

CLAIMS

What is claimed is:

1. A method of purifying a viral vector composition comprising the steps of:
 - (a) feeding a composition comprising a viral vector through an SPTFF system, the system comprising:
 - (i) a feed pump; and
 - (ii) an SPTFF filtration module, wherein the SPTFF filtration module purifies the viral vector composition; and
 - (b) collecting a purified viral vector composition.
2. A method of concentrating viral vector composition comprising the steps of:
 - (a) feeding a composition comprising viral vector through an SPTFF system, the system comprising:
 - (i) a feed pump; and
 - (ii) an SPTFF filtration module, wherein the SPTFF filtration module concentrates the viral vector composition; and
 - (b) collecting a concentrated viral vector composition.
3. The method of claim 1 or claim 2, wherein the viral vector is derived from an adeno-associated virus or a lentivirus.
4. The method of any one of the preceding claims, wherein the viral vector is derived from a lentivirus.
5. The method of any one of the preceding claims, wherein the SPTFF system does not comprise an affinity chromatography component.

6. The method of any one of the preceding claims, wherein the SPTFF system comprises one or more SPTFF filtration modules.
7. The method of any one of the preceding claims, wherein the SPTFF filtration module(s) comprise three or more tangential flow filtration (TFF) cassettes or hollow fiber cartridges.
8. The method of any one of the preceding claims, wherein the SPTFF filtration module(s) comprise four or more tangential flow filtration (TFF) cassettes or hollow fiber cartridges.
9. The method of any one of the preceding claims, wherein the SPTFF filtration module(s) comprise five or more tangential flow filtration (TFF) cassettes or hollow fiber cartridges.
10. The method of any one of the preceding claims, wherein the SPTFF filtration module(s) comprise six or more tangential flow filtration (TFF) cassettes or hollow fiber cartridges.
11. The method of any one of the preceding claims, wherein the SPTFF filtration module(s) comprise seven or more tangential flow filtration (TFF) cassettes or hollow fiber cartridges.
12. The method of any one of the preceding claims, wherein the SPTFF filtration module(s) comprise eight or more, nine or more, ten or more, eleven or more, twelve or more, or thirteen or more, tangential flow filtration (TFF) cassettes or hollow fiber cartridges.
13. The method of any one of claims 7-12, wherein the TFF cassettes comprise one or more flat sheet membranes.
14. The method of any one of claims 7-12, wherein the hollow fiber cartridges comprise one or more hollow fiber membranes.
15. The method of claim 13 or claim 14, wherein the one or more membranes comprise an average molecular weight cut-off (MWCO) selected from the group consisting of: about 1 kDa,

about 5 kDa, about 10 kDa, about 20 kDa, about 30 kDa, about 40 kDa, about 50 kDa, about 60 kDa, about 70 kDa, about 80 kDa, about 90 kDa, about 100 kDa, about 200 kDa, about 300 kDa, about 400 kDa, and about 500 kDa.

16. The method of claim 13 or claim 14, wherein the one or more membranes comprises an MWCO of about 30 kDa.

17. The method of claim 13 or claim 14, wherein the one or more membranes comprises an MWCO of about 300 kDa.

18. The method of any one of claims 7-17, wherein two or more TFF cassettes or hollow fiber cartridges are configured for processing in parallel.

19. The method of any one of claims 7-18, wherein two or more TFF cassettes or hollow fiber cartridges are configured for processing in serial.

20. The method of any one of claims 7-19, wherein the TFF cassettes or hollow fiber cartridges are configured for processing in parallel and serial.

21. The method of any one of the preceding claims, wherein the viral vector composition follows a flow path through the SPTFF system and/or SPTFF filtration module.

22. The method of any one of claims 7-21, wherein the TFF cassettes or hollow fiber cartridges have an effective membrane area.

23. The method of claim 22, wherein the effective membrane area decreases along the flow path within the SPTFF filtration module.

