**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 ~~about~~ 15 days to ~~about~~ 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).

~~2.~~3. The method according to any of the preceding claims, wherein the first and second laminin are coated at an amount of ~~about~~ 5 µg/cm$^2$ to ~~about~~ 20 µg/cm$^2$.

~~3.~~4. The method according to any of the preceding claims, wherein the first and/or second substrate is/are xeno-free.

~~4.~~5. The method according to any of the preceding claims, wherein the first and/or second substrate contain only a single type of laminin.

~~5.~~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.

~~6.~~7. The method according to any of the preceding claims, wherein the first laminin is LN-111 and the second laminin is LN-521.

~~7.~~8. The method according to claim 5, wherein the first and second laminins are both LN-521.

~~8.~~9. The method according to any of the preceding claims, wherein the culture of pluripotent stem cells is provided at a cell density of ~~about~~ $1.2x10^4$ cells/$cm^2$ to ~~about~~ $4.8x10^4$ cells/$cm^2$.

~~9.~~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.

~~10.~~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 ~~about~~ 12 hours to ~~about~~ 48 hours prior to the initiation of the exposure to differentiation medium.

~~11.~~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.

~~12.~~13. The method according to any of the preceding claims, wherein the differentiation medium is devoid of bFGF and TGFbeta.

~~13.~~14. The method according to any of the preceding claims, wherein Activin A is included in the differentiation medium.

~~14.~~15. The method according to any of the preceding claims, wherein Activin A is included in the differentiation medium at a concentration of ~~about~~ 50 ng/ml to ~~about~~ 200 ng/ml.

~~15.~~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 ~~about~~ 4 days to ~~about~~ 8 days from the exposure, preferably for a time period starting from ~~about~~ 5 days to ~~about~~ 7 days from the exposure and ending at the time of the dissociation

step, most preferably ~~about~~ 6 days from the exposure and ending at the time of the dissociation step.

~~16.~~17. The method according to any of the preceding claims, wherein the time period is ~~about~~ 15 days to ~~about~~ 40 days, preferably ~~about~~ 15 days to ~~about~~ 33 days, more preferably ~~about~~ 18 days to ~~about~~ 30 days, most preferably ~~about~~ 18 days to ~~about~~ 22 days.

~~17.~~18. The method according to any of the preceding claims, comprising quantitating the number or fraction of cells expressing a marker selected from ~~CD140b,~~ CD56, CD104, CD164, CD220, or EGFR, preferably ~~CD140b or~~ CD56~~, most preferably CD140b~~.

~~18.~~19. The method according to claim ~~17~~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.

~~19.~~20. The method according to any of claims ~~17-18~~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.

~~20.~~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.

~~21.~~22. The method according to claim ~~20~~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.

~~22.~~23. The method according to any of claims ~~20-21~~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.

~~23. The method according to any of the preceding claims, wherein the dissociation of the first population of cells from the first substrate is performed indiscriminately.~~

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 ~~about~~ $1.4x10^4$ cells/$cm^2$ to ~~about~~ $1.4x10^6$ 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.

~~34. A method of enriching RPE cells or progenitors of RPE cells in a mixture of cells, comprising:~~

~~a. labeling a cell surface marker in a starting mixture of RPE cells or progenitors of RPE cells; and~~

b. separating the cells that are positive for said cell surface marker from cells that are negative for said cell surface marker to obtain a final mixture of RPE cells or progenitors of RPE cells that is more enriched in RPE cells or progenitors of RPE cells compared to the starting mixture;

wherein said cell surface marker is CD140b, CD56, CD104, CD164, CD220, CD165, EGFR, GD2, CD184, CD10, CD30, CD49a, CD49b, CD50, CD171, TRA-1-60, or CD326.

35. The method according to claim 34, comprising selecting cells positive for CD140b, CD56, CD104, CD164, CD220, CD165 and/or EGFR, preferably CD140b and/or CD56, for the final mixture.

