We claim:

1. ~~——~~A method for producing an ultra-high density frozen cell bank directly from a population of cultured cells, the method comprising:

a) culturing cells in a perfusion bioreactor to obtain an ultra-high density cell population ~~with a concentration of at least about 1.0 x 10^8 cells/mL~~, wherein said perfusion bioreactor is coupled to a cell retention system~~;~~ comprising an alternating tangential flow filtration system (ATF) comprising a filter, wherein the dissolved oxygen (DO) in the perfusion bioreactor is maintained at ≥30% by setting the rock angle to 10° and the rock speed to 22 rpm for a first time period, manually setting the rock angle to 12° and the rock speed to 25 rpm for a second time period; and

b) adding a cryoprotectant to the ultra-high density cell population to produce an ultra-high density frozen cell bank, wherein the ultra-high density frozen cell bank has a concentration of at least ~~about~~ 1.0 x ~~10^8~~10^8 cells/mL~~,~~ and at least 95% post-thaw viability;

wherein no additional concentrating step is performed between culturing the cells and adding the cryoprotectant to the ultra-high density cell population.

~~2.~~ 2. The method of claim 1, wherein the ~~cell retention system comprises an alternating tangential flow filtration system comprising a filter~~filter has a surface area of at least 0.08 m^2.

~~3.~~ 3. The method of claim 2, wherein the filter has a surface area of ~~at least about 0.08 m^2.~~

~~4.~~ ~~The method of claim 3, wherein the filter has a surface area of about~~ 0.3 m^2 to ~~about~~ 0.5 m^2, ~~about~~ 0.5 ~~m^2~~ to ~~about~~ 1.0 m^2, ~~about~~ 0.7 m^2 to ~~about~~ 0.8 m^2, ~~about~~ 1.0 m^2 to ~~about~~ 2.0 m^2, ~~about~~ 2.0 m^2 to ~~about~~ 3.0 m^2, ~~about~~ 3.0 m^2 to ~~about~~ 4.0 m^2, or ~~about~~ 4.0 m^2 to ~~about~~ 5.5 m^2.

~~5.~~ 4. The method of any one of ~~the preceding~~ claims 1-3, wherein the filter has a pore size selected from the group consisting of 0.7 µm, 1.2 µm, and 7 µm.

~~6.~~ 5. The method of any one of ~~the preceding~~ claims 1-4, wherein the ultra-high density cell population has a cell density selected from the group consisting of ~~about~~ 1.0 x ~~10^8~~10^8 cells/mL, ~~about~~ 1.1 x ~~10^8~~10^8 cells/mL, ~~about~~ 1.2 x ~~10^8~~10^8 cells/mL, ~~about~~ 1.3 x ~~10^8~~10^8 cells/mL, ~~about~~ 1.4 x ~~10^8~~10^8 cells/mL, ~~about~~ 1.5 x ~~10^8~~10^8 cells/mL, ~~about~~ 1.6 x ~~10^8~~10^8 cells/mL, ~~about~~ 1.7 x ~~10^8~~10^8 cells/mL, ~~about~~ 1.8 x ~~10^8~~10^8 cells/mL, ~~about~~ 1.9 x ~~10^8~~10^8 cells/mL, and ~~about~~ 2.0 x ~~10^8~~10^8 cells/mL.

~~7.~~ 6. The method of any one of ~~the preceding~~ claims 1-5, wherein ~~said cryopreserving~~the step of adding the cryoprotectant comprises adding dimethyl sulphoxide (DMSO) to the ultra-high density cell population at a final concentration of ~~about~~ 5% to ~~about~~ 10%, vol/vol.

~~8.~~ 7. The method of any one of ~~the preceding~~ claims~~, wherein said cryopreserving comprises~~ 1-6, further comprising freezing at least a portion of the ultra-high density cell population in a container appropriate for storage under cryopreservation conditions.

~~9.~~ 8. The method of claim ~~8~~7, wherein the container is a vial.

~~10.~~ 9. The method of claim ~~9~~8, wherein the ultra-high density frozen cell bank comprises ~~about~~ 4.5 x ~~10^8~~10^8 cells/vial.

~~11.~~ 10. The method of claim ~~8~~7, wherein the container is a cryobag.

~~12.~~ 11. The method of claim ~~11~~10, wherein the ultra-high density frozen cell bank comprises ~~about~~ 100 x ~~10^8~~10^8 cells/cryobag.

~~13.~~ 12. The method of any one of ~~the preceding~~ claims 1-11, wherein the ultra-high density frozen cell bank has a cell density of at least ~~about 1.~~1.1 x ~~10^8~~10^8 cells/mL.

