Claims:

1. An isolated population of human pluripotent stem cells comprising at least 50% human pluripotent stem cells characterized by an endogenous $OCT4^{+}$/TRA1-60^{-}/TRA1-81^{-}/$SSEA1^{+}$/$SSEA4^{-}$ expression signature, wherein said human pluripotent stem cells are capable of differentiating into endoderm, ectoderm and mesoderm embryonic germ layers.

2. A method of generating, expanding and maintaining in an undifferentiated state the isolated population of human pluripotent stem cells (PSCs) of claim 1, the method comprising:

(a) passaging human PSCs in a suspension culture by mechanical dissociation of PSC clumps to single cells for at least 2 and no more than 10 passages, to thereby obtain a suspension culture of PSCs devoid of clumps, and subsequently;

(b) passaging said suspension culture of human PSCs devoid of said clumps without dissociation of said clumps,

wherein following step (b) at least 50% of said human pluripotent stem cells are characterized by an endogenous $OCT4^{+}$/TRA1-60^{-}/TRA1-81^{-}/$SSEA1^{+}$/$SSEA4^{-}$ expression signature.

3. The method of claim 2, wherein culturing in said suspension culture is performed under culture conditions which allow expansion of the human pluripotent stem cells in the undifferentiated state.

4. A method of deriving a human embryonic stem cell line, the method comprising:

(a) obtaining human embryonic stem cells (ESCs) from a pre-implantation stage blastocyst, post-implantation stage blastocyst and/or a genital tissue of a fetus; and

(b) expanding and maintaining said human ESCs according to the method of claim 2,

thereby deriving the human embryonic stem cell line.

5. The method of claim 2 or 4, wherein said passaging is performed under conditions devoid of an enzymatic dissociation.

6. A method of cloning human pluripotent stem cells, comprising:

culturing a single human pluripotent stem cell obtained according to the method of claim 2, 3 or 5, or a single human embryonic stem cell obtained according to the method of claim 4 or 5, in a suspension culture under conditions which allow expansion of said single human pluripotent stem cell or of said single human embryonic stem cell in the undifferentiated state, thereby expanding said single human pluripotent stem cell or said embryonic stem cell into a clonal culture,

thereby cloning the human pluripotent stem cells.

7. A method of generating lineage-specific cells from human pluripotent stem cells, the method comprising:

(a) culturing the human pluripotent stem cells in a suspension culture according to the method of claim 2, 3, or 5, to thereby obtain expanded, undifferentiated pluripotent stem cells devoid of clumps; and

(b) subjecting said expanded, undifferentiated pluripotent stem cells devoid of clumps to culturing conditions suitable for differentiating and/or expanding lineage specific cells,

thereby generating lineage-specific cells from human pluripotent stem cells.

8. A method of generating embryoid bodies from human pluripotent stem cells, the method comprising:

(a) culturing the human pluripotent stem cells in a suspension culture according to the method of claim 2, 3, or 5, to thereby obtain expanded, undifferentiated human pluripotent stem cells devoid of clumps; and

(b) subjecting said expanded, undifferentiated human pluripotent stem cells devoid of clumps to culturing conditions suitable for differentiating said human pluripotent stem cells to embryoid bodies;

thereby generating the embryoid bodies from the human pluripotent stem cells.

9. A method of generating lineage-specific cells from human pluripotent stem cells, the method comprising:

(a) culturing the human pluripotent stem cells in a suspension culture according to the method of claim 2, 3, or 5, to thereby obtain expanded, undifferentiated human pluripotent stem cells devoid of clumps;

(b) subjecting said expanded, undifferentiated human pluripotent stem cells devoid of clumps to culturing conditions suitable for differentiating said human pluripotent stem cells to embryoid bodies; and

(c) subjecting cells of said embryoid bodies to culturing conditions suitable for differentiating and/or expanding lineage specific cells;

thereby generating the lineage-specific cells from the pluripotent stem cells.