36. The method according to any of claims 34-35, comprising selecting cells negative for GD2, CD184, CD10, CD30, CD49a, CD49b, CD50, CD171, TRA-1-60 and/or CD326, preferably GD2 and/or CD184, for the final mixture.

37. The method according to any of claims 34-36, comprising selecting cells that are positive for CD140b and negative for GD2 for the final mixture.

38. The method according to any of claims 34-37, comprising selecting cells that are positive for CD140b and negative for CD184 for the final mixture.

39. The method according to any of claims 34-38, wherein the labelling is performed using an antibody targeting the cell surface marker.

40. A method for analyzing an *in vitro* cell culture comprising mature RPE cells, progenitors of RPE cells and/or undifferentiated stem cells, comprising quantitating the number or fraction of cells expressing one or more cell surface markers selected from CD140b, CD56, CD104, CD164, CD220, EGFR, GD2, CD184, CD10, CD30, CD49a, CD49b, CD50, CD171, TRA-1-60 and CD326, preferably CD140b, CD56, GD2 and/or CD184.

41. The method according to claim 40, wherein the expression of CD140b, CD56, CD104, CD164, CD220 and EGFR is indicative of the presence of mature RPE cells or progenitors of RPE cells.

42. The method according to claim 40 or 41, wherein the expression of GD2, CD184, CD10, CD30, CD49a, CD49b, CD50, CD171, TRA-1-60 and/or CD326 is indicative of undifferentiated stem cells.

43. A method of obtaining Retinal Pigment Epithelium (RPE) cells, comprising:

culturing one or more human pluripotent stem cells on a first substrate comprising a single first laminin, wherein the laminin is an intact protein or protein fragment;

exposing the stem cells to a first cell culture medium for a first time period, wherein the first cell culture medium maintains pluripotency;

removing the first cell culture medium and exposing the stem cells to a second cell culture medium for a second time period of 3 days to 12 days, wherein the second cell culture medium is a differentiation medium and does not contain growth factors;

removing the second cell culture medium and exposing the stem cells to a third cell culture medium that contains a growth factor being Activin A, for a third time period of 18 days to 35 days to obtain induced RPE cells;

replating the induced RPE cells on a second substrate comprising a single second laminin; and

culturing the induced RPE cells on the second substrate for 14 days to 28 days to obtain the RPE cells, wherein the total time for differentiation ranges from 42 days to 70 days, and

wherein the single first laminin and the single second laminin are independently laminin-521, laminin-511, laminin-121, or laminin-111.

44. The method of claim 43, wherein the single first laminin and the single second laminin are coated at a concentration of about 5 $\mu g/cm^2$ to about 20 $\mu g/cm^2$.

45. The method of claim 43 or 44, wherein the first laminin and the second laminin are recombinant laminins.

46. The method of any of claims 43-45, wherein the first cell culture medium comprises a Rho-kinase inhibitor that is present in the first cell culture medium at a concentration of about 5 μM to about 20 μM.

47. The method of any of claims 43-46, wherein the first cell culture medium comprises at least one growth factor.

48. The method of claim 47, wherein the at least one growth factor in the first cell culture medium comprises bFGF and TGFβ.

~~49. The method of any of claims 43-48, wherein the second cell culture medium does not contain growth factors or a Rho-kinase inhibitor.~~

~~50. The method of any of claims 43-49, wherein the Activin A is present at a concentration of about 50 ng/mL to about 200 ng/mL.~~

~~51. The method of any of claims 43-50, wherein the substrate, the first cell culture medium, the second cell culture medium, and the third cell culture medium do not contain any differentiation inhibitors, feeder cells, or apoptosis inhibitors.~~

~~52. The method of any of claims 43-51, wherein the stem cells are plated onto the first substrate at a cell density of about $1.2x10^4$ cells/$cm^2$ to $4.8x10^4$ cells/$cm^2$.~~