

CLAIMS

1. A method for producing a composition of neuronal precursor cells, or of cells capable of proliferation that express neural lineage biomarkers including:
- 5 - treating a sample of hair follicle cells in conditions enabling the transition of hair follicle cells to a growth phase, thereby forming a sample of conditioned cells;
- treating the sample of conditioned cells in conditions enabling enrichment of the number of cells containing neuronal lineage biomarkers in the sample;
- thereby producing the composition of neuronal precursor or cells capable of proliferation that express neural lineage biomarkers.
- 10 2. The method of claim 1 wherein the hair follicle cells include hair follicular precursors.
3. The method of claim 2 wherein a subpopulation of hair follicular precursor cells express neuro-ectodermal biomarkers.
4. The method of any one of the preceding claims wherein hair follicle cells are
- 15 provided in a sample of skin.
5. The method of any one of the preceding claims wherein the hair follicle cells are treated in conditions enabling the majority of the hair follicle precursor cells to transition to a growth phase.
6. The method of any one of the preceding claims wherein the hair follicle cells
- 20 are treated with an anti-refractory hair follicle factor for promoting transition of hair follicle cells from telogen to anagen phase, thereby enabling the retention or transition of hair follicle cells in the skin to a growth phase.
7. The method of claim 6 wherein the factor is noggin or sonic hedgehog (SHH).
- 25 8. The method of any one of the preceding claims wherein the hair follicle cells are treated with a pro-growth factor for promoting transition of hair follicle cells from

telogen to anagen phase, thereby enabling the retention or transition of hair follicle cells in the skin to a growth phase.

9. The method of claim 8 wherein the pro-growth factor is TGF- β 2.

10. The method of any one of the preceding claims wherein the hair follicle cells
5 are cultured in cell culture medium for hair follicle cells.

11. The method of claim 10 wherein the medium is Williams medium E.

12. The method of any one of the preceding claims wherein the skin is human skin, or wherein the hair follicles are human hair follicles.

13. The method of any one of the preceding claims wherein the skin or hair
10 follicles are of the scalp.

14. The method of any one of the preceding claims wherein the skin or hair follicles are of the midline occipital scalp.

15. The method of any one of the preceding claims including:

- treating a sample of skin, the skin including hair follicle cells, in conditions
15 enabling the transition of hair follicle cells in the skin to a growth phase, thereby forming a sample of conditioned skin;

- releasing cells from the conditioned skin into the sample;

- depleting terminally differentiated cells or apoptotic cells or cell debris from the sample, thereby forming a sample of non-terminally differentiated cells; and optionally

20 - treating the sample of non-terminally differentiated cells in conditions enabling expansion of the number of non-terminally differentiated cells in the sample;

thereby producing the composition of neuronal precursor cells or cells capable of proliferation that express neural lineage biomarkers.

16. The method of claim 15 wherein the cells are released from the skin by
25 contacting the skin with one or more enzymes in conditions enabling degradation of the extracellular matrix of the conditioned skin for release of the cells into the sample.

17. The method of claim 16 wherein the enzyme is selected from the group consisting of: trypsin, Dnase, dispase, and collagenase.

18. The method of claim 15 wherein the cells are released from the conditioned skin by mechanically disrupting the extracellular matrix of the conditioned skin for release
5 of the cells into the sample.

19. The method of claim 18 wherein the mechanically disruption includes manual trituration.

20. The method of claim 15 including the step of removing non cellular components from the sample after release of cells from the conditioned skin into the
10 sample and prior to depletion of terminally differentiated cells from the sample.

21. The method of claim 15 wherein the terminally differentiated cells are depleted from the sample by contacting the sample with a reagent for selectively depleting terminally differentiated cells from the sample in conditions enabling selective depletion of terminally differentiated cells from the sample.

15 22. The method of claim 21 wherein the agent is an antibody that binds to terminally differentiated cells but not to non-terminally differentiated cells.

23. The method of claim 22 wherein the agent is an antibody that does not bind to neural precursor cells.

24. The method of claim 23 wherein the antibody binds to cells of the epidermis
20 or dermis.

25. The method of claim 24 wherein the antibody binds to epithelial cells or keratinocytes, or to fibroblasts.

26. The method of claim 22 wherein the antibody binds to fibroblast-specific antigen 1 or to CD45.

25 27. The method of any one of the preceding claims wherein at the completion of the depletion step, the sample contains no more than about 5% by number of terminally differentiated cells.

28. The method of any one of claims 1 to 14 including:

- treating a sample of hair follicle cells in conditions enabling the transition of hair follicle cells to a growth phase, thereby forming a sample of conditioned cells;

5 - providing conditions to the sample of conditioned cells to enable dissociation of cells into single cells;

- treating the sample of conditioned cells in conditions enabling the formation of a sample of neurospheres from the cells of the sample of conditioned cells;

- providing conditions to the sample of neurospheres to expand the number of cells of the neurospheres;

10 thereby producing the composition of neuronal precursor cells or cells capable of proliferation that express neural lineage biomarkers.

29. The method of any one of the preceding claims wherein the composition produced by the method has the following characteristics:

15 <5% of cells express astroglial GFAP, adiponectin, oligodendrocyte 04, myofibroblast SMA.

>90% of cells are positive for nestin, CD133 and β III-tubulin.

30. The method of any one of the preceding claims wherein the composition produced by the method has a neuronal yield of 90 to 100% following in vitro neuronal differentiation.

20 31. The method of any one of the preceding claims wherein the cells of the composition are expandable to produce in the order of 10^7 homogenous and unipotent neuronal precursor cells within 4 weeks.