CLAIMS

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 x 10^6 cells/mL to 5.0 x 10^6 cells/mL;

(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 x 10^6 cells/mL to 0.5 x 10^6 cells/mL;

(d) perfusion culturing the second cell culture to a cell density of 5 x 10^6 cells/mL to 120 x 10^6 cells/mL;

(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 x 10^6 cells/mL to 8 x 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

(g) harvesting the recombinant protein from the production cell culture.

2. A method of producing a recombinant protein consisting of:

(a) thawing a frozen cell bank comprising recombinant mammalian cells;

(b) disposing a volume of the thawed cell bank into a first culture medium comprised within a vessel to provide a first cell culture;

(c) batch culturing the first cell culture to a cell density of 1.0 x 10^6 cells/mL to 5.0 x 10^6 cells/mL;

(d) disposing a volume of the first cell culture of step (c) into a second culture medium comprised within a perfusion bioreactor to provide a second cell culture with an initial cell density of 0.25 x 10^6 cells/mL to 0.5 x 10^6 cells/mL;

(e) perfusion culturing the second cell culture to a cell density of 5 x 10^6 cells/mL to 120 x 10^6 cells/mL;

(f) disposing a volume of the second cell culture of step (e) into a third culture medium comprised within a production bioreactor to provide a production cell culture with an initial cell density of 0.25 x 10^6 cells/mL to 8 x 10^6 cells/mL;

(g) perfusion culturing the production cell culture under conditions that allow the recombinant mammalian cells to secrete a recombinant protein; and

(h) harvesting the recombinant protein from the production cell culture.

3. The method of claim 2, wherein the frozen cell bank comprises a cell density of 10 x 10^7 cells/mL to 50 x 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. A method of producing a recombinant protein consisting of:

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

(b) batch culturing the third cell culture to a cell density of 1.0 x 10^6 cells/mL to 5.0 x 10^6 cells/mL;

(c) disposing a volume of the third cell culture of step (b) into a first culture medium comprised within a vessel to provide a first cell culture;

(d) batch culturing the first cell culture to a cell density of 1.0 x 10^6 cells/mL to 5.0 x 10^6 cells/mL;

(e) disposing a volume of the first cell culture of step (d) into a second culture medium comprised within a perfusion bioreactor to provide a second cell culture with an initial cell density of 0.25 x 10^6 cells/mL to 0.5 x 10^6 cells/mL;

(f) perfusion culturing the second cell culture to a cell density of 5 x 10^6 cells/mL to 120 x 10^6 cells/mL;

(g) disposing a volume of the second cell culture of step (f) into a third culture medium comprised within a production bioreactor to provide a production cell culture with an initial cell density of 0.25 x 10^6 cells/mL to 8 x 10^6 cells/mL;

(h) perfusion culturing the production cell culture under conditions that allow the recombinant mammalian cells to secrete a recombinant protein; and

(i) harvesting the recombinant protein from the production cell culture.

7. The method of claim 6, wherein one or both of the vessel in (a) or the vessel in (c) is a disposable single-use bioreactor.

8. The method of claim 7, wherein the disposable single- use bioreactor comprises a plastic sterile bag.

9. A method of producing a recombinant protein consisting of:

(a) thawing a frozen cell bank comprising recombinant mammalian cells;

(b) disposing a volume of the thawed cell bank into a fourth culture medium comprised within a vessel to provide a third cell culture;

(c) batch culturing the third cell culture to a cell density of 1.0 x 10^6 cells/mL to 5.0 x 10^6 cells/mL;

(d) disposing a volume of the third cell culture of step (c) into a first culture medium comprised within a vessel to provide a first cell culture, wherein the vessel in one or both of (b) and (d) is a plastic sterile bag;

(e) batch culturing the first cell culture to a cell density of 1.0 x 10^6 cells/mL to 5.0 x 10^6 cells/mL;

(f) disposing a volume of the first cell culture of step (e) into a second culture medium comprised within a perfusion bioreactor to provide a second cell culture with an initial cell density of 0.25 x 10^6 cells/mL to 0.5 x 10^6 cells/mL;

(g) perfusion culturing the second cell culture to a cell density of 5 x 10^6 cells/mL to 120 x 10^6 cells/mL;

(h) disposing a volume of the second cell culture of step (g) into a third culture medium comprised within a production bioreactor to provide a production cell culture with an initial cell density of 0.25 x 10^6 cells/mL to 8 x 10^6 cells/mL;

(i) perfusion culturing the production cell culture under conditions that allow the recombinant mammalian cells to secrete a recombinant protein; and

(j) harvesting the recombinant protein from the production cell culture.

10. The method of claim 9, wherein the frozen cell bank comprises a cell density of 10 x 10^7 cells/mL to 50 x 10^7 cells/mL.

11. The method of claim 10, wherein the thawed cell bank contains a percentage of viable cells of at least 60%.

12. The method of claim 11, wherein the thawed cell bank contains a percentage of viable cells of at least 90%.

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

14. The method of claim 13, wherein the first cell culture in (a) has a volume of 5.0 L to 10.0 L.

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

16. The method of claim 15, wherein the second cell culture in (c) has a volume of 10 L to 300 L.

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

18. The method of claim 17, wherein the production cell culture in (e) has a volume of 100 L to-10,000 L.

19. The method of claim 6, wherein the fourth culture medium in (a) has a volume 500 mL to 20 L.

20. The method of claim 6, wherein the fourth culture medium in (a) has a volume of 500 mL to 10 L.

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

22. The method of claim 21, wherein the vessel in (a) has an internal volume of 1.5 L to 50 L.

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

24. The method of claim 23, wherein the perfusion bioreactor in (c) has an internal volume of 50 L to 1000 L.

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

26. The method of claim 25, wherein the production bioreactor in (e) has an internal volume of 150 L to 10,000 L.

27. The method of claim 6, wherein the vessel in (a) has an internal volume of 1 L to 40 L.

28. The method of claim 27, wherein the vessel in (a) has an internal volume of 1 L to 20 L.

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

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

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

32. The method of claim 31, wherein the initial cell density in (e) is at least 20% of the steady state production cell density.

33. The method of claim 31, wherein the steady state production cell density is 5 x 10^6 cells/mL to 50 x 10^6 cells/mL.

34. The method of claim 33, wherein the steady state production cell density is 15 x 10^6 cells/mL to 50 x 10^6 cells/mL.

35. 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.

36. The method of claim 35, 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.

37. 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 x 10^6 cells/mL to 5.0 x 10^6 cells/mL;

(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 x 10^6 cells/mL to 0.5 x 10^6 cells/mL;

(d) perfusion culturing the second cell culture to a cell density of 5 x 10^6 cells/mL to 120 x 10^6 cells/mL;

(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 x 10^6 cells/mL to 8 x 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

(g) continuously removing culture medium from the production bioreactor.