

What Is Claimed:

1. A process for manufacturing pluripotent stem cells, comprising
placing a plurality of microcarriers in a bioreactor;
inoculating the bioreactor with pluripotent stem cells ;
incubating the pluripotent stem cells in the bioreactor for a period of time
sufficient to yield a fold expansion of about 50 times or greater to give expanded
pluripotent stem cells;
concentrating the expanded pluripotent stem cells; and
cryopreserving the expanded pluripotent stem cells;
wherein the pluripotent stem cells are inoculated at a seeding density of
about 0.2×10^6 cells/mL or less, and
wherein the process is a closed and/or automated process.
2. The process of claim 1, wherein the pluripotent stem cells are not
passaged during incubation.
3. The process of claim 1, wherein the pluripotent stem cells used for
inoculating the bioreactor are inoculated into the bioreactor as cryopreserved
pluripotent stem cells.
4. The process of claim 1, wherein the pluripotent stem cells are not
incubated in a 2D process prior to inoculating the bioreactor.
5. The process of claim 1, wherein the plurality of microcarriers have a
particle size of about 125 μm or greater.
6. The process of claim 1, wherein the plurality of microcarriers are
coated with a growth matrix prior to being placed in the bioreactor.
7. The process of claim 1, further comprising a harvesting step after
incubation.
8. The process of claim 7, wherein a non-enzymatic passaging solution
is used to separate the microcarriers from the expanded pluripotent stem cells.
9. The process of claim 8, wherein, after passaging with the non-
enzymatic passaging solution, the pluripotent stem cells and plurality of
microcarriers are run through a mesh having a mesh size sufficient to allow the
pluripotent stem cells to pass through while restricting passage of the
microcarriers.

10. The process of claim 9, wherein the mesh size is about 10 μm to about 100 μm .
11. The process of claim 1, wherein concentrating is performed by a continuous centrifugation device.
12. The process of claim 11, wherein a flow rate into the continuous centrifugation device is selected that allows formation of a fluidized bed in about 15 minutes or less.
13. The process of claim 12, wherein cell retention in the fluidized bed is about 80% or greater.
14. The process of claim 1, wherein cell retention after cryopreservation is about 70% or greater.
15. The process of claim 1, wherein during incubation, the microcarriers and pluripotent stem cells are subject to agitation.
16. The process of claim 15, wherein the agitation has an initial speed, and wherein the initial speed is increased after about 1 to 5 days to a second speed.
17. The process of claim 16, wherein the second speed is increased after about 1 to 5 days to a third speed.
18. The process of claim 15, wherein the agitation has an initial speed, and wherein the initial speed is increased to a second speed when the cell density reaches about 1×10^5 cells/cm² to about 10×10^5 cells/cm².
19. The process of claim 15, wherein, during a first 24 hours or less after inoculation, the agitation is discontinuous agitation.
20. The process of claim 19, wherein the bioreactor is a perfusion bioreactor.