

CLAIMS

1. A viral vector construct, wherein the construct comprises a barcoded concatemer molecule comprising one or more copies of an expression cassette encoding a gene of interest (GOI).
- 5 2. The viral vector construct of claim 1, wherein the one or more copies of the expression cassette comprises one or more unique molecular identifiers.
3. The viral vector construct of claim 1, wherein the one or more expression cassettes is monocistronic.
4. The viral vector construct of claim 2, wherein the one or more unique molecular
10 identifiers comprises one or more spacers.
5. The viral vector construct of claim 2, wherein the one or more unique molecular identifiers does not comprise a spacer.
6. The viral vector construct of claim 2, wherein the one or more unique molecular identifiers is a single-stranded nucleic acid or a double-stranded nucleic acid.
- 15 7. The viral vector construct of claim 2, wherein the one or more unique molecular identifiers comprise equivalent numbers of nucleotides or different numbers of nucleotides.
8. The viral vector construct of claim 2, wherein the one or more unique molecular identifiers is flanked by one or more restriction enzyme sites.
- 20 9. The viral vector construct of claim 2, wherein the one or more unique molecular identifiers comprise a unique oligonucleotide sequence or a known oligonucleotide sequence.
10. The viral vector construct of claim 9, wherein the unique oligonucleotide sequence comprises randomly incorporated oligonucleotides.
- 25 11. The viral vector construct of claim 1, wherein the barcoded concatemer comprises about 25 to about 50 unique molecular identifiers.
12. The viral vector construct of claim 2, wherein the one or more unique molecular identifiers is linked to the one or more copies of the expression cassette.
13. The viral vector construct of claim 12, wherein the linkage occurs via ligation.
- 30 14. The viral vector construct of claim 13, wherein the ligation comprises directional ligation.
15. The viral vector construct of claim 13, wherein the ligation comprises sequential ligation.

16. The viral vector construct of claim 1, wherein the barcoded concatemer molecule comprises one or more expression cassettes encoding a transcription factor.
17. The viral vector construct of claim 1, wherein the barcoded concatemer molecule comprises one or more expression cassettes encoding an antibiotic selection gene.
- 5 18. The viral vector construct of claim 1, wherein the barcoded concatemer molecule comprises one or more insulator sequences.
19. The viral vector construct of claim 1, wherein the barcoded concatemer molecule comprises two or more restriction enzyme sites.
20. The viral vector construct of claim 1, wherein the barcoded concatemer molecule
10 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.
21. The viral vector construct of claim 1, wherein the barcoded concatemer molecule does not comprise a 5' long terminal repeat, a 3' long terminal repeat, a packaging signal,
15 or a central polypurine tract.
22. The barcoded concatemer molecule of claim 20, wherein the 5' long terminal repeat is chimeric.
23. The viral vector construct of claim 1, wherein the viral vector construct is target-specific.
- 20 24. The viral vector construct of claim 1, wherein the viral vector construct is derived from a retrovirus, herpesvirus, adenovirus, or adeno-associated virus.
25. The viral vector construct of claim 1, wherein the viral vector construct is derived from a lentivirus.
26. The viral vector construct of claim 1, wherein the viral vector construct comprises a
25 self-inactivating long terminal repeat.
27. The viral vector construct of claim 1, wherein the viral vector construct comprises one or more selectable or reporter elements.
28. The viral vector construct of claim 27, wherein the one or more selectable or reporter elements is a reporter gene, an epitope tag, or both.
- 30 29. The viral vector construct of claim 28, wherein the one or more selectable or reporter elements is selected or detected by luminescence, absorbance, fluorescence, antibiotics, antigen-antibody interactions, or a combination thereof.
30. The viral vector construct of claim 1, wherein the viral vector construct comprises a promoter and a polyadenylation sequence.

