

CLAIMS

We claim:

1. A method of determining potency of a recombinant viral vector encoding at least one SMN polypeptide comprising:

(a) transducing modified host cells with the recombinant viral vector, wherein the modified host cells comprise decreased expression of the SMN polypeptide relative to an unmodified reference host cell of the same type;

(b) contacting the modified host cells with a first agent for detection of the SMN polypeptide;

(c) contacting the modified host cells with a second agent comprising a detection moiety for detection of the first agent; and

(d) detecting presence of Gemini of coiled bodies (GEMs), thereby determining potency of the at least one SMN polypeptide.

2. The method of claim 1, wherein the recombinant viral vector comprises an adeno-associated viral (AAV) vector, an adenoviral vector, or a retroviral vector.

3. The method of claim 2, wherein the retroviral vector comprises a lentiviral vector or a gammaretroviral vector.

4. The method of claim 2, wherein the AAV vector comprises AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, or a variant thereof.

5. The method of claim 4, wherein the AAV vector comprises AAVhu68.

6. The method of claim 4 or 5, wherein the AAV vector comprises a *SMN1* gene operably linked to a chicken- β actin promoter (CB7).

7. The method of any one of claims 4-6, wherein the AAV vector comprises two ITRs flanking the *SMN1* gene.

8. The method of any one of claims 4-7, wherein the AAV vector comprises a rabbit β -globin polyA signal.

9. The method of any one of claims 1-8, wherein the modified host cells comprise a conditional knockdown or a knockout of an *SMN1* gene.

10. The method of any one of claims 1-9, wherein the modified host cells comprise at least one shRNA for conditional knockdown of an *SMN1* gene.

11. The method of claim 9, wherein the at least one shRNA:

- (i) comprises shRNA120 or shRNA 128; and/or
- (ii) does not target the recombinant viral vector.

12. The method of any one of claims 1-11, wherein the modified host cells comprise or are mammalian host cells.

13. The method of claim 12, wherein the modified host cells comprise or are human cells.

14. The method of claim 13, wherein the modified host cells comprise or are SH-SY5Y cells.

15. The method of claim 14, wherein the modified host cells comprise or are SH-SY5Y KD cells.

16. The method of any one of claims 1-15, wherein, prior to transduction, one or more of the following occurs:

- (i) host cells are frozen and thawed at least once;
- (ii) host cells are passaged at least 3 times;
- (iii) host cells are treated with doxycycline; and/or
- (iv) host cells are seeded at a density of about 5.0×10^3 to about 5.0×10^4 cells/well.

17. The method of any one of claims 1-15, wherein the modified host cells are seeded and transduced within a 24 hour period.
18. The method of any one of claims 1-17, wherein the transduction step (b) is performed at about 5 different MOIs achieved by serial dilution.
19. The method of claim 18, wherein the transduction step (b) is performed with an MOI of about 6.1×10^5 VG/cell to about 4×10^6 VG/cell.
20. The method of any one of claims 1-19, wherein a signal to noise ratio is greater than or about 2.5.
21. The method of any one of claims 1-20, wherein the first agent comprises an anti-SMN1 antibody or an antigen-binding fragment thereof or an aptamer.
22. The method of any one of claims 1-21, wherein the detection moiety comprises or is a fluorescent, colorimetric, or enzymatic label.
23. The method of claim 22, wherein the second agent comprises a fluorescently labeled secondary antibody or an antigen-binding fragment thereof.
24. The method of any one of claims 1-23, wherein the presence of GEMs is detected by immunofluorescence.
25. The method of one of claims 1-24, wherein the presence of GEMs is detected by imaging.
26. The method of claim 25, wherein the imaging comprises or is High-Content Imaging (HCI).

27. The method of any one of claims 1-26, wherein the method is performed without or substantially without use of at least one helper function.

28. The method of claim 27, wherein the at least one helper function comprises an Ad2 or Ad5 helper virus.

29. The method of any one of claims 1-28, wherein lower amounts of recombinant viral vector are required for transduction than transducing with an unmodified reference host cells of the same type or a different type.

30. The method of any one of claims 1-29, wherein the method has a low standard deviation.

31. The method of any one of claims 1-30, wherein potency is determined with an accuracy of about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more.

32. The method of claim 31, wherein potency is determined with a precision of about 5%, about 6%, about 7%, about 8%, about 9%, or about 10%.

33. The method of any one of claims 1-32, wherein the method indicates stability of the recombinant viral vector, *e.g.*, following thermal stress of the recombinant viral vector.

34. The method of any one of claims 1-33, wherein potency of the recombinant viral vector is not affected by presence of empty capsids.

35. The method of any one of claims 1-34, wherein the recombinant viral vector comprises a plurality of empty virus capsids.