24. The method of any one of the preceding claims, wherein the system comprises (a) a viral vector composition feed flow rate entering the SPTFF filtration module and (b) a viral vector composition retentate flow rate exiting the SPTFF filtration module.
25. The method of claim 24, wherein the viral vector composition feed flow rate entering the SPTFF filtration module is at least about 10x greater than the viral vector composition retentate flow rate exiting the SPTFF filtration module.
26. The method of claim 24, wherein the viral vector composition retentate flow rate exiting the SPTFF filtration module is at least about 10x less than the viral vector composition feed flow rate entering the SPTFF filtration module.
27. The method of any one of the preceding claims, wherein the SPTFF filtration module has an average transmembrane pressure (TMP) of about 10 psi or lower, about 9 psi or lower, about 8 psi or lower, about 7 psi or lower, about 6 psi or lower, about 5 psi or lower, about 4 psi or lower, about 3 psi or lower, or about 2 psi or lower.
28. The method of claim 27, wherein the SPTFF filtration module has an average transmembrane pressure (TMP) of about 5 psi or lower.
29. The method of any one of the preceding claims, wherein the feed pump is positioned immediately before the SPTFF filtration module and continuous with the flow path.
30. The method of any one of the preceding claims, wherein the system further comprises a retentate pump after the SPTFF filtration module(s) and continuous with the flow path.
31. The method of any one of the preceding claims, wherein the system further comprises a waste or permeate pump.

32. The method of any one of the preceding claims, wherein the SPTFF system does not comprise centrifugation.
33. The method of any one of the preceding claims, wherein the viral vector composition is not recirculated through the SPTFF system, SPTFF filtration module(s), and/or any TFF cassettes or hollow fiber cartridges within the SPTFF filtration module(s).
34. The method of any one of the preceding claims, wherein the system further comprises a single-pass diafiltration (SPDF) component following the SPTFF filtration module(s).
35. The method of any one of the preceding claims, wherein the system further comprises a polishing chromatography component after the SPTFF filtration module(s).
36. The method of claim 35, wherein the polishing chromatography component comprises a hydrophobic interaction resin, a size exclusion resin, and/or an ion exchange resin.
37. The method of claim 35, wherein the polishing chromatography component comprises an anion exchange resin.
38. The method of claims 35-37, wherein the polishing chromatography component removes host cell protein and/or host gDNA from the viral vector composition.
39. The method of any one of the preceding claims, wherein the SPTFF system further comprises a 0.22 μ M final filter.
40. The method of any one of the preceding claims, wherein the SPTFF system further comprises a mechanism to add a nuclease to the viral vector composition.
41. The method of claim 40, wherein the nuclease is added to the viral vector composition prior to feeding the viral vector composition through the SPTFF filtration module.

42. The method of claim 40, wherein the nuclease is added to the viral vector composition after the viral vector composition has passed through the SPTFF filtration module.
43. The method of any one of claims 40-42, wherein the nuclease is a denarase DNA endonuclease or the like.
44. The method of any one of the preceding claims, wherein the system further comprises a bioreactor.
45. The method of claim 44, wherein the bioreactor comprises viral vector producer cells.
46. The method of claim 45, wherein the viral vector producer cells are lentiviral vector producer cells.
47. The method of claim 45 or claim 46, wherein the producer cells are maintained in suspension.
48. The method of any one of claims 45-47, wherein the producer cells are HEK 293 cells.
49. The method of claim 48, wherein the HEK 293 cells are HEK 293T or HEK 293F cells.
50. The method of any one of the preceding claims, wherein the viral vector comprises a polynucleotide encoding a therapeutic protein.
51. The method of claim 50, wherein the therapeutic protein is an engineered $\alpha\beta$ TCR, an engineered $\gamma\delta$ TCR, a dimerizing agent regulated immunoreceptor complex (DARIC), a chimeric antigen receptor (CAR), a chimeric costimulatory receptor (CCR), a bispecific T cell engager (BiTE), a zetakine receptor, a β -globin protein, an ABCD1 polypeptide, an erythropoietin receptor or fragment thereof, an endonuclease, or a megaTAL.

52. The method of any one of the preceding claims, wherein the viral vector comprises a polynucleotide encoding an shRNA, a shmiR, or a guide RNA.