CLAIMS

1. An automated method for large-scale production of retinal pigment epithelium (RPE) cells and for promoting directed differentiation of human pluripotent stem cells into retinal pigment epithelium (RPE) cells, characterized in that said ~~the~~ method ~~comprising or consisting~~ comprises or consists in the sequential steps of:

(a) culturing human pluripotent stem cells in a medium supplemented with a first differentiating agent, at least one Nicotinamide (NA) mimetic compound to generate differentiating cells;

(b) culturing said differentiating cells obtained in step a) in a medium supplemented with a second differentiating agent, at least one member of transforming growth factor β (TGF β) superfamily to further differentiating said differentiating cells

(c) culturing said further differentiating cells obtained in step b) in a medium supplemented with a third differentiating agent, at least one activator of the Wnt canonical pathway to induce said further differentiating cells to differentiate into RPE cells,

wherein the method uses an automated platforms for cell culture adapted for large-scale production of stem cells or differentiated cells derived from stem cells.

2. The automated method of claim 1, ~~wherein~~ characterized in that culturing said human pluripotent stem cells in a medium supplemented with at least one Nicotinamide (NA) mimetic compound is effected for at least 3 days, preferably for 3 to 10 days and more preferably for 7 days.

3. The automated method of claim 1 or 2, ~~wherein~~ characterized in that the Nicotinamide (NA) mimetic compound is Nicotimamide.

4. The automated method of any one of claims 1 to 3, ~~wherein~~ characterized in that culturing said differentiating cells obtained in step a) in a medium supplemented with at least one member of transforming growth factor β (TGF β) superfamily is effected for at least 3 days, preferably for 3 to 10 days and more preferably for 7 days.

5. The automated method of any one of claims 1 to 4, ~~wherein~~ characterized in that the at least one member of transforming growth factor β (TGF β) superfamily is selected from the group consisting of the transforming growth factor-like (TGF-like) group with the TGFβ subfamily, Activin,

Nodal and some growth and differentiation factors (GDF), the bone morphogenetic protein like (BMP-like) group with the BMP, GDF and antiMullerian hormone (AMH).

6. The automated method of of any one of claims 1 to 5, ~~wherein~~ characterized in that the medium in step (b) is substantially or completely free of the at least one Nicotinamide (NA) mimetic compound used in step a).

7. The automated method of any one of claims 1 to 6, ~~wherein~~ characterized in that culturing said further differentiating cells obtained in step b) in a medium supplemented with at least one activator of the Wnt canonical pathway is effected for at least 20 to 50 days.

8. The automated method of any one of claims 1 to 7, ~~wherein~~ characterized in that the at least one activator of the Wnt canonical pathway is a GSK-3 inhibitor selected from the group consisting of 3F8, 1 -Azakenpaullone, 10Z-Humenialdisine, Alsterpaullone, A1 070722~~Al 070722~~, AR-A014418, AZD1080, AZD2858, Bikinin, BIO, Cazpaullone, CT98014, CT98023, CT99021 (Chir99021), Chir98014, Dibromocantharelline, GSKJ2, HMK-32, Hymenialdesine, Indirubin, Indirubin-3'-~~omime~~ oxime , IM-12, Kenpaullone ~~KenpauUone~~, L803, L803-mts, Lithium carbonate, LY2090314, Manzamine A, Meridianin, NSC693868 ~~NCS693868~~, NP031115, Palinurine, SB216763, SB415286, TCS21311, TC-G-24, TCS2002, TDZD-8, Tideglusib, Tricantine and TWS119.

9. The automated method of any one of claims 1 to 8, ~~wherein~~ characterized in that the medium in step (c) is substantially or completely free of the at least one Nicotinamide (NA) mimetic compound and the at least one member of transforming growth factor β (TGF β) superfamily respectively used in steps a) and b).

10. The automated method of any one of claims 1 to 9, ~~wherein~~ characterized in that the method comprises or consists in the sequential steps of:

(a) culturing human pluripotent stem cells in a medium supplemented with Nicotinamide to generate differentiating cells;

(b) culturing said differentiating cells obtained in step a) in a medium supplemented with Activin A to further differentiating said differentiating cells;

(c) culturing said further differentiating cells obtained in step b) in a medium supplemented with CHIR99021 to induce said further differentiating cells to differentiate into RPE cells.

11. The automated method of any one of claims 1 to 10, ~~wherein~~ characterized in that the method further comprises the step of:

(d) treating the population of cells obtained in step c) to remove the non-adherent cells.

12. The automated method of claim 11, ~~wherein~~ characterized in that the step d) is a two-step dissociation procedure comprising or consisting in washing and treating the cells enzymatically.

13. The automated method of any one of claims 1 to 12, wherein the method further comprises the step of

(e) expanding the cells obtained in step d) over at least two passages.

14. The automated method of claim 13, ~~wherein~~ characterized in that the passages comprises (i) dissociating the RPE cells and/or the differentiating cells in a first vessel to form a suspension; (ii) transferring the RPE cells and/or the differentiating cells to at least two further culture vessels; and (iii) culturing the RPE cells and/or the differentiating cells until the RPE cells and/or the differentiating cells are 50 to 100% confluent, wherein the passages does not comprise a centrifugation step.

15. The automated method of claim 1, characterized in that
-the Nicotinamide (NA) mimetic compound is Nicotimamide,
-the at least one member of transforming growth factor β (TGF β) superfamily is Activin A,
-the at least one activator of the Wnt canonical pathway is Chir99021,
-the step a) of the method is effected for 3 to 10 days,
-the step b) of the method is effected for 3 to 10 days, and
-the step c) of the method is effected for at least 20 to 50 days.

~~15~~ 16. The automated method of any one of claims 1 to ~~14~~ 15, ~~wherein the method is carried out with an apparatus for large-scale automated production of cells comprising~~ characterized in that said method comprises: a) robotic means for handling culture vessels; b) means for inoculating cells into a culture; c) means for changing or adding medium to a culture; and d) programmable control means; wherein the apparatus is adapted to the phase of directed differentiation of hPSCs toward RPE cells and the phase of passage the cells when they reach a predetermined percentage confluence.