

CLAIMS

1. 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 ~~said the~~ construct further comprises two recombina-
5 recombina-
recombination sites in opposite orientations.
2. The construct of claim 1, wherein ~~said the~~ promoter sequence becomes operably linked to more or fewer copies of ~~said the~~ coding sequences relative to ~~said the~~ first set of one or more copies of coding sequences upon a recombination event via ~~said the~~ two recombina-
10 recombination sites.
3. The construct of claim 1, wherein ~~said the two~~ recombina-
recombination sites flank ~~said the~~ promoter sequence.
- 15 4. The construct of claim 1, wherein ~~said the~~ recombina-
recombination sites flank at least one coding sequence encoding a viral accessory protein, ~~or flank~~ a recombinant viral vector genome, flank the one or more copies of the viral accessory protein coding sequence, flank the one or more copies of the recombinant viral vector genome coding sequence, flank the promoter and the one or more copies of the viral accessory protein coding sequence, or flank a promoter and the one or more copies of the recombinant viral vector genome coding sequence.
- 20 5. ~~The construct of claim 1, wherein said recombina-
recombination sites flank said one or more copies of said viral accessory protein coding sequence.~~
- 25 6. ~~The construct of claim 1, wherein said recombina-
recombination sites flank said one or more copies of said recombinant viral vector genome coding sequence.~~
7. ~~The construct of claim 1, wherein said recombina-
recombination sites flank said promoter and said one or more copies of said viral accessory protein coding sequence.~~
- 30 8. ~~The construct of claim 1, wherein said recombina-
recombination sites flank a promoter and said one or more copies of said recombinant viral vector genome coding sequence.~~

9.5. The construct of any one of claims 1 to 84, further comprising a second set of one or more copies of ~~said the~~ coding sequences, wherein, upon inversion via the two ~~opposing~~ recombinase recognition sites, ~~said the~~ promoter becomes operably linked to ~~said the~~ second set of coding sequences.

10.6. The construct of claim 1, further comprising one or more endonuclease recognition motifs.

11.7. The construct of claim 106, wherein ~~said the~~ one or more endonuclease recognition motifs are protospacer adjacent motifs.

~~12. 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.~~

~~13. The construct of claim 12, wherein said promoter is flanked by two recombinase recognition sites in opposite orientations.~~

~~14. 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.~~

~~15. 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 16.8. The construct of claim ~~9-or-125~~, wherein ~~said-the~~ 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.

10 17.9. The construct of claim ~~9-or-125~~, wherein ~~said-the~~ first and second sets of one or more copies of coding sequences are capable of providing different levels of expression of ~~said-the~~ coding sequences depending on an orientation of ~~said-the~~ promoter.

15 18.10. The construct of claim ~~9-or-125~~, wherein ~~said-the~~ first and second sets of one or more copies of coding sequences comprise different copy numbers.

19.11. The construct of claim ~~1-or-12~~, wherein ~~said-the~~ promoter comprises an inducible, recombined, or edited promoter.

20 20.12. The construct of claim ~~1-or-13~~, wherein ~~said-the~~ recombinase recognition sites comprise two palindromic recognition regions flanking a spacer region.

21.13. The construct of claim ~~1-or-13~~, wherein each of ~~said-the~~ recombinase recognition sites comprises a lox site.

25 22.14. The construct of claim ~~21-13~~, wherein ~~said-the~~ lox site comprises a wildtype lox site or a mutant lox site.

30 23.15. The construct of claim ~~20-12~~, wherein ~~said-the~~ spacer region is selected from the group consisting of loxP, lox511, lox2272, lox5171, m2, m3, and m7.

24.16. The construct of any one of claims 1 to ~~15-23~~, wherein ~~said-the~~ recombinase is a Cre-recombinase.

25.17. The construct of claim ~~1-or-13~~, wherein each of ~~said-the~~ recombina-
se recognition sites are a Flippase recombina-
se target sequence.

26.18. The construct of claim ~~25.17~~, wherein ~~said-the~~ recombina-
se is a Flippase recombina-
se.

27.19. The construct of claim ~~1-or-12~~, wherein ~~said-the~~ first set of ~~said-the~~ one or
more copies of coding sequences comprises two or more copies of ~~said-the~~ coding
sequences arranged as a polycistronic expression cassette.

28.20. The construct of claim ~~27.19~~, wherein ~~said-the~~ polycistronic expression
cassette is in the sense orientation downstream of ~~said-the~~ promoter prior to promoter
inversion.