31. The viral vector construct of claim 30, wherein the promoter is constitutive or inducible.
32. The viral vector construct of claim 31, wherein the inducible promoter comprises a tetracycline-dependent promoter.
- 5 33. The viral vector construct of claim 30, wherein the promoter is synthetic.
34. A cell line comprising a viral vector construct comprising a barcoded concatemer molecule.
35. The cell line of claim 34, wherein the cell line is in a cell culture comprising a volume of medium.
- 10 36. The cell line of claim 34, wherein the cell line is adapted for adherent culturing or culturing in suspension.
37. The cell line of claim 34, wherein the cell line is cultured in a serum-supplemented or a serum-free medium.
38. The cell line of claim 34, wherein the cell line is immortalized.
- 15 39. The cell line of claim 34, wherein the cell line is eukaryotic.
40. The cell line of claim 34, wherein the cell line is mammalian.
41. The cell line of claim 34, wherein the cell line is human.
42. The cell line of claim 34, wherein the cell line is a HEK293 cell line or a derivative thereof.
- 20 43. The cell line of claim 42, wherein the HEK293 cell line is a HEK293T cell line.
44. The cell line of claim 34, wherein the cell line produces psuedotyped viral particles.
45. The cell line of claim 44, wherein the psuedotyped viral particles comprise one or more envelope proteins of a virus selected from the group consisting of Vesiculovirus, Gammaretrovirus, and Morbillivirus.
- 25 46. The cell line of claim 34, wherein the cell line produces mosaic viral particles.
47. The cell line of claim 34, wherein the cell line produces chimeric viral particles.
48. The cell line of claim 34, wherein the barcoded concatemer molecule comprises one or more expression cassettes encoding a gene of interest (GOI) and one or more unique molecular identifiers.
- 30 49. The cell line of claim 48, wherein the one or more expression cassettes is monocistronic.
50. The cell line of claim 48, wherein the unique molecular identifier is a single-stranded nucleic acid.

51. The cell line of claim 48, wherein the unique molecular identifier is a double-stranded nucleic acid.
52. The cell line of claim 48, wherein the one or more unique molecular identifiers comprises one or more spacers.
- 5 53. The cell line of claim 52, wherein the one or more unique molecular identifiers does not comprise a spacer.
54. The cell line of claim 52, wherein the one or more unique molecular identifiers comprise equivalent numbers of nucleotides.
55. The cell line of claim 52, wherein the one or more unique molecular identifiers
10 comprise different numbers of nucleotides.
56. The cell line of claim 52, wherein the one or more unique molecular identifiers is flanked by one or more restriction enzyme sites.
57. The cell line of claim 52, wherein the barcoded concatemer comprises about 25 to about 50 unique molecular identifiers.
- 15 58. The cell line of claim 52, wherein the unique molecular identifier is linked to the expression cassette.
59. The cell line of claim 58, wherein the linkage occurs via ligation.
60. The cell line of claim 59, wherein the ligation comprises directional ligation.
61. The cell line of claim 59, wherein the ligation comprises sequential ligation.
- 20 62. The cell line of claim 34, wherein the barcoded concatemer molecule comprises one or more expression cassettes encoding a transcription factor.
63. The cell line of claim 34, wherein the barcoded concatemer molecule comprises one or more expression cassettes encoding an antibiotic selection gene.
64. The cell line of claim 34, wherein the barcoded concatemer molecule comprises one
25 or more insulator sequences.
65. The cell line of claim 34, wherein the barcoded concatemer molecule comprises two or more restriction enzyme sites.
66. The cell line of claim 34, wherein the barcoded concatemer molecule comprises one or more elements selected from the group consisting of a 5' long terminal repeat, a 3'
30 long terminal repeat, a packaging signal, and a central polypurine tract.
67. The cell line of claim 34, wherein the barcoded concatemer molecule does not comprise a 5' long terminal repeat, a 3' long terminal repeat, a packaging signal, or a central polypurine tract.
68. The cell line of claim 66, wherein the 5' long terminal repeat is chimeric.

