

We claim:

1. A method for the purification of a recombinant flavivirus viral particle without loss of infectivity from a host cell culture comprising the steps of:

- a. recovering from a host cell culture flavivirus viral particles from the host cells;
- 5 b. subjecting the solution obtained from step (a) to tangential flow filtration, comprising filtration through one or more hollow fiber TFF cassettes;
- c. applying the retentate from the tangential flow filtration step to an anion exchange chromatography resin comprising quaternary amino groups or diethylaminoethane groups on one or more anionic exchange monolithic columns;
- 10 d. eluting the flavivirus viral particles from the anion exchange chromatography resin; and
- e. recovering the purified flavivirus viral particles.

2. The method of claim 1 wherein the flavivirus viral particles comprise REPLIVAX®
15 PIVs.

3. The method of claim 1 wherein the flavivirus viral particles comprise chimeric flaviviruses (CHIMERIVAX®).

20 4. The method of claim 1 wherein the flavivirus viral particles comprise flaviviruses transmitted via an arthropod vector.

5. The method of claim 4 wherein the flaviviruses are selected from the group comprising yellow fever viruses, Japanese encephalitis viruses, dengue viruses, West Nile
25 viruses, and tick-borne encephalitis viruses.

6. The method of claim 1 wherein the recombinant flaviviruses comprise yellow fever viruses.

30 7. The method of claim 6 wherein the recombinant yellow fever viruses comprises YF-17D virus.

8. The method of claim 1 wherein the host cell culture comprises mammalian cell culture.
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9. The method of claim 1 wherein the one or more hollow fiber TFF cassettes comprises a media selected from the following polysulfone, polyethersulfone, modified polyethersulfone or mixed cellulose ester medias.

5 10. The method of claim 9 wherein the hollow fiber TFF cassettes media is a polysulfone.

11. The method of claim 1 wherein the one or more anionic exchange monolithic columns comprise CONVECTIVE INTERACTIVE MEDIA® columns.

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12. The method of claim 1 wherein the recovering of flavivirus viral particles from the host cells comprises decanting medium from the host cell culture.

13. A pharmaceutically acceptable dosage form of infectious recombinant flavivirus viral particles produced in a host cell culture, said flavivirus viral particles isolated by the method comprising the steps of:

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a. recovering from a host cell culture flavivirus viral particles from the host cells;

b. subjecting the solution obtained from step (a) to tangential flow filtration, comprising filtration through one or more hollow fiber TFF cassettes;

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c. applying the retentate from the tangential flow filtration step to an anion exchange chromatography resin comprising quaternary amino groups or diethylaminoethane groups on one or more anionic exchange monolithic columns;

d. eluting the flavivirus viral particles from the anion exchange column;

e. recovering the purified flavivirus viral particles; and

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f. suspending the purified flavivirus viral particles in a pharmaceutically acceptable carrier.

14. The pharmaceutically acceptable dosage form of claim 13 wherein the flavivirus viral particles comprise REPLIVAX® PIVs.

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15. The pharmaceutically acceptable dosage form of claim 13 wherein the flavivirus viral particle comprise CHIMERIVAX® PIVs

16. The pharmaceutically acceptable dosage form of claim 13 wherein the flavivirus viral particle are flaviviruses transmitted via an arthropod vector.

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17. The pharmaceutically acceptable dosage form of claim 16 wherein the flaviviruses are selected from the group comprising yellow fever viruses, Japanese encephalitis viruses, dengue viruses, West Nile viruses, and tick-borne encephalitis viruses.

5 18. The pharmaceutically acceptable dosage form of claim 13 wherein the recombinant flaviviruses comprise yellow fever viruses.

19. The pharmaceutically acceptable dosage form of claim 18 wherein recombinant yellow fever viruses comprises YF-17D virus.

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20. The pharmaceutically acceptable dosage form of claim 13 wherein the host cell culture comprises mammalian cell culture.

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21. The pharmaceutically acceptable dosage form of claim 13 wherein the one or more hollow fiber TFF cassettes comprises a media selected from the following polysulfone, polyethersulfone, modified polyethersulfone or mixed cellulose ester medias.

22. The pharmaceutically acceptable dosage form of claim 21 wherein the hollow fiber TFF cassettes media is a polysulfone.

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23. The pharmaceutically acceptable dosage form of claim 13 wherein the one or more anionic exchange monolithic columns comprise CONVECTIVE INTERACTIVE MEDIA[®] columns.

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24. The pharmaceutically acceptable dosage form of claim 13 wherein the quantity of host cell DNA in said composition is less than 10 ng host cell DNA per dose, one dose being defined as 500 μ L of 2E+7 PFU/mL.

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25. The method of claim 1, wherein the purified flavivirus viral particles have an infectivity titer greater than 1×10^6 focus forming units (FFU) per mL.

26. The method of claim 1, wherein the hollow fiber TFF cassettes have a molecular weight cutoff of about 500 kDa.

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27. The method of claim 1, wherein the method results in about 50% or greater recovery of infectious virus titer.

We claim:

1. A method for the purification of a flavivirus viral particle from a host cell culture comprising the steps of:

- 5 a. recovering from a host cell culture flavivirus viral particles from the host cells;
- b. subjecting the solution obtained from step (a) to tangential flow filtration;
- c. applying the retentate from the tangential flow filtration step to an anion exchange chromatography resin;
- 10 d. eluting the flavivirus viral particles from the anion exchange chromatography resin; and
- e. recovering the purified flavivirus viral particles.