29.21. The construct of claim ~~27.19~~, wherein ~~said-the~~ polycistronic expression
cassette further comprises one or more viral skip sequences, internal ribosome entry
site elements, or both.

30.22. The construct of claim ~~29.21~~, wherein ~~said-the~~ viral skip sequences are selected
from the group consisting of P2A, T2A, E2A, and F2A.

31.23. The construct of claim ~~59-or-12~~, wherein ~~said-the~~ second set of ~~said-the~~ one or
more copies of coding sequences comprises a monocistronic expression cassette.

32.24. The construct of claim ~~31.23~~, wherein ~~said-the~~ monocistronic expression
cassette is in the antisense orientation upstream of ~~said-the~~ promoter prior to promoter
inversion.

33.25. The construct of any of claims 1 to ~~24.32~~, wherein ~~said-the~~ viral accessory
protein is a fusion protein.

34.26. The construct of any of claims 1 to ~~24.32~~, wherein ~~said-the~~ viral accessory
protein comprises a sequence encoding a structural viral protein, a regulatory viral
protein, or both.

35:27. The construct of claim 3426, wherein ~~said the~~ structural and regulatory proteins are selected from the group consisting of Gag, Pol, Rev, Env, Tat, Nef, Vpr, Vif, Vpu, and Vpx.

36:28. The construct of any of claims 1 to 3224, wherein ~~said the~~ viral accessory protein comprises a sequence encoding one or more viral accessory protein domains.

37:29. The construct of claim 3628, wherein ~~said the~~ one or more viral accessory protein domains are selected from the group consisting of CA, MA, NC, p6, SP1, RT, IN, PR, and DU.

38:30. The construct of any one of claims 1 to 3729, wherein a sequence encoding ~~said the~~ viral accessory protein or one or more viral accessory protein domains comprise a wild-type sequence, a codon optimized sequence, or both.

~~39. 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.~~

~~40. A cell comprising the construct of any of claims 1 to 39.~~

~~41. The cell of claim 40, wherein said cell is a eukaryotic cell.~~

~~42. The cell of claim 40, wherein said cell is a mammalian cell.~~

~~43. The cell of claim 40, wherein said cell is a human cell.~~

~~44. The cell of claim 40, wherein said cell is a viral vector producer cell.~~

~~45. The cell of claim 40, wherein said construct is integrated in the genome of said cell.~~

~~46. The cell of claim 44, wherein said viral vector producer cell is adherent or in suspension.~~

47. The cell of claim 44, wherein said viral vector producer cell is cultured in serum-supplemented or serum-free media.
- 5 48. The cell of claim 44, wherein said viral vector producer cell is immortalized.
49. The cell of claim 44, wherein said viral vector producer cell is a HEK293 cell, or a derivative thereof.
- 10 50. The cell of claim 49, wherein said HEK293 cell is a HEK293T cell.
51. 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:
- 15 a. introducing the construct of any of claims 1 to 39 into a first clonal population of cells;
- b. transiently providing a recombinase and/or a CRISPR-based complex to said first clonal population; and
- 20 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.
52. The method of claim 51, wherein said second clonal population comprises a viral vector genome construct and one or more accessory constructs encoding one or more viral accessory proteins.
- 25 53. The method of claim 51, wherein said method further comprises generating from said second clonal population a stable viral vector producer cell line.
- 30 54. The method of claim 51, wherein said invertible sequence comprises a promoter sequence, a viral accessory protein sequence, a recombinant viral vector genome sequence, or a combination thereof.

55. The method of claim 53, wherein said stable viral vector producer cell line exhibits a higher viral titer relative to said first clonal population.
56. The method of claim 55, wherein said viral titer is determined by physical titration, functional titration, or both.
57. The method of claim 55, 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.
58. The method of claim 51, 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.
59. The method of claim 51, 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.
60. The method of claim 53, wherein said method further comprises storing said stable viral vector producer cell line by cryopreservation.
61. The method of claim 53, wherein said method further comprises expanding cells from said cryopreserved cell line to produce viral vectors.
62. The method of claim 51, wherein said recombinase recognition sites comprise two palindromic recognition regions flanking a spacer region.
63. The method of claim 51, wherein each of said recognition sites comprises a lox site.
64. The method of claim 63, wherein said lox site comprises a wildtype lox site or a mutant lox site.