69. The cell line of claim 34, wherein the viral vector construct is target-specific.
70. The cell line of claim 34, wherein the viral vector construct is derived from a retrovirus.
71. The cell line of claim 34, wherein the viral vector construct is derived from a lentivirus.
72. The cell line of claim 34, wherein the viral vector construct is derived from a herpesvirus.
73. The cell line of claim 34, wherein the viral vector construct is derived from an adenovirus.
74. The cell line of claim 34, wherein the viral vector construct is derived from an adeno-associated virus.
75. The cell line of claim 34, wherein the viral vector construct comprises a self-inactivating long terminal repeat.
76. The cell line of claim 34, wherein the viral vector construct comprises one or more selectable or reporter elements.
77. The cell line of claim 76, wherein the one or more selectable or reporter elements is a reporter gene, an epitope tag, or both.
78. The cell line of claim 77, wherein the one or more selectable or reporter elements is selected or detected by luminescence, absorbance, fluorescence, antibiotics, antigen-antibody interactions, or a combination thereof.
79. The cell line of claim 34, wherein the viral vector construct comprises a promoter and a polyadenylation sequence.
80. The cell line of claim 79, wherein the promoter is constitutive or inducible.
81. The cell line of claim 80, wherein the inducible promoter comprises a tetracycline-dependent promoter.
82. The cell line of claim 80, wherein the promoter is synthetic.
83. A method of making a viral vector construct comprising a barcoded concatemer molecule, the method comprising:
- ligating a unique molecular identifier to a viral vector genome construct to generate a barcoded viral vector genome construct; and
 - ligating a concatemer to the barcoded viral vector genome construct to generate the barcoded concatemer molecule.
84. The method of claim 83, wherein the viral vector construct comprises one or more unique molecular identifiers.

85. The method of claim 83, wherein the viral vector construct comprises about 25 to about 50 unique molecular identifiers.
86. The method of claim 83, wherein the unique molecular identifier is flanked by one or more restriction enzyme sites.
- 5 87. The method of claim 86, wherein the one or more restriction enzyme sites is located at a 5' end of the unique molecular identifier.
88. The method of claim 86, wherein the one or more restriction enzyme sites is located at a 3' end of the unique molecular identifier.
89. The method of claim 83, wherein the viral vector genome construct comprises one or more restriction enzyme sites.
- 10 90. The method of claim 83, wherein the unique molecular identifier is ligated to the viral vector genome construct prior to concatemer ligation.
91. The method of claim 83, wherein the ligation of the unique molecular identifier to the viral vector genome construct occurs via beads.
- 15 92. The method of claim 83, wherein the beads comprise magnetic beads.
93. The method of claim 83, wherein the ligation of the unique molecular identifier to the viral vector genome construct occurs without use of beads.
94. The method of claim 91, wherein the beads comprise one or more streptavidin molecules.
- 20 95. The method of claim 83, wherein the unique molecular identifier is biotinylated.
96. The method of claim 95, wherein the unique molecular identifier is biotinylated at a 5' end.
97. The method of claim 83, wherein the unique molecular identifier is a single-stranded nucleic acid.
- 25 98. The method of claim 83, wherein the unique molecular identifier is a double-stranded nucleic acid.
99. A method of making a stable viral vector producer cell line comprising a barcoded concatemer, the method comprising:
- 30 a) introducing into a population of cells a viral vector genome construct, wherein the viral vector genome construct comprises a barcoded concatemer, comprising an expression cassette encoding one or more copies of a gene of interest (GOI) and one or more unique molecular identifiers;
- b) producing a population of transgenic cells comprising integrated sequences encoding the GOI and the unique molecular identifiers;

c) selecting from the population of transgenic cells a cell clone producing a desired viral titer; and

d) generating from the cell clone a stable viral vector producer cell line.

