CLAIMS

What is claimed is:

1. A method of manufacturing lentiviral particles, comprising:

a) culturing a plurality of mammalian cells that do not express a large T antigen, at a first pH,

b) contacting the plurality of mammalian cells with a transfection complex comprising:

i) FectoVIR®-AAV transfection reagent; and

ii) a nucleic acid encoding a transgene and one or more LTR sequences for packaging into a viral particle;

c) culturing the plurality of mammalian cells under conditions suitable for production of the lentiviral particles;

d) harvesting the lentiviral particles; and

e) purifying the lentiviral particles to produce a filtrate.

2. The method of claim 1, wherein the transfection complex comprises one or more nucleic acids encoding a retroviral envelope protein and a retroviral packaging protein.

3. The method of claim 1 or 2, wherein the transgene encodes a CAR comprising an antigen binding domain, a transmembrane domain, and one or more signaling domains.

4. The method of any one of claims 1-3, wherein:

a) the transfection complex comprises FectoVIR®-AAV at a concentration of 0.3 – 0.6 μL FectoVIR®-AAV per million cells;

b) the transfection complex comprises the nucleic acid encoding the transgene and the one or more nucleic acids encoding a retroviral envelope protein and a retroviral packaging protein at a concentration of 0.3 – 0.6 μg per million cells; or

c) the transfection complex comprises a ratio of FectoVIR®-AAV to nucleic acid of 1:0 to 1:2.

5. The method of any one of claims 1-4, wherein the first pH is lowered to a second pH prior to contacting the cells with the transfection complex.

6. The method of claim 5, wherein the second pH is 0.1 – 1.0 pH units lower than the first pH.

7. The method of claim 5 or 6, wherein the second pH is 0.5 pH units lower than the first pH.

8. The method of any one of claims 1-7, wherein a nuclease is added prior to step d).

9. The method of claim 8, wherein the nuclease is benzonase.

10. The method of any one of claims 1-9, wherein a solution comprising arginine is added prior to step e).

11. The method of claim 10, wherein the arginine concentration prior to step e) is 25-50 mM or 50-100 mM.

12. The method of claim 10 or 11, wherein the solution further comprises a buffer, a salt, a non-reducing carbohydrate, and/or a pH of 6.0 to 7.0.

13. The method of claim 12, wherein:

(a) the buffer is PIPES at a concentration of 10 mM to 50 mM;

(b) the salt is at a concentration of 25-150 mM; or

(c) the salt is selected from the group consisting of sodium chloride, magnesium chloride, and calcium chloride.

14. The method of claim 12 or 13, wherein the non-reducing carbohydrate is present in the solution at a concentration of from 1 % to 10% by weight per volume of said solution or at a concentration of 30-150 mM or 150-300 mM.

15. The method of any one of claims 12-14, wherein the non-reducing carbohydrate is selected from the group consisting of sucrose and trehalose.

16. The method of any one of claims 1-15, wherein a solution comprising arginine is added following step e).

17. The method of claim 16, wherein the arginine concentration in the filtrate is at a concentration of 100-200 mM or 200-400 mM.

18. The method of any one of claims 1-17, wherein:

(a) the method yields at least $1x10^7$ lentiviral particles;

(b) the filtrate has a reduced aggregation of the lentiviral particles; or

(c) the filtrate has a total particle concentration per mL of less than 400,000, as measured by micro-flow imaging.

19. The method of any one of claims 1-18, wherein the mammalian cells are Expi293F cells.