CLAIMS

1. A method of expanding pluripotent stem cells (PSC) in suspension culture in a bioreactor, the method comprising
   (i) adding an inhibitor of ROCK (ROCKi) to pluripotent stem cells being cultivated in suspension in the bioreactor;
   (ii) adding a cell dissociation agent, thereby dissociating aggregates of the pluripotent stem cells;
   (iii) diluting the cell dissociation agent added in step (ii) by adding an excess volume of culture medium sufficient to decrease the concentration of the cell dissociation agent to a concentration at which cell aggregates can form again; and
   (iv) culturing of the mixture obtained in step (iii) under suitable conditions that allow the expansion of the PSCs.

2. The method of claim 1, wherein the cell dissociation reagent is a chelating agent, preferably the chelating agent is selected from the group consisting of ethylenediaminetetraacetate (EDTA), ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), iminodisuccinic acid (IDS), polyaspartic acid, ethylenediamine-N,N′-disuccinic acid (EDDS), citrate, citric acid, 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), methylglycinediacetic acid (MGDA) and combinations thereof.

3. The method of claim 2, wherein the cell dissociation reagent is EDTA.

4. The method of claim 3, wherein the final concentration of EDTA in step (ii) is at least 100 µM EDTA, in a range of about 100 to about 1000 µM EDTA, in a range of about 250 to about 750 µM EDTA, in a range of about 400 to about 600 µM EDTA or is about 500 µM EDTA, preferably about 500 µM EDTA.

5. The method of any one of claims 3 or 4, wherein the concentration of EDTA in step (iii) after adding the excess volume of culture medium is about 100 µM or less, about 95 µM or less, about 90 µM or less, about 80 µM or less, about 70 µM or less, in a range of about 100 to about 1 µM EDTA, or in a range of about 90 to about 1 µM EDTA.

6. The method of any one of the preceding claims, wherein the excess volume exceeds the volume of the cell dissociation agent by at least 5 times.

7. The method of any one of the preceding claims, wherein the culture medium in (iii) comprises a ROCKi.

8. The method of any one of the preceding claims, wherein the method further comprises:
(v) exchanging the medium to a medium essentially free of the ROCKi.

9. The method of any one of the preceding claims, wherein step (iv) is performed for about 1 to about 3 days, preferably about 2 days.

10. The method of 8, wherein step (v) starts about 1 to about 3 days, preferably about 2 days, after step (iii).

11. The method of any one of the preceding claims, wherein the ROCKi is selected from the group consisting of AS1892802, fasudil hydrochloride, GSK 269962, GSK 429286, H 1152, HA 1100, OXA 06, RKI 1447, SB 772077B, SR 3677, TC-S 7001, thiazovivin Y27632 and combinations thereof.

12. The method of any one of the preceding claims, wherein the ROCKi is Y27632.

13. The method of claim 12, wherein Y27632 is added to a final concentration of about 10 µM.

14. The method of any one of the preceding claims, wherein the ROCKi is added in step (i) about 2 to about 4 hours prior to step (ii).

15. The method of any one of the preceding claims, wherein the addition of an excess volume of the culture medium in step (iii) results in a cell number of about $1x10^5$ to about $1x10^6$ cells/ml, about 1.5 to about $7.5x10^5$ cells/ml, about $2x10^5$ to about $5x10^5$ cells/ml, about $2x10^5$ to about $3x10^5$ cells/ml or about $2.5x10^5$ cells/ml in the culture medium.

16. The method of any one of the preceding claims, wherein the culture medium is selected from the group consisting of IPS-Brew, E8, StemFlex, mTeSR1, and PluriSTEM

17. The method of claim 16, wherein the culture medium is iPSC-Brew.

18. The method of any one of the preceding claims, wherein the culture medium in steps (i) and (iii) is essentially identical.

19. The method of any one of the preceding claims, wherein the temperature of the culture medium is about 30 to 50 °C, about 35 to 40 °C, about 36 to 38 °C or about 37 °C, preferably 37 °C.

20. The method of any one of the preceding claims, wherein steps (i) to (iv) or (i) to (v) are repeated once, twice, 3 times, 4 times, 5 times, at least 5 times, or at least 10 times.

21. The method of any one of the preceding claims, wherein the pluripotent stem cells are selected from the group consisting of induced pluripotent stem cells (iPSC), embryonic stem cells (ESC), parthenogenetic stem cells (pPSC) and nuclear transfer derived PSCs (ntPSC).

22. The method of claim 20, wherein the PSCs maintain their pluripotency after each repetition of steps (i) to (iv) or (v).

23. The method of any one of the preceding claims, wherein the pluripotent stem cells are TC-1133 cells.

24. The method of any one of the preceding claims, wherein the aggregates in step (ii) have an average diameter of about 180 µm to about 250 µm, preferably about 200 µm to about 250 µm, most preferably about 200 µm.

25. The method of any one of the preceding claims, wherein the aggregates are dissociated in step (ii) for at least about 1 min, at least about 2 min, at least about 3 min, at least about 5 min, at least about 10 min, for 1 to 20 min, for about 10 to about 20 min, for about 10 to about 15 min or for up to about 15 min, preferably for about 15 min.