

5

CLAIMS

What is claimed:

1. A method of purifying a viral vector from a host cell, the method comprising:
 - (i) contacting a composition comprising the viral vector and a transgene protein contaminant with a first chromatography matrix capable of selectively binding the transgene protein contaminant; and
 - (ii) recovering the viral vector in the flow-through of the chromatography matrix, thereby separating the viral vector from the transgene protein contaminant.
2. The method of claim 1, further comprising
 - (iii) contacting the composition comprising the viral vector and the transgene protein contaminant with a second chromatography matrix capable of selectively binding the viral vector, and
 - (iv) eluting the viral vector from the second chromatography matrix, wherein steps (iii) and (iv) are performed prior to step (i).
3. The method of claim 1, further comprising
 - (iii) contacting the viral vector recovered from step (ii) with a second chromatography matrix capable of selectively binding the viral vector, and
 - (iv) eluting the viral vector from the second chromatography matrix.
4. The method of any one of the preceding claims, wherein step (i) and (ii) are repeated multiple times.
5. The method of any one of the preceding claims, wherein step (i) is conducted in the presence of an agent that stabilizes the transgene protein contaminant.
6. The method of claim 5, wherein the agent is CaCl_2 .

- 5 7. The method of any one of the preceding claims, further comprising the step of (v)
adjusting the concentration of a salt of the composition comprising the viral vector and
the transgene protein contaminant to a target salt concentration.
8. The method of claim 7, wherein step (v) is performed prior to step (i).
- 10 9. The method of claim 7 or 8, wherein if steps (iii) and (iv) are performed prior to step (i),
then step (v) is performed between steps (iv) and (i).
10. The method of any one of claims 7-9, wherein the salt is NaCl.
- 15 11. The method of any one of claims 7-10, wherein the target salt concentration is from about
0.2M to about 0.6M.
12. The method of any one of claims 7-11, wherein the target salt concentration is about
- 20 0.4M.
13. The method of claim 12, wherein the target salt concentration is from about 200 mM
NaCl to about 600mM NaCl.
- 25 14. The method of any one of the previous claims, wherein step (i) is performed by loading
the first chromatography matrix with the composition comprising the viral vector and the
transgene protein contaminant in a loading buffer comprising Tris-HCl buffer or
Phosphate buffer at a pH about 7.0 to about 7.5.
- 30 15. The method of any one of the previous claims, step (iv) is performed by eluting the viral
vector from the second chromatography matrix with an elution buffer comprising Tris-
HCl buffer or Phosphate buffer at a pH about 7.0 to about 7.5.
- 35 16. The method of any one of the preceding claims, further comprising the step of (vi)
combining the viral vector separated from the transgene protein contaminant with one or
more pharmaceutical excipients to produce a pharmaceutical composition comprising the

- 5 viral vector, wherein the pharmaceutical composition is substantially free of the transgene protein contaminant.
17. The method of claim 16, wherein step (vi) is performed by ultrafiltration / diafiltration (UF/DF) of the viral vector separated from the transgene protein contaminant with a
10 formulation buffer.
18. The method of claim 17, wherein the UF/DF step comprises tangential flow filtration (TFF).
- 15 19. The method of claim 17, wherein the formulation buffer is a phosphate or histidine buffer comprising NaCl and Sucrose.
- 20 20. The method of any one of claims 16-19, wherein the pharmaceutical composition contains less than 20% of the transgene protein contaminant present in a reference viral vector composition purified with the second chromatography matrix but not the first chromatography matrix.
21. The method of any one of the preceding claims, wherein the viral vector is an enveloped viral vector.
25
22. The method of claim 21, wherein the enveloped viral vector is a lentiviral vector.
23. The method of any one of the preceding claims, wherein the viral vector encodes the transgene protein contaminant.
30
24. The method of any one of the preceding claims, wherein the transgene protein contaminant is produced in the host cell by expression from a transfer plasmid.
25. The method of any one of the preceding claims, wherein the first chromatography matrix
35 is selected from the group consisting of an affinity chromatography column, a cationic

- 5 exchange (CEX) chromatography column, a multimodal chromatography (MMC) column
and a hydrophobic interaction chromatography (HIC) column.
26. The method of any one of the preceding claims, wherein the first chromatography matrix
is an affinity chromatography column comprising an affinity ligand that specifically
10 binds to the transgene protein contaminant.
27. The method of any one of the preceding claims, wherein the second chromatography
matrix is an anionic exchange (AEX) chromatography matrix.
- 15 28. The method of any one of the preceding claims, wherein the second chromatography
matrix is an anionic exchange (AEX) membrane.
29. The method of any one of the preceding claims, wherein the transgene protein is a
clotting factor.
- 20 30. The method of claim 29, wherein the clotting factor is FVIII or FVIII_{XTEN}.
31. The method of claim 30, wherein the affinity chromatography matrix is VIIISelect.
- 25 32. The method of any one of the preceding claims, wherein the host cell is a mammalian cell
or an insect cell.
33. The method of claim 32 wherein the mammalian cell is a CHO cell, a HEK293 cell, or a
HeLa cell.
- 30 34. The method of any one of the preceding claims, wherein the composition comprising the
viral vector and the transgene protein contaminant is a cell culture supernatant generated
by culturing the host cell and separating and clarifying the cell culture supernatant from
the host cell.
- 35

