

**CLAIMS**

1. A method of making a stable viral vector producer cell line, said method comprising:
  - a. introducing into a population of cells a viral vector genome construct encoding a gene of interest (GOI) and one or more viral accessory constructs encoding one or more viral accessory proteins;
  - b. producing a population of transgenic cells comprising integrated or episomal sequences encoding said GOI and said one or more viral accessory proteins;
  - c. selecting from said population of transgenic cells a cell clone producing a desired viral titer; and
  - d. generating from said cell clone a stable viral vector producer cell line,wherein the introduction of said one or more accessory constructs occurs concurrently.
2. A method of making a stable viral vector producer cell line, said method comprising:
  - a. introducing into a population of cells a viral vector genome construct encoding a gene of interest (GOI) and one or more viral accessory constructs encoding one or more viral accessory proteins;
  - b. producing a population of transgenic cells comprising integrated or episomal sequences encoding said GOI and said one or more viral accessory proteins;
  - c. selecting from said population of transgenic cells a cell clone producing a desired viral titer; and
  - d. generating from said cell clone a stable viral vector producer cell line,wherein the introduction of said one or more accessory constructs occurs via one or more sequential steps with no intervening cell culturing.
3. The method of claim 1 or 2, wherein said transgenic cells comprise polyclonal cells.
4. The method of claim 1 or 2, wherein said selecting further comprises polyclonal to monoclonal selection of said transgenic cells.
5. The method of claim 1 or 2, wherein said method further comprises storing said selected cell line by cryopreservation.

6. The method of claim 5, wherein said method further comprises expanding cells from said cryopreserved cell line to produce viral vectors.
- 5 7. The method of claim 1 or 2, wherein said method further comprises quantifying the level of said viral vector genome and said one or more accessory proteins in said selected cell clone, said generated cell line, or both.
- 10 8. The method of claim 1 or 2, wherein said method further comprises determining the stoichiometric ratio of viral vector genome RNA and one or more accessory proteins in said selected cell clone, said generated cell line, or both.
- 15 9. The method of claim 1 or 2, wherein said method further comprises determining an integration profile of said selected cell clone, said generated cell line, or both.
10. The method of claim 1 or 2, wherein said method further comprises harvesting viral vector from said selected cell clone, said generated cell line, or both.
- 20 11. The method of claim 1 or 2, wherein said method further comprises determining a viral titer of said selected cell clone, said generated cell line, or both.
12. The method of claim 1 or 2, wherein said viral vector producer cell line produces a viral vector derived from a retrovirus.
- 25 13. The method of claim 1 or 2, wherein said viral vector producer cell line produces a viral vector derived from a lentivirus.
14. The method of claim 1 or 2, wherein said viral vector producer cell line produces a viral vector derived from an adeno-associated virus.
- 30 15. The method of claim 1 or 2, wherein said viral vector producer cell line produces a viral vector comprising one or more capsid proteins.
16. The method of claim 1 or 2, wherein said viral vector producer cell line produces a viral vector comprising one or more envelope proteins.

17. The method of claim 1 or 2, 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.
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18. The method of claim 1 or 2 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.
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19. The method of claim 1 or 2, wherein said viral vector genome construct comprises a self-inactivating long terminal repeat.
20. The method of claim 1 or 2, wherein said one or more viral accessory proteins comprise sequences encoding structural viral proteins, regulatory viral proteins, or both.
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21. The method of claim 20, wherein said structural proteins and/or regulatory proteins are selected from the group consisting of Gag, Pol, Rev, Env, Tat, Nef, Vpr, Vif, Vpu, and Vpx.
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22. The method of claim 1 or 2, wherein said introducing step comprises a transduction.
23. The method of claim 1 or 2, wherein said introducing step comprises a transfection.
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24. The method of claim 1 or 2, wherein said stable viral vector producer cell line is adapted for adherent culturing or culturing in suspension.
25. The method of claim 1 or 2, wherein said stable viral vector producer cell line is cultured in a serum-supplemented or serum-free medium.
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26. The method of claim 1 or 2, wherein said stable viral vector producer cell line is a HEK293 cell or a derivative thereof.

27. The method of claim 1 or 2, wherein said stable viral vector producer cell line produces chimeric viral particles.

28. The method of claim 1 or 2, wherein a predetermined or pre-selected ratio of the viral  
5 vector genome construct and the one or more viral accessory constructs are used.

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