10. The method of claim 2 or 4, wherein said suspension culture devoid of clumps comprises single cells or small clusters, each of said clusters comprising no more than about 200 human pluripotent stem cells.

11. The method of any of claims 2-6, wherein said suspension culture is devoid of substrate adherence.

12. The method of any of claims 2-6, wherein said culture conditions being devoid of a Rho-associated kinase (ROCK) inhibitor.

13. The method of any of claims 2-3 and 5-12, wherein said human pluripotent stem cells are embryonic stem cells.

14. The method of any of claims 2-3 and 5-12, wherein said human pluripotent stem cells are induced pluripotent stem cells.

15. The isolated population of cells of claim 1 wherein said human pluripotent stem cells are embryonic stem cells.

16. The isolated population of cells of claim 1, wherein said human pluripotent stem cells are induced pluripotent stem cells.

17. The method of any of claims 2-14, wherein said culture conditions comprise a culture medium which comprises interleukin 11 (IL11) and Ciliay Neurotrophic Factor (CNTF).

18. The method of any of claims 2-14, wherein said culture conditions comprise a culture medium which comprises basic fibroblast growth factor (bFGF) at a concentration of at least 50 ng/ml and an IL6RIL6 chimera.

19. The method of any of claims 2-14, wherein said culture conditions comprise a culture medium which comprises an animal contaminant-free serum replacement and an IL6RIL6 chimera.

20. The method of any of claims 2-14, wherein said culture conditions comprise a serum-free culture medium which comprises a soluble interleukin 6 receptor (sIL6R) and interleukin 6 (IL6), wherein a concentration of said sIL6R is at least 5 ng/ml, and wherein a concentration of said IL6 is at least 3 ng/ml.

21. The method of any of claims 2-14, wherein said culture conditions comprise a culture medium which comprises interleukin 11 (IL11) and oncostatin.

22. The isolated population of human pluripotent stem cells of claim 1, wherein an expression level of said OCT4 in said human pluripotent stem cells is at least 8-folds higher than in said human ESCs grown on said MEFs.

23. The method of claim 2, wherein the cells of step (a) and the cells of step (b) are passaged for at least a total of 15 passages.

24. The method of claim 2, wherein said suspension culture is a three-dimensional (3D) suspension culture.

Claims:

1. An isolated population of human pluripotent stem cells comprising at least 50% human pluripotent stem cells characterized by an endogenous $OCT4^{+}$/TRA1-60^{-}/TRA1-81^{-}/$SSEA1^{+}$/$SSEA4^{-}$ expression signature, wherein said human pluripotent stem cells are capable of differentiating into endoderm, ectoderm and mesoderm embryonic germ layers.

2. A method of generating, expanding and maintaining in an undifferentiated state the isolated population of human pluripotent stem cells (PSCs) of claim 1, the method comprising:

(a) passaging human PSCs in a suspension culture by mechanical dissociation of PSC clumps to single cells for at least 2 and no more than 10 passages, to thereby obtain a suspension culture of PSCs devoid of clumps, and subsequently;

(b) passaging said suspension culture of human PSCs devoid of said clumps without dissociation of said clumps,

wherein following step (b) at least 50% of said human pluripotent stem cells are characterized by an endogenous $OCT4^{+}$/TRA1-60^{-}/TRA1-81^{-}/$SSEA1^{+}$/$SSEA4^{-}$ expression signature.

3. The method of claim 2, wherein culturing in said suspension culture is performed under culture conditions which allow expansion of the human pluripotent stem cells in the undifferentiated state.

4. A method of deriving a human embryonic stem cell line, the method comprising:

(a) obtaining human embryonic stem cells (ESCs) from a pre-implantation stage blastocyst, post-implantation stage blastocyst and/or a genital tissue of a fetus; and

(b) expanding and maintaining said human ESCs according to the method of claim 2,

thereby deriving the human embryonic stem cell line.

