CLAIMS

1. A method for obtaining Retinal Pigment Epithelium (RPE) cells, comprising:
    a. providing a culture of human pluripotent stem cells adherent on a first substrate comprising a first laminin (LN);
    b. exposing the stem cells to a differentiation medium for a first time period of 15 days to 50 days to obtain a first population of adherent cells comprising RPE cells and/or progenitors thereof;
    c. at the end of the first time period, dissociating the first population from the first substrate;
    d. replating the dissociated first population of cells on a second substrate comprising a second laminin; and
    e. culturing the replated first population of cells on the second substrate for second time period to obtain an expanded and matured second population of cells comprising the RPE cells;
    wherein the first and second laminins are independently selected from LN-521, LN-511, LN-111 and LN-121, and are an intact protein or protein fragment having essentially the same activity as an intact protein;
    wherein the method comprises quantitating the fraction of cells expressing the marker CD140b; and
    wherein the dissociation step (c) is initiated when at least 30% of the cells are **positive** for the marker and/or wherein the culturing step (e) is continued until at least 80% of the cells are **positive** for the marker.
2. The method according to any of the preceding claims, wherein the laminin(s) is/are (an) intact protein(s).
3. The method according to any of the preceding claims, wherein the first and second laminin are coated at an amount of 5 μg/cm^2 to 20 μg/cm^2.
4. The method according to any of the preceding claims, wherein the first and/or second substrate is/are xeno-free.
5. The method according to any of the preceding claims, wherein the first and/or second substrate contain only a single type of laminin.

6. The method according to any of the preceding claims, wherein the first laminin is LN-111 or LN-521 and the second laminin is LN-521.
7. The method according to any of the preceding claims, wherein the first laminin is LN-111 and the second laminin is LN-521.
8. The method according to claim 5, wherein the first and second laminins are both LN-521.
9. The method according to any of the preceding claims, wherein the culture of pluripotent stem cells is provided at a cell density of $1.2x10^4$ cells/cm^2 to $4.8x10^4$ cells/cm^2.
10. The method according to any of the preceding claims, wherein the pluripotent stem cells are cultured in a culture medium comprising bFGF or TGFbeta prior to exposure to differentiation medium.
11. The method according to any of the preceding claims, wherein the stem cells are cultured in a stem cell culture medium comprising bFGF or TGFbeta, and a Rho-kinase inhibitor is included in the stem cell culture medium for a time period of 12 hours to 48 hours prior to the initiation of the exposure to differentiation medium.
12. The method according to any of the preceding claims, wherein the differentiation medium is a stem cell culture medium devoid of any composition maintaining the stem cells in a pluripotent state.
13. The method according to any of the preceding claims, wherein the differentiation medium is devoid of bFGF and TGFbeta.
14. The method according to any of the preceding claims, wherein Activin A is included in the differentiation medium.
15. The method according to any of the preceding claims, wherein Activin A is included in the differentiation medium at a concentration of 50 ng/ml to 200 ng/ml.
16. The method according to any of the preceding claims, wherein Activin A is included in the differentiation medium no earlier than a time point at 4 days to 8 days from the exposure, preferably for a time period starting from 5 days to 7 days from the exposure and ending at the time of the dissociation step, most preferably 6 days from the exposure and ending at the time of the dissociation step.

17. The method according to any of the preceding claims, wherein the time period is 15 days to 40 days, preferably 15 days to 33 days, more preferably 18 days to 30 days, most preferably 18 days to 22 days.
18. The method according to any of the preceding claims, comprising quantitating the number or fraction of cells expressing a marker selected from CD56, CD104, CD164, CD220, or EGFR, preferably CD56.
19. The method according to claim 18, wherein the dissociation step (c) is initiated when at least 30%, preferably at least 40%, more preferably at least 50%, most preferably at least 60% of the cells are **positive** for the marker.
20. The method according to any of claims 18-19, wherein the culturing step (e) is continued until at least 80%, preferably at least 85%, more preferably at least 90%, most preferably at least 95% of the cells are **positive** for the marker.
21. The method according to any of the preceding claims, comprising quantitating the number or fraction of cells expressing a marker selected from GD2, CD184, CD10, CD30, CD49a, CD49b, CD50, CD171, TRA-1-60, or CD326, preferably GD2 or CD184.
22. The method according to claim 21, wherein the dissociation step (c) is initiated when at least 30%, preferably at least 40%, more preferably at least 50%, most preferably at least 60% of the cells are **negative** for the marker.
23. The method according to any of claims 21-22, wherein the culturing step (e) is continued until at least 80%, preferably at least 85%, more preferably at least 90%, most preferably at least 95% of the cells are **negative** for the marker.
24. The method according to any of the preceding claims, wherein the dissociation of the first population of cells from the first substrate involves dissociating the substantially the entire population into a single suspension of cells.
25. The method according to any of the preceding claims, wherein the dissociated first population of cells are replated without any selection.
26. The method according to any of claims 1-25, wherein the dissociated first population of cells is enriched prior to replating by the steps of:
    F1. labelling a cell surface marker on the dissociated first population of cells;
    F2. separating the cells that are positive for said cell surface marker from cells that are negative for said cell surface marker to obtain an enriched first

population of cells that is more enriched in RPE cells and/or progenitors thereof than the dissociated first population cells; and

F3. using the enriched first population of cells in the replating step.

27. The method according to claim 26, wherein the cell surface marker is selected from CD140b, CD56, CD104, CD164, CD220, CD165 and EGFR, preferably CD140b or CD56.

28. The method according to claim 27, wherein cells positive for said cell surface marker are selected for the enriched first population of cells.

29. The method according to claim 26, wherein the cell surface marker is selected from GD2, CD184, CD10, CD30, CD49a, CD49b, CD50, CD171, TRA-1-60, and CD326, preferably GD2 or CD184.

30. The method according to claim 29, wherein cells negative for said cell surface marker are selected for the enriched first population.

31. The method according to any of the preceding claims, wherein the dissociation from the first substrate involves forming a suspension of single cells of the first population of cells.

32. The method according to any of the preceding claims, wherein the dissociated cells are replated on a second substrate at a cell density from $1.4x10^4$ cells/cm^2 to $1.4x10^6$ cells/cm^2.

33. The method according to any of the preceding claims, wherein the RPE cells are obtained after a total of 6-12 weeks, preferably 8-10 weeks, more preferably about 9 weeks or 60-66 days.