~~14.~~ 13. The method of any one of ~~the preceding~~ claims 1-12, wherein ~~the~~a perfusion rate in the perfusion bioreactor is between ~~about~~ 0.02 nL/cell/day ~~to about~~and 0.5 nL/cell/day.

~~15.~~ 14. The method of any one of ~~the preceding~~ claims 1-13, wherein ~~the~~a perfusion rate in the perfusion bioreactor is between 0 and 15 reactor volumes per day.

~~16.~~ 15. The method of any one of ~~the preceding~~ claims 1-14, wherein the perfusion bioreactor cell culture has a pH of between ~~about~~ 6.8 ~~to about~~and 7.2.

~~17.~~ 16. The method of any one of ~~the preceding~~ claims~~, wherein the perfusion bioreactor cell culture has a dissolved oxygen concentration (DO) of at least about 30%.~~

~~18. The method of any one of the preceding claims~~ 1-15, wherein the bioreactor is a flexible bag bioreactor.

~~19. The method of any one of the preceding claims, wherein the ultra-high density frozen cell bank has a post-thaw cell viability of at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95%.~~

~~20. The method of any one of the preceding claims~~17. The method of any one of claims 1-16, wherein the cells are mammalian cells.

~~21.~~ 18. The method of claim ~~20~~17, wherein the mammalian cells are selected from the group consisting of: CHO, CHO-DBX11, CHO-DG44, CHO-S, CHO-K1, Vero, BHK, HeLa, COS, MDCK, HEK-293, NIH-3T3, W138, BT483, Hs578T, HTB2, BT20, T47D, ~~NS0~~NSO, CRL7030, HsS78Bst cells, PER.C6, SP2/0-~~Ag14~~Ag14, and hybridoma cells.

~~22.~~ 19. The method of any one of ~~the preceding~~ claims 1-18, wherein the cells are transfected cells.

~~23.~~ 20. The method of any one of ~~the preceding~~ claims 1-19, wherein the cells express a therapeutic protein.

~~24.~~ 21. The method of claim 1, wherein:

~~a)~~ a) the perfusion bioreactor comprises a flexible bag bioreactor;

~~b)~~ b) the filter has a filter surface area of at least 0.08 m^2 and a molecular mass cut off (MWCO) size of at least 50 kDa;

~~c)~~ c) the cryoprotectant added to the ultra-high density cell population is DMSO; and

~~d)~~ d) the ultra-high density frozen cell bank comprises ~~about~~ 5% to ~~about~~ 10%, vol/vol DMSO.

~~25.~~ 22. The method of any one of ~~the preceding~~ claims 1-21, wherein the pH and DO of the culture are controlled by automated methods.

~~26.~~ 23. The method of any one of ~~the preceding~~ claims 1-21, wherein the pH and DO of the culture are controlled by non-automated methods.

~~27.~~ 24. The method of ~~claim 26, wherein the pH and DO are controlled through~~ any one ~~or more~~ of ~~the following: adjustment of the mixture of gases that are introduced to the culture, adjustment of the rock rate of the bioreactor, or adjustment of the rock angle of the bioreactor.~~

~~28. The method of claim 27, wherein the bioreactor is rocked at 25 rpm with a rock angle of 12°.~~

~~29. The method of claim 27, wherein the bioreactor is rocked at 22 rpm with a rock angle of 10°.~~

~~30.~~ ~~The method of any one of the preceding~~ claims 1-23, wherein the ultra-high density cell population is cooled to and maintained at a temperature of ~~about 4ºC~~4 °C prior to and during the addition of the cryoprotectant ~~and dispensing~~.

~~31.~~ 25. The method of any one of ~~the preceding~~ claims 1-23, wherein the ultra-high density cell population is maintained at a temperature of ~~about 20ºC~~20 °C to ~~about 26ºC~~26 °C prior and during the addition of the cryoprotectant ~~and dispensing~~.

~~32.~~ 26. The method of any one of ~~the preceding~~ claims 1-23, wherein the ultra-high density cell population is maintained at an uncontrolled cold temperature by using an ice water bath prior to and during the addition of the cryoprotectant ~~and dispensing~~.

~~33.~~ 27. The method of any one of ~~the preceding~~ claims 1-26, wherein the bioreactor ~~comprises~~is further rocked at a ~~built-in filter.~~rock speed of 30 rpm and a rock angle of 12° following the rock speed of 25 rpm and rock angle of 12°.