Claims

1. A method for producing retinal pigment epithelial (RPE) cells comprising the steps of:
(a) culturing pluripotent cells in the presence of 4-(6-(4-(piperazin-1-yl)phenyl)pyrazolo[1,5-a]pyrimidin-3-yl)quinoline (LDN193189) and 4-(4-(benzo[d][1,3]dioxol-5-yl)-5-(pyridin-2-yl)-1H-imidazol-2-yl)benzamide (SB-431542);
(b) culturing the cells of step (a) in the presence of a BMP4/7 heterodimer and in the absence of LDN193189 and SB-431542; and,
(c) replating the cells of step (b).

2. The method according to claim 1, wherein in step (a), the cells are cultured as a monolayer or in a suspension culture, and wherein in step (b), the cells are cultured as a monolayer or in a suspension culture.

3. The method according to claim 1, wherein the pluripotent cells are selected from embryonic stem cells or induced pluripotent stem cells.

4. The method according to claim 1 wherein in step (a), the concentration of LDN193189 is between 0.5nM and 10μM.

5. The method according to claim 1, wherein in step (a), the concentration of SB-431542 is between 0.5nM and 100μM.

6. The method according to claim 1 wherein in step (a), the pluripotent cells are cultured for at least 1, 3, 4, 5, 6, 7, 8, 9, or 10 days.

7. The method according to claim 1 wherein before step (a), the cells are cultured as a monolayer at an initial density of at least 1000 cells/cm^2, or at an initial density of between 100000 and 500000 cells/cm^2.

8. The method according to claim 1 wherein in step (b), the concentration of BMP4/7 heterodimer is between 1 ng/mL and 10 μg/mL.

9. The method according to claim 1 wherein, in step (b), said cells are cultured for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days.

10. The method according to claim 1 wherein in step (c), said cells are replated at a density of at least 1000 cells/cm^2.

11. The method according to claim 1 wherein in step (c), said cells are replated on Matrigel®, fibronectin or Cellstart®.

12. The method according to claim 1, wherein said method further comprises:
(d) culturing the replated cells of step (c) in the presence of an activin pathway activator;
(e) replating the cells of step (d); and,
(f) culturing the replated cells of step (e).

13. The method according to claim 12 wherein in step (d), the concentration of activin pathway activator is between 1 ng/mL and 10 µg/mL.

14. The method according to claim 12 wherein, in step (d), the cells are cultured in the presence of cAMP.

15. The method according to claim 1, wherein step (b) further comprises, after culturing the cells in the presence of the BMP4/7 heterodimer, culturing the cells for at least 10 days in the absence of the BMP4/7 heterodimer; and said method further comprises a step of:
(d) culturing the replated cells of step (c).

16. The method according to claim 15, comprising the following additional steps:
(e) replating the cells of step (d);
(f) culturing the replated cells of step (e).

17. The method according to claim 16 wherein, in step (e), the cells are replated at a density of at least 1000 cells/cm^2.

18. The method according to claim 17 wherein, in step (f), the cells are cultured for at least 10 days.

19. The method according to claim 1, wherein said method further comprises the step of harvesting the RPE cells.

20. The method according to claim 1, wherein said method further comprises the step of purifying the RPE cells.

21. The method according to claim 1, wherein said method further comprises the step of purifying the RPE cells by Fluorescence Activated Cell Sorting (FACS) or Magnetic Activated Cell Sorting (MACS).

22. The method according to claim 1, wherein the RPE cells are expanded by a method comprising:
replating RPE cells; and,
culturing the replated RPE cells.

23. The method according to claim 22 wherein the cells are replated at a density between 1000 and 100000 cells/cm^2.

24. The method according to claim 22, wherein the cells are cultured for at least 7 days, at least 14 days, at least 28 days or at least 42 days.

25. The method according to claim 22, the RPE cells are expanded by a method comprising the following steps:
replating RPE cells at a density of at least 1000 cells/cm^2, and,
culturing the replated RPE cells in the presence of SMAD inhibitor, cAMP or an agent which increases the intracellular concentration of cAMP.

26. The method according to claim 20, wherein the step of purifying the RPE cells comprises:
providing a cell population comprising RPE cells and non RPE cells;
increasing the percentage of RPE cells in the cell population by enriching the cell population for cells expressing CD59 by contacting the cells with an anti-CD59 antibody conjugated to a

fluorophore or a magnetic particle and selecting the cells that bind to the anti-CD59 antibody using FACS or MACS.

27.The method according to claim 26, wherein the non RPE cells are pluripotent cells or RPE progenitors.