## CLAIMS

1. A method of manufacturing an AAV viral vector, comprising:

   a. culturing adherent cells;

   b. transfecting the adherent cells with plasmid(s) to enable production of the AAV viral vector;

   c. lysing the adherent cells to isolate the AAV viral vector;

   d. acidifying and clarifying the cell lysate of (c);

   e. purifying the product of (d) using cation exchange chromatography (CEX);

   f. filtering the product of (e) using tangential flow filtration (TFF);

   g. ultracentrifuging the product of (f) in a cesium chloride (CsCl) buffer; and

   h. collecting the AAV viral vectors from the product of (g).

2. The method of claim 1, wherein the AAV is AAV9.

3. The method of claims 1 or 2, wherein the AAV is self-complementary (scAAV).

4. The method of any one of claims 1-3, wherein the adherent cells are HEK293 cells.

5. The method of claim 4, wherein step (a) comprises seeding the cells in a large-scale bioreactor that can provide continuous circulation of cell culture media.

6. The method of claim 5, wherein the seeding density is 8,000-12,000 cells/$cm^2$.

7. The method of any one of claims 1-6, wherein the transfecting step (b) comprises contacting the adherent cell with an adenovirus helper plasmid (pHELP), and a plasmid encoding AAV rep and cap genes.

8. The method of claim 7, wherein the AAV rep gene is rep2 and the AAV cap gene is cap9.

9. The method of any one of claims 1-8, wherein the transfecting step (b) comprises contacting the adherent cells with a plasmid comprising a polynucleotide encoding a survival motor neuron (SMN) protein, a polynucleotide encoding MeCP2 or a polynucleotide encoding a SOD1 shRNA.

10. The method of claim 9, wherein the plasmid comprising the polynucleotide encoding SMN1 protein comprises a modified AAV2 ITR, a chicken beta-actin (CB) promoter,

a cytomegalovirus (CMV) immediate/early enhancer, a modified SV40 late 16s intron, a Bovine growth hormone (BGH) polyadenylation signal, and an AAV2 ITR.

11. The method of claim 9 or 10, wherein the polynucleotide encoding SMN encodes the SMN protein of SEQ ID NO: 2.

12. The method of any one of claims 1-11, wherein the AAV viral vector comprises SEQ ID NO: 1.

13. The method of any one of claims 1-12, wherein the transfecting step comprises contacting the adherent cell with the transfection agent polyethylenimine (PEI).

14. The method of any one of claims 1-13, wherein the transfecting step comprises contacting the adherent cells with a transfection medium that does not contain serum, calcium, or glutamine.

15. The method of any one of claims 1-14, wherein the lysing step comprises a lysis buffer supplemented with benzonase and Tween.

16. .The method of any one of claims 1-15, further comprising freezing the cell lysate of step (c) prior to the acidification step of (d).

17. A method of purifying an AAV viral vector from a cell culture lysate, comprising the steps of:

    a. acidifying and clarifying the cell lysate;

    b. purifying the product of (a) using cation exchange chromatography (CEX);

    c. filtering the product of (b) through a tangential flow filtration;

    d. ultracentrifuging the product of (c) using a 2 – 4 M cesium chloride (CsCl) buffer;

    e. collecting the AAV viral vectors from the product of (d);

    f. filtering the product of (e) through a tangential flow filtration.

18. The method of any one of claims 1-17, wherein the acidification step comprises acidifying the cell lysate to a pH of 3.0 – 4.0.

19. The method of any one of claims 1-18, wherein the ultracentrifugation is performed between 40,000 – 50,000 rpm.

20. The method of any one of claims 1-19, wherein the CsCl buffer comprises 3 M CsCl, Tris, $MgCl_2$, and Poloxamer 188, and is between pH 7.5 and 8.5.

21. The method of any one of claims 1-20, wherein the cell lysate is incubated with Tween prior to the acidification step.

22. The method of any one of claims 1-21, wherein the clarification step comprises filtering the cell lysate through a depth filter.

23. The method of any one of claims 1-22, wherein the CEX comprises a sulfonyl resin.

24. The method of any one of claims 1-23, wherein at least one TFF step comprises using cellulose membranes with a molecular weight cutoff of 300 kDa MW.

25. The method of any one of claims 1-24, wherein the number of empty viral capsid is less than 7%, less than 5%, less than 3% or less than 1 % of the total viral capsids after collecting the AAV viral vectors from the ultracentrifuged cell lysate.

26. The method of any one of claims 17-25, wherein the AAV viral vectors collected after the second TFF step are stored in a solution comprising Tris, $MgCl_2$, NaCl, and Poloxamer 188, and the pH is between 7.5 and 8.5.

27. The method of any one of claims 17-26, wherein the AAV viral vectors collected after the second TFF contain less than 30 μg/g or less than 20 μg/g of CsCl.

28. The method of any one of claims 17-27, wherein the concentration of AAV viral vectors collected after the second TFF is greater than or equal to $3x10^{13}$ vg/ml.

29. The method of any one of claims 1-28, wherein the produced yield of AAV is more than $5 \times 10^{15}$ vg, or more than $8 \times 10^{15}$ vg or more than $1 \times 10^{16}$ vg per manufacturing batch.

30. The method of any one of claims 1-29, wherein the AAV vector comprises a polynucleotide encoding a survival motor neuron (SMN) protein, a polynucleotide encoding MeCP2 or a polynucleotide encoding a SOD1 shRNA.