- 5 100. The method of claim 99, wherein the transgenic cells comprise polyclonal cells.
101. The method of claim 99, wherein the selecting further comprises polyclonal to monoclonal selection of the transgenic cells.
102. The method of claim 101, wherein the polyclonal to monoclonal selection comprises limiting dilution, single cell sorting, or both.
- 10 103. The method of any one of claims 99 to 102, wherein the generation of the stable vector producer cell line occurs by expansion of the selected cell clone.
104. The method of claim 99, wherein the method further comprises storing the selected cell line by cryopreservation.
- 15 105. The method of claim 104, wherein the method further comprises expanding cells from the cryopreserved cell line to produce viral vectors.
106. The method of claim 99, wherein the method further comprises quantifying the level of the viral vector genome in the selected cell clone, the generated cell line, or both.
107. The method of claim 99, wherein the method further comprises harvesting viral vector from the selected cell clone, the generated cell line or both.
- 20 108. The method of claim 99, wherein the method further comprises determining a viral titer of the selected cell clone, the generated cell line, or both.
109. The method of claim 108, wherein determining the viral titer comprises physical titration, functional titration, or both.
- 25 110. The method of claim 108, wherein determining the viral titer comprises PCR, immunodetection, ELISA, or any combination thereof.
111. The method of claim 109, wherein the method further comprises determining an infectivity of the viral titer of the selected cell clone or the generated cell line.
112. The method of claim 99, wherein the viral vector producer cell line produces a target-specific viral vector.
- 30 113. The method of claim 99, wherein the viral vector producer cell line produces a viral vector derived from a lentivirus.

114. The method of claim 99, wherein the viral vector producer cell line produces a viral vector derived from a retrovirus, a herpesvirus, an adenovirus, or an adeno-associated virus.
115. The method of claim 99, wherein the viral vector producer cell line produces a viral vector comprising one or more capsid proteins or one or more envelope proteins.
116. The method of claim 115, wherein the one or more capsid proteins are heterologous.
117. The method of claim 115, wherein the one or more capsid proteins are genetically modified or chemically modified.
118. The method of claim 115, wherein the one or more envelope proteins are heterologous.
119. The method of claim 99, wherein the viral vector genome construct comprises 5' and 3' long terminal repeats, a packaging signal, and a central polypurine tract.
120. The method of claim 119, wherein the 5' long terminal repeat is chimeric.
121. The method of claim 99, wherein the viral vector genome construct comprises a self-inactivating long terminal repeat.
122. The method of claim 99, wherein the viral vector genome construct comprises one or more selectable or detectable elements.
123. The method of claim 122, wherein the one or more selectable or detectable elements is a reporter gene, an epitope tag, or both.
124. The method of claim 122, wherein the one or more selectable or detectable elements is selected or detected by luminescence, absorbance, fluorescence, antibiotics, antigen-antibody interactions, or a combination thereof.
125. The method of claim 99, wherein the viral vector genome construct comprises a promoter and a polyadenylation sequence.
126. The method of claim 125, wherein the promoter of the viral vector genome construct is constitutive or inducible.
127. The method of claim 125, wherein the promoter of the viral vector genome construct is synthetic.
128. The method of claim 99, wherein the concatemer comprises multiple copies of an expression cassette encoding the GOI.
129. The method of claim 99, wherein the concatemer comprises one or more expression cassettes encoding a transcription factor.

