Claims:

1. An isolated population of human pluripotent stem cells comprising at least 50% human pluripotent stem cells characterized by an $OCT4^{+}/TRA1\text{-}60^{-}/TRA1\text{-}81^{-}/SSEA1^{+}/SSEA4^{-}$ expression signature, wherein an expression level of said OCT4 in said human pluripotent stem cells is higher than in human embryonic stem cells (ESCs) grown on mouse embryonic fibroblasts (MEFs) and wherein said human pluripotent stem cells are capable of differentiating into endoderm, ectoderm and mesoderm embryonic germ layers.

2. A method of expanding and maintaining pluripotent stem cells (PSCs) in an undifferentiated state, the method comprising:

(a) passaging the 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;

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

thereby expanding and maintaining the PSCs in the undifferentiated state.

3. The method of claim 2, further comprising culturing said PSCs under conditions which allow expansion of the pluripotent stem cells in the undifferentiated state.

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

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

(b) passaging said ESCs in a suspension culture by mechanical dissociation of ESC clumps to single cells for at least 2 and no more than 10 passages, to thereby obtain a suspension culture of ESCs devoid of clumps, and;

(c) passaging said suspension culture of ESCs devoid of said clumps without dissociation of said clumps,

thereby deriving the embryonic stem cell line.

5. The method of claim 4, further comprising culturing said ESCs under conditions which allow expansion of the embryonic stem cells in the undifferentiated state.

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

7. A method of cloning pluripotent stem cells, comprising:

culturing a single pluripotent stem cell obtained according to the method of claim 2 or 3, or a single 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 pluripotent stem cell or of said single embryonic stem cell in the undifferentiated state, thereby expanding said single pluripotent stem cell or said embryonic stem cell into a clonal culture,

thereby cloning the pluripotent stem cells.

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

(a) culturing the pluripotent stem cells in a suspension culture according to the method of claim 2, 3, 5 or 6, 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 pluripotent stem cells.

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

(a) culturing the pluripotent stem cells in a suspension culture according to the method of claim 2, 3, 5 or 6, 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 said pluripotent stem cells to embryoid bodies;

thereby generating the embryoid bodies from the pluripotent stem cells.

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

(a) culturing the pluripotent stem cells in a suspension culture according to the method of claim 2, 3, 5 or 6, to thereby obtain expanded, undifferentiated pluripotent stem cells devoid of clumps;

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

11. 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 pluripotent stem cells.

12. The method of any of claims 2-7, wherein said culturing is effected under culturing conditions devoid of substrate adherence.

13. The method of any of claims 2-7, wherein said culturing conditions being devoid of a Rho-associated kinase (ROCK) inhibitor.

14. The method of any of claims 2-3 and 6-13, wherein said pluripotent stem cells are human pluripotent stem cells.

15. The isolated population of cells of claim 1 or the method of claim 14, wherein said human pluripotent stem cells are embryonic stem cells.

16. The isolated population of cells of claim 1 or the method of claim 14, wherein said human pluripotent stem cells are induced pluripotent stem cells.

17. An isolated population of pluripotent stem cells devoid of cell clumps generated according to the method of any of claims 2-7, said isolated population comprises at least 50% human pluripotent stem cells characterized by an $OCT4^{+}/TRA1\text{-}60^{-}/TRA1\text{-}81^{-}/SSEA1^{+}/SSEA4^{-}$ expression signature, wherein an expression level of said OCT4 in said human pluripotent stem cells is higher than in human embryonic stem cells (ESCs) grown on

mouse embryonic fibroblasts (MEFs), and being capable of differentiating into the endoderm, ectoderm and mesoderm embryonic germ layers.

18. The method of any of claims 2-16, wherein said culture conditions comprise a culture medium which comprises interleukin 11 (IL11) and Ciliay Neurotrophic Factor (CNTF).

19. The method of any of claims 2-16, 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.

20. The method of any of claims 2-16, wherein said culture conditions comprise a culture medium which comprises an animal contaminant-free serum replacement and an IL6RIL6 chimera.

21. The method of any of claims 2-16, 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.

22. The method of any of claims 2-16, wherein said culture conditions comprise a culture medium which comprises interleukin 11 (IL11) and oncostatin.

23. The isolated population of human pluripotent stem cells of claim 1, wherein said 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.

Claims:

1. An isolated population of human pluripotent stem cells comprising at least 50% human pluripotent stem cells characterized by an endogenous $OCT4^{+}/TRA1\text{-}60^{-}/TRA1\text{-}81^{-}/SSEA1^{+}/SSEA4^{-}$ 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 $OCT4^{+}/TRA1\text{-}60^{-}/TRA1\text{-}81^{-}/SSEA1^{+}/SSEA4^{-}$ 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.