5. The method of claim 2 or 4, wherein said passaging is performed under conditions devoid of an enzymatic dissociation.

6. A method of cloning human pluripotent stem cells, comprising:

culturing a single human pluripotent stem cell obtained according to the method of claim 2, 3 or 5, or a single human embryonic stem cell obtained according to the method of claim 4 or 5, in a suspension culture under conditions which allow expansion of said single human pluripotent stem cell or of said single human embryonic stem cell in the undifferentiated state, thereby expanding said single human pluripotent stem cell or said embryonic stem cell into a clonal culture,

thereby cloning the human pluripotent stem cells.

7. A method of generating embryoid bodies from human pluripotent stem cells, the method comprising:

(a) culturing the human pluripotent stem cells in a suspension culture according to the method of claim 2, 3, or 5, to thereby obtain expanded, undifferentiated human pluripotent stem cells devoid of clumps; and

(b) subjecting said expanded, undifferentiated human pluripotent stem cells devoid of clumps to culturing conditions suitable for differentiating said human pluripotent stem cells to embryoid bodies;

thereby generating the embryoid bodies from the human pluripotent stem cells.

8. A method of generating lineage-specific cells from human pluripotent stem cells, the method comprising:

(a) culturing the human pluripotent stem cells in a suspension culture according to the method of claim 2, 3, or 5, to thereby obtain expanded, undifferentiated human pluripotent stem cells devoid of clumps;

(b) subjecting said expanded, undifferentiated human pluripotent stem cells devoid of clumps to culturing conditions suitable for differentiating said human pluripotent stem cells to embryoid bodies; and

(c) subjecting cells of said embryoid bodies to culturing conditions suitable for differentiating and/or expanding lineage specific cells;

thereby generating the lineage-specific cells from the pluripotent stem cells.

9. The method of claim 2 or 4, wherein said suspension culture devoid of clumps comprises single cells or small clusters, each of said clusters comprising no more than about 200 human pluripotent stem cells.

10. The method of any of claims 2-6, wherein said suspension culture is devoid of substrate adherence.

11. The method of any of claims 2-6, wherein said culture conditions being devoid of a Rho-associated kinase (ROCK) inhibitor.

12. The method of any of claims 2-3 and 5-11, wherein said human pluripotent stem cells are embryonic stem cells.

13. The method of any of claims 2-3 and 5-11, wherein said human pluripotent stem cells are induced pluripotent stem cells.

14. The isolated population of cells of claim 1 wherein said human pluripotent stem cells are embryonic stem cells.

15. The isolated population of cells of claim 1, wherein said human pluripotent stem cells are induced pluripotent stem cells.

16. The method of any of claims 3-6 and 10-13, wherein said culture conditions comprise a culture medium which comprises interleukin 11 (IL11) and Ciliay Neurotrophic Factor (CNTF).

17. The method of any of claims 3-6 and 10-13, wherein said culture conditions comprise a culture medium which comprises basic fibroblast growth factor (bFGF) at a concentration of at least 50 ng/ml and an IL6RIL6 chimera.

18. The method of any of claims 3-6 and 10-13, wherein said culture conditions comprise a culture medium which comprises an animal contaminant-free serum replacement and an IL6RIL6 chimera.

19. The method of any of claims 3-6 and 10-13, wherein said culture conditions comprise a serum-free culture medium which comprises a soluble interleukin 6 receptor (sIL6R) and interleukin 6 (IL6), wherein a concentration of said sIL6R is at least 5 ng/ml, and wherein a concentration of said IL6 is at least 3 ng/ml.

20. The method of any of claims 3-6 and 10-13, wherein said culture conditions comprise a culture medium which comprises interleukin 11 (IL11) and oncostatin.

21. The isolated population of human pluripotent stem cells of claim 1, wherein an expression level of said OCT4 in said human pluripotent stem cells is at least 8-folds higher than in said human ESCs grown on said MEFs.

22. The method of claim 2, wherein the cells of step (a) and the cells of step (b) are passaged for at least a total of 15 passages.

23. The method of claim 2, wherein said suspension culture is a three-dimensional (3D) suspension culture.