- 5 35. The method of claim 34, wherein the cell culture supernatant is subjected to nuclease treatment.
36. A method of purifying a lentiviral vector (LVV) from a host cell, the lentiviral vector comprising a FVIII transgene, the method comprising:
- 10 (i) contacting a composition comprising the lentiviral vector and a FVIII transgene protein contaminant with a first chromatography matrix capable of selectively binding FVIII transgene protein contaminant; and
- (ii) recovering the lentiviral vector in the flow-through of the chromatography matrix, thereby separating the lentiviral vector from the FVIII protein contaminant.
- 15 37. The method of claim 36, further comprising
- (iii) contacting the composition comprising the lentiviral vector and the FVIII transgene protein contaminant with a second chromatography matrix capable of selectively binding the lentiviral vector, and
- 20 (iv) eluting the lentiviral vector from the second chromatography matrix, wherein steps (iii) and (iv) are performed prior to step (i), thereby separating the lentiviral vector from host cell contaminants.
38. The method of claim 37, further comprising
- 25 (iii) contacting the lentiviral vector recovered from step (ii) with a second chromatography matrix capable of selectively binding the lentiviral vector, and
- (iv) eluting the lentiviral vector from the second chromatography matrix.
39. The method of any one of claims 36-38, wherein step (i) and (ii) are repeated multiple
- 30 times.
40. The method of any one of claims 36-39, wherein step (i) is conducted in the presence an agent that stabilizes the transgene protein contaminant.
- 35 41. The method of claim 40, wherein the agent is CaCl_2 .

5

42. The method of any one of claims 36-41, further comprising the step of (v) adjusting the concentration of a salt of the composition comprising the lentiviral vector and a FVIII transgene protein contaminant to a target salt concentration.

10

43. The method of claim 42, wherein step (v) is performed prior to step (i).

44. The method of claim 42 or 43, wherein if steps (iii) and (iv) are performed prior to step (i), then step (v) is performed between steps (iv) and (i).

15

45. The method of any one of claims 42-44, wherein the salt is NaCl.

46. The method of any one of claims 42-45, wherein the target salt concentration is from about 0.2M to about 0.6M.

20

47. The method of any one of claims 42-46, wherein the target salt concentration is about 0.4M.

25

48. The method of any one of claims 36-47, further comprising the step of (vi) combining the lentiviral vector separated from the transgene protein contaminant with one or more pharmaceutical excipients to make a pharmaceutical composition comprising the lentiviral vector, wherein the pharmaceutical composition is substantially free of the FVIII transgene protein contaminant.

30

49. The method of claim 48, wherein step (vi) is performed by ultrafiltration / diafiltration (UF/DF) of the lentiviral vector separated from the transgene protein contaminant with a formulation buffer.

35

50. The method of claim 49, wherein the formulation buffer is a phosphate or histidine buffer comprising NaCl and Sucrose.

- 5 51. The method of claim 49, wherein the UF/DF step comprises tangential flow filtration (TFF).
52. The method of any one of claims 36-51, wherein the pharmaceutical composition contains less than 20% of the FVIII transgene protein contaminant present in a reference
10 viral vector composition purified with the second chromatography matrix but not the first chromatography matrix.
53. The method of any one of claim 36-52, wherein the first chromatography matrix is selected from the group consisting of affinity chromatography column, cationic exchange
15 (CEX) chromatography column, multimodal chromatography (MMC) column and hydrophobic interaction chromatography (HIC) column.
54. The method of any one of claims 36-53, wherein the first chromatography matrix is an affinity chromatography column comprising an affinity ligand that specifically binds to
20 the recombinant FVIII protein.
55. The method of claim 54, wherein the affinity ligand is VIIISelect.
56. The method of any one of claims 36-55, wherein the FVIII transgene protein contaminant
25 is a B-domain deleted FVIII protein.
57. The method of any one of claims 36-55, wherein the FVIII transgene protein contaminant is a human FVIII protein comprising the amino acid sequence of SEQ ID NO:4.
- 30 58. The method of any one of claims 36-55, wherein the FVIII transgene protein contaminant is a FVIIIXTEN molecule.
59. The method of claim 58, wherein the FVIIIXTEN molecule comprises the amino acid
35 sequence of SEQ ID NO:5.

- 5 60. The method of any one of claims 36-59, wherein the second chromatography matrix is an anionic exchange (AEX) chromatography matrix.
61. The method of any one of claims 36-59, wherein the second chromatography matrix is an anionic exchange (AEX) membrane.
- 10 62. The method of any one of claims 36-59, wherein the host cell is a mammalian cell or an insect cell.
63. The method of claim 62, wherein the mammalian cell is a CHO cell, a HEK293 cell, or a HeLa cell.
- 15 64. The method of any one of claims 36-63, wherein the composition comprising the viral vector and the FVIII transgene protein contaminant is a cell culture supernatant generated by culturing the host cell and separating and clarifying the cell culture supernatant from the host cell.
- 20 65. The method of claim 64, wherein the cell culture supernatant is subjected to nuclease treatment.
- 25 66. A composition comprising a viral vector produced according the method of any one of claims 1-65.
67. The composition of claim 66, wherein the composition is a pharmaceutical composition comprising the viral vector and one or more pharmaceutical excipients, and wherein the composition is substantially free of transgene protein contaminant.
- 30 68. The composition of claim 66 or 67, wherein the transgene protein is a clotting factor.
69. The composition of claim 68, wherein the clotting factor is FVIII, FVIIIXTEN, or FIX.

35

- 5 70. The composition of claim 68, wherein the clotting factor is a B-domain deleted FVIII protein.
71. The composition of claim 68, wherein the clotting factor is a human FVIII protein comprising the amino acid sequence of SEQ ID NO:4.
- 10 72. The composition of claim 68, wherein the clotting factor is a FVIII-XTEN molecule.
73. The composition of claim 72, wherein the FVIII-XTEN molecule comprises the amino acid sequence of SEQ ID NO:5.
- 15 74. The composition of any one of claims 66-73, wherein the composition contains less than 20% of the transgene protein present in a reference viral vector composition purified with the second chromatography matrix but not the first chromatography matrix.