

WHAT IS CLAIMED IS:

- 5 1. A method of producing a recombinant protein consisting of:
- (a) disposing a plurality of recombinant mammalian cells into a first culture medium
comprised within a vessel to provide a first cell culture;
- (b) batch culturing the first cell culture to a cell density of 1.0×10^6 cells/mL to 5.0×10^6
cells/mL;
- 10 (c) disposing a volume of the first cell culture of step (b) into a second culture medium
comprised within a perfusion bioreactor to provide a second cell culture with an initial cell
density of 0.25×10^6 cells/mL to 0.5×10^6 cells/mL;
- (d) perfusion culturing the second cell culture to a cell density of 5×10^6 cells/mL to $120 \times$
 10^6 cells/mL; and
- 15 (e) disposing a volume of the second cell culture of step (d) into a third culture
medium comprised within a production bioreactor to provide a production cell culture with an
initial cell density of 0.25×10^6 cells/mL to 8×10^6 cells/mL;
- (f) perfusion culturing the production cell culture under conditions that allow the
recombinant mammalian cells to secrete a recombinant protein; and
- 20 (g) harvesting the recombinant protein from the production cell culture.
2. The method of claim 1, wherein disposing the plurality of recombinant mammalian
cells in (a) to provide the first cell culture comprises:
- thawing a frozen cell bank; and
- 25 disposing a volume of the thawed cell bank into the first culture medium.
3. The method of claim 2, wherein the frozen cell bank comprises a cell density of 10
 $\times 10^7$ cells/mL to 50×10^7 cells/mL.

4. The method of claim 3, wherein the thawed cell bank contains a percentage of viable cells of at least 60%.

5. The method of claim 4, wherein the thawed cell bank contains a percentage of viable cells of at least 90%.

6. The method of claim 1, wherein disposing the plurality of recombinant mammalian cells in (a) to provide the first cell culture comprises disposing a volume of a third cell culture comprising the plurality of recombinant mammalian cells into the first culture medium.

7. The method of claim 6, wherein the third cell culture is provided by:

(1) disposing a plurality of the recombinant mammalian cells in a fourth culture medium comprised within a vessel to provide the third cell culture; and

(2) batch culturing the third cell culture to a cell density of 1.0×10^6 cells/mL to 5.0×10^6 cells/mL.

8. The method of claim 7, wherein one or both of the vessel in (a) or the vessel in (1) is a disposable single-use bioreactor.

9. The method of claim 8, wherein the disposable single-use bioreactor comprises a plastic sterile bag.

10. The method of claim 9, wherein disposing the plurality of the recombinant mammalian cells in (1) to provide the third cell culture comprises:

thawing a frozen cell bank; and

disposing a volume of the thawed cell bank into the fourth culture medium.

11. The method of claim 10, wherein the frozen cell bank comprises a cell density of 10×10^7 cells/mL to 50×10^7 cells/mL.

12. The method of claim 11, wherein the thawed cell bank contains a percentage of viable cells of at least 60%.

13. The method of claim 12, wherein the thawed cell bank contains a percentage of viable cells of at least 90%.

14. The method of claim 1, wherein the first cell culture in (a) has a volume of 1.0 L to 50 L.

15. The method of claim 14, wherein the first cell culture in (a) has a volume of 5.0 L to 10.0 L.

16. The method of claim 1, wherein the second cell culture in (c) has a volume of 5 L to 600 L.

17. The method of claim 16, wherein the second cell culture in (c) has a volume of 10 L to 300 L.

18. The method of claim 1, wherein the production cell culture in (e) has a volume of 50 L to 20,000 L.

19. The method of claim 18, wherein the production cell culture in (e) has a volume of 100 L to 10,000 L.

20. The method of claim 7, wherein the fourth culture medium in (1) has a volume of 500 mL to 20 L.

21. The method of claim 7, wherein the fourth culture medium in (1) has a volume of 500 mL to 10 L.

22. The method of claim 1, wherein the vessel in (a) has an internal volume of 1.5 L to 100 L.

23. The method of claim 22, wherein the vessel in (a) has an internal volume of 1.5 L to 50 L.

24. The method of claim 1, wherein the perfusion bioreactor in (c) has an internal volume of 7.5 L to 1,000 L.

25. The method of claim 24, wherein the perfusion bioreactor in (c) has an internal volume of 50 L to 1,000 L.

26. The method of claim 1, wherein the production bioreactor in (e) has an internal volume of 150 L to 25,000 L.

27. The method of claim 26, wherein the production bioreactor in (e) has an internal volume of 150 L to 10,000 L.

28. The method of claim 7, wherein the vessel in (1) has an internal volume of 1 L to 40 L.

29. The method of claim 28, wherein the vessel in (1) has an internal volume of 1 L to 20 L.

30. The method of claim 1, wherein the perfusion bioreactor in (c) is equipped with an alternating tangential flow filtration device.

31. The method of claim 1, wherein the initial cell density in (e) is 2.0×10^6 cells/mL to 8×10^6 cells/mL.

32. The method of claim 1, wherein the initial cell density in (e) is at least 10% of a steady state production cell density.

33. The method of claim 32, wherein the initial cell density in (e) is at least 20% of the steady state production cell density.

34. The method of claim 32, wherein the steady state production cell density is 5×10^6 cells/mL to 50×10^6 cells/mL.

35. The method of claim 34, wherein the steady state production cell density is 15×10^6 cells/mL to 50×10^6 cells/mL.

36. The method of claim 1, wherein the perfusion culturing in (f) results in the production cell culture reaching a steady state production cell density in a period of 1 day to 10 days.

37. The method of claim 36, wherein the perfusion culturing in (f) results in the production cell culture reaching the steady state production cell density in a period of 2 days to 5 days.

38. The method of claim 1, wherein the harvesting in (g) comprises removing culture medium from the production bioreactor.

39. The method of claim 38, wherein the culture medium is continuously removed from the production bioreactor.