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65. ~~The method of claim 62, wherein said spacer region is selected from the group consisting of loxP, lox511, lox2272, lox5171, m2, m3, and m7.~~
66. ~~The method of claim 51, wherein said recombinase is a Cre recombinase.~~
67. ~~The method of claim 51, wherein each of said recombinase recognition sites is a Flippase recombinase target sequence.~~
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68. ~~The method of claim 51, wherein said recombinase is a Flippase recombinase.~~
69. ~~The method of claim 51, 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.~~
- 15
70. ~~The method of claim 51, 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.~~
- 20
71. ~~The method of claim 51, wherein said viral vector genome construct comprises a self-inactivating long terminal repeat.~~
72. ~~The method of claim 51, wherein said viral vector genome construct comprises a promoter and a polyadenylation sequence.~~
- 25
73. ~~The method of claim 72, wherein said promoter of said viral vector genome construct is constitutive or inducible.~~
74. ~~The method of claim 51, wherein said viral vector genome construct comprises a concatemer.~~
- 30
75. ~~The method of claim 74, wherein said concatemer comprises multiple copies of a viral vector genome construct.~~

76. ~~The method of claim 74, wherein said concatemer comprises one or more selection genes.~~
- 5 77. ~~The method of claim 74, wherein said concatemer comprises one or more transcription factors.~~
78. ~~The method of claim 51, wherein said first clonal population or said second clonal population comprises viral vector producer cells.~~
- 10 79. ~~The method of claim 51, wherein said introducing step may be chemical, biological or physical.~~
80. ~~The method of claim 51, wherein said introducing step comprises optical, magnetic, biolistic, polymer based, liposome based, nanoparticle based methods, or a combination thereof.~~
- 15 81. ~~The method of claim 51, wherein said introducing step comprises a transduction.~~
82. ~~The method of claim 51, wherein said introducing step comprises a transfection.~~
- 20 83. ~~The method of claim 79, wherein said chemical introducing step comprises a cationic polymer, calcium phosphate, cationic lipid, or a combination thereof.~~
84. ~~The method of claim 79, wherein said biological introducing step comprises introduction via retrovirus, lentivirus, transposon, CRISPR/Cas9, or recombinase.~~
- 25 85. ~~The method of claim 79, wherein said physical introducing step is selected from the group consisting of electroporation, sonoporation, mechanoporation, and photoporation.~~
- 30 86. ~~The method of claim 51, wherein said recombinase is an inducible recombinase.~~
87. ~~The method of claim 51, wherein said recombinase comprises a Cre recombinase, a Flippase recombinase, or a modified version thereof.~~

88. ~~The construct of claim 86, wherein said inducible recombinase is chemical or light inducible.~~
- 5 89. ~~The method of claim 51, wherein said cell is a eukaryotic cell.~~
90. ~~The method of claim 51, wherein said cell is a mammalian cell.~~
91. ~~The method of claim 51, wherein said cell is a human cell.~~
- 10 92. ~~The method of claim 51, wherein said promoter comprises an inducible, recombined, or edited promoter.~~
93. ~~The method of claim 51, wherein said viral accessory proteins comprise sequences encoding structural viral proteins, regulatory viral proteins, or both.~~
- 15 94. ~~The method of claim 51, wherein said structural and regulatory proteins are selected from the group consisting of Gag, Pol, Rev, Env, Tat, Nef, Vpr, Vif, Vpu, and Vpx.~~
95. ~~The method of claim 51, wherein said viral accessory proteins comprise sequences encoding a partial viral accessory protein.~~
- 20 96. ~~The method of claim 51, wherein said partial viral accessory protein comprises one or more viral accessory protein domains.~~
- 25 97. ~~The method of claim 96, 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.~~
98. ~~The method of any one of claims 93 to 97, wherein a sequence encoding said one or more viral accessory proteins or domains comprises a wild type sequence, a codon optimized sequence, or both.~~
- 30 99. ~~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.~~

~~100. The method of claim 99, wherein said engineering is via one or more recombination
events.~~

~~101. The method of claim 99, wherein said engineering comprises introducing a
recombinase.~~

~~102. The method of claim 99, wherein said engineering comprises transiently expressing a
recombinase.~~

~~103. The method of claim 99, wherein said engineering is via gene editing.~~

~~104. The method of claim 99, wherein said engineering comprises introducing a CRISPR-
based complex.~~

~~105. The method of claim 99, 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.~~

~~106. The method of claim 99, wherein said engineering comprises both a recombination
event and gene editing.~~