130. The method of claim 99, wherein the introducing step comprises a chemical, biological, or physical step.
131. The method of claim 99, wherein the introducing step comprises an optical method, a magnetic method, a biolistic method, a polymer-based method, a liposome-based method, a nanoparticle-based method, or a combination thereof.
132. The method of claim 99, wherein the introducing step comprises transduction.
133. The method of claim 99, wherein the introducing step comprises transfection.
134. The method of claim 130, wherein the chemical introducing step comprises the use of a cationic polymer, calcium phosphate, cationic lipid, or a combination thereof.
135. The method of claim 130, wherein the biological introducing step comprises introduction via a retrovirus, lentivirus, transposon, TALEN, Zinc Finger nuclease, meganuclease, transposase, CRISPR-related nuclease, or recombinase.
136. The method of claim 135, wherein the recombinase comprises Cre-recombinase or Flippase recombinase.
137. The method of claim 130, wherein the physical introducing step is selected from the group consisting of electroporation, sonoporation, mechanoporation, and photoporation.
138. The method of claim 99, wherein the integrated sequences exhibit random integration.
139. The method of claim 99, wherein the integrated sequences exhibit site-specific integration.
140. The method of claim 99, wherein the stable viral vector producer cell line is in a cell culture that comprises a volume of medium.
141. The method of claim 99, wherein the stable viral vector producer cell line is adapted for adherent culturing or culturing in suspension.
142. The method of claim 99, wherein the stable viral vector producer cell line is cultured in a serum-supplemented or a serum-free medium.
143. The method of claim 99, wherein the stable viral vector producer cell line is immortalized.
144. The method of claim 99, wherein the stable viral vector producer cell line is eukaryotic.
145. The method of claim 99, wherein the stable viral vector producer cell line is mammalian.

146. The method of claim 99, wherein the stable viral vector producer cell line is human.
147. The method of claim 99, wherein the stable viral vector producer cell line is a HEK293 cell or a derivative thereof.
- 5 148. The method of claim 147, wherein the HEK293 cell line is a HEK293 T cell line.
149. The method of claim 99, wherein the stable viral vector producer cell line produces psuedotyped viral particles.
- 10 150. The method of claim 149, wherein the psuedotyped viral particles comprise one or more envelope proteins of a virus selected from the group consisting of Vesiculovirus, Gammaretrovirus, and Morbillivirus.
151. The method of claim 99, wherein the stable viral vector producer cell line produces mosaic viral particles.
152. The method of claim 99, wherein the stable viral vector producer cell line produces chimeric viral particles.
- 15 153. A method of characterizing a viral vector comprising a barcoded concatemer molecule, wherein the viral vector is integrated, and wherein the barcoded concatemer comprises one or more unique molecular identifiers, the method comprising:
- a. sequencing the barcoded concatemer molecule to generate sequence reads;
 - 20 b. assembling the generated sequence reads; and
 - c. mapping the assembled sequence reads.
154. The method of claim 153, wherein the assembling of the generated sequence reads enables quantifying a number of viral vector genome constructs comprised in the barcoded concatemer.
- 25 155. The method of claim 153, wherein the mapping of the assembled sequence reads enables identification of a genomic location of an integrated viral vector comprising the barcoded concatemer molecule.
156. The method of claim 153, wherein the method comprises generating sequencing reads that comprise one or more unique molecular identifier sequences.
- 30 157. The method of claim 153, wherein the method comprises generating sequencing reads that comprise one or more vector genome sequences and one or more cell genomic sequences.

158. The method of claim 153, wherein the method comprises generating sequencing reads that comprise one or more vector genome sequences.
159. The method of claim 156, wherein the sequencing reads comprising the one or more unique molecular identifier sequences facilitates quantification of a number of viral vector genome constructs comprised in a concatemer.
160. The method of claim 157, wherein the sequencing reads comprising the one or more vector genome sequences and the one or more cell genomic sequences facilitates determination of a locus of integration of the barcoded concatemer molecule.
161. The method of claim 153, wherein the method comprises quantifying one or more selectable or reporter elements of a viral vector construct.
162. The method of claim 161, wherein the one or more selectable or reporter elements is a reporter gene, an epitope tag, or both.
163. The method of claim 161, wherein the one or more selectable or reporter elements is selected or detected by luminescence, absorbance, fluorescence, antibiotics, antigen-antibody interactions, or a combination thereof.
164. The method of claim 154, wherein the quantifying comprises quantifying a number of vector-vector junctions.
165. The method of claim 154, wherein the quantifying comprises quantifying a number of vector-cellular genome junctions.
166. The method of claim 154, wherein the quantifying comprises quantifying a number of vector-transgene junctions.
167. The method of claim 166, wherein the transgene comprises a gene of interest (GOI).