2. The method of claim 1 wherein the flavivirus viral particles are recombinant flavivirus viral particles.

15 3. The method of claim 2 wherein the flavivirus viral particles comprise REPLIVAX[®] PIVs.

4. The method of claim 2 wherein the flavivirus viral particles comprise chimeric flaviviruses (CHIMERIVAX[®]).

5. The method of claim 1 wherein the flavivirus viral particles comprise flaviviruses transmitted via an arthropod vector.

25 6. The method of claim 5 wherein the flaviviruses are selected from the group comprising yellow fever viruses, Japanese encephalitis viruses, dengue viruses, West Nile viruses, and tick-borne encephalitis viruses.

7. The method of claim 2 wherein the recombinant flaviviruses comprise yellow fever viruses.

8. The method of claim 7 wherein the recombinant yellow fever viruses comprises
5 YF-17D virus.

9. The method of claim 1 wherein the host cell culture comprises mammalian cell culture.

10. The method of claim 1 wherein the tangential flow filtration comprises filtration through one or more hollow fiber TFF cassettes.

11. The method of claim 10 wherein the one or more hollow fiber TFF cassettes comprises a media selected from the following polysulfone, polyethersulfone, modified
15 polyethersulfone or mixed cellulose ester medias.

12. The method of claim 11 wherein the hollow fiber TFF cassettes media is a polysulfone.

13. The method of claim 1 wherein the tangential flow filtration comprises filtration through a flat sheet media.

14. The method of claim 1 wherein the anion exchange chromatography resin comprises immobilized positively charged moieties.

15. The method of claim 14 wherein the immobilized positively charged moieties comprise quaternary amino groups or diethylaminoethane groups.

16. The method of claim 14 wherein the immobilized positively charged moieties are quaternary amino groups.

17. The method of claim 1 wherein the anion exchange chromatography resin
5 comprises one or more anionic exchange monolithic columns.

18. The method of claim 17 wherein the one or more anionic exchange monolithic columns comprise CONVECTIVE INTERACTIVE MEDIA® columns.

10 19. The method of claim 1 wherein the recovering of flavivirus viral particles from the host cells comprises decanting medium from the host cell culture.

20. A pharmaceutically acceptable dosage form of flavivirus viral particles produced in a host cell culture said flavivirus viral particles isolated by the method comprising the
15 steps of:

- a. recovering from a host cell culture flavivirus viral particles from the host cells;
- b. subjecting the solution obtained from step (a) to tangential flow filtration;
- c. applying the retentate from the tangential flow filtration step to an anion
20 exchange chromatography resin;
- d. eluting the flavivirus viral particles from the anion exchange column;
- e. recovering the purified flavivirus viral particles; and
- f. suspending the purified flavivirus viral particles in a pharmaceutically acceptable carrier.

25 21. The pharmaceutically acceptable dosage form of claim 20 wherein the flavivirus viral particles are recombinant flavivirus viral particles.

22. The pharmaceutically acceptable dosage form of claim 22 wherein the flavivirus viral particles comprise REPLIVAX® PIVs.

23. The pharmaceutically acceptable dosage form of claim 21 wherein the flavivirus viral particle comprise CHIMERIVAX® PIVs

24. The pharmaceutically acceptable dosage form of claim 21 wherein the flavivirus viral particle are flaviviruses transmitted via an arthropod vector.

25. The pharmaceutically acceptable dosage form of claim 24 wherein the flaviviruses are selected from the group comprising yellow fever viruses, Japanese encephalitis viruses, dengue viruses, West Nile viruses, and tick-borne encephalitis viruses.

26. The pharmaceutically acceptable dosage form of claim 21 wherein the recombinant flaviviruses comprise yellow fever viruses.

27. The pharmaceutically acceptable dosage form of claim 26 wherein recombinant yellow fever viruses comprises YF-17D virus.

28. The pharmaceutically acceptable dosage form of claim 20 wherein the host cell culture comprises mammalian cell culture.

29. The pharmaceutically acceptable dosage form of claim 20 wherein the tangential flow filtration comprises filtration through one or more hollow fiber TFF cassettes.

30. The pharmaceutically acceptable dosage form of claim 29 wherein the one or more hollow fiber TFF cassettes comprises a media selected from the following polysulfone, polyethersulfone, modified polyethersulfone or mixed cellulose ester medias.

31. The pharmaceutically acceptable dosage form of claim 30 wherein the hollow fiber TFF cassettes media is a polysulfone.

5 32. The pharmaceutically acceptable dosage form of claim 20 wherein the tangential flow filtration comprises filtration through a flat sheet media.

33. The pharmaceutically acceptable dosage form of claim 20 wherein the anion exchange chromatography resin comprises immobilized positively charged moieties.

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34. The pharmaceutically acceptable dosage form of claim 33 wherein the immobilized positively charged moieties comprise quaternary amino groups or diethylaminoethane groups.

15 35. The pharmaceutically acceptable dosage form of claim 34 wherein the immobilized positively charged moieties are quaternary amino groups.

20 36. The pharmaceutically acceptable dosage form of claim 20 wherein the anion exchange chromatography resin comprises one or more anionic exchange monolithic columns.

37. The pharmaceutically acceptable dosage form of claim 36 wherein the one or more anionic exchange monolithic columns comprise CONVECTIVE INTERACTIVE MEDIA® columns.

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38. The pharmaceutically acceptable dosage form of claim 20 wherein the quantity of host cell DNA in said composition is less than 10 ng host cell DNA per dose, one dose being defined as 500 µL of 2E+7 PFU/mL.