biolistic, polymer-based, liposome-based, nanoparticle-based methods, or a combination thereof.

70. The method of claim 40, wherein said introducing step comprises a transduction.

71. The method of claim 40, wherein said introducing step comprises a transfection.

72. The method of claim 68, wherein said chemical introducing step comprises a cationic polymer, calcium phosphate, cationic lipid, or a combination thereof.

73. The method of claim 68, wherein said biological introducing step comprises introduction via retrovirus, lentivirus, transposon, CRISPR/Cas9, or recombinase.

74. The method of claim 68, wherein said physical introducing step is selected from the group consisting of electroporation, sonoporation, mechanoporation, and photoporation.

75. The method of claim 40, wherein said recombinase is an inducible recombinase.

76. The method of claim 40, wherein said recombinase comprises a Cre-recombinase, a Flippase recombinase, or a modified version thereof.

77. The construct of claim 75, wherein said inducible recombinase is chemical or light inducible.

78. The method of claim 40, wherein said cell is a eukaryotic cell.

79. The method of claim 40, wherein said cell is a mammalian cell.

80. The method of claim 40, wherein said cell is a human cell.

81. The method of claim 40, wherein said promoter comprises an inducible, recombined, or edited promoter.

82. The method of claim 40, wherein said viral accessory proteins comprise sequences encoding structural viral proteins, regulatory viral proteins, or both.

83. The method of claim 40, wherein said structural and regulatory proteins are selected from the group consisting of Gag, Pol, Rev, Env, Tat, Nef, Vpr, Vif, Vpu, and Vpx.

84. The method of claim 40, wherein said viral accessory proteins comprise sequences encoding a partial viral accessory protein.

85. The method of claim 40, wherein said partial viral accessory protein comprises one or more viral accessory protein domains.

86. The method of claim 85, wherein said one or more viral accessory protein domains is selected from the group consisting of CA, MA, NC, p6, SP1, RT, IN, PR, and DU.

87. The method of any one of claims 82 to 86, wherein a sequence encoding said one or more viral accessory proteins or domains comprises a wild-type sequence, a codon optimized sequence, or both.

88. A method comprising:

a. obtaining a first stable viral vector producer cell line;

b. determining a viral titer from said first stable viral vector producer cell line; and

c. engineering one or more elements in said stable viral vector producer cell line to produce a second stable viral vector producer cell line with a stoichiometric ratio of viral vector genome and one or more viral accessory proteins different from the corresponding stoichiometric ratio in said first stable viral vector producer cell line.

89. The method of claim 88, wherein said engineering is via one or more recombination events.

90. The method of claim 88, wherein said engineering comprises introducing a recombinase.

91. The method of claim 88, wherein said engineering comprises transiently expressing a recombinase.

92. The method of claim 88, wherein said engineering is via gene editing.

93. The method of claim 88, wherein said engineering comprises introducing a CRISPR-based complex.

94. The method of claim 88, wherein said engineering comprises introducing a CRISPR-based complex specific to some but not all copies of coding sequences for said one or more viral accessory proteins.

95. The method of claim 88, wherein said engineering comprises both a recombination event and gene editing.