WHAT IS CLAIMED IS:

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 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

WHAT IS CLAIMED IS:

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 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 method of claim 14, wherein said human pluripotent stem cells are embryonic stem cells.

16. 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, and 11-16 and being capable of differentiating into the endoderm, ectoderm and mesoderm embryonic germ layers.

18. A method of generating a mesenchymal stem cell in a suspension culture, comprising culturing the pluripotent stem cells of claim 1 or 17 in a suspension culture under conditions suitable for differentiation of pluripotent stem cells to mesenchymal stem cells,

thereby generating the mesenchymal stem cell in the suspension culture.

19. An isolated population of mesenchymal stem cells (MSCs) in a suspension culture generated by the method of claim 18.

20. The isolated population of mesenchymal stem cells of claim 19, wherein at least 40% of the cells are characterized by a CD73+/CD31-/CD105+ expression signature.

21. The isolated population of MSCs of claim 19 or 20, wherein said MSCs are capable of differentiation in a suspension culture into a cell lineage selected from the group consisting of an adipogenic lineage, an osteoblastic lineage, and a chrondrogenic lineage.

22. A method of generating a neuronal progenitor cell in a suspension culture, comprising culturing the pluripotent stem cells of claim 1 or 17 in a suspension culture under conditions suitable for differentiation of neuronal progenitor cell,

thereby generating the neuronal progenitor cell in the suspension culture.

23. An isolated population of neuronal progenitor cells in a suspension culture generated by the method of claim 22.

24. A culture medium comprising interleukin 11 (IL11) and Ciliary Neurotrophic Factor (CNTF).

25. The culture of claim 24, wherein said IL11 is provided at a concentration of at least 0.1 ng/ml.

26. The cell culture of claim 24, wherein said CNTF is provided at a concentration of 1 ng/ml.

27. A culture medium comprising basic fibroblast growth factor (bFGF) at a concentration of at least 50 ng/ml and an IL6RIL6 chimera.

28. The culture medium of claim 27, wherein said concentration of said bFGF is selected from the range of between 50 ng/ml to 150 ng/ml.

29. The culture medium of any of claims 24-28, further comprising serum replacement.

30. The culture medium of claim 29, wherein said serum replacement is devoid of animal contaminants.

31. A culture medium comprising an animal contaminant-free serum replacement and an IL6RIL6 chimera.

32. The culture medium of claim 31, wherein said IL6RIL6 chimera is provided at a concentration of 50-150 ng/ml.

33. The culture medium of claim 31, wherein said IL6RIL6 chimera is provided at a concentration of 50-150 pg/ml.

34. The culture medium of any of claims 24-26 and 31-33 further comprising basic fibroblast growth factor (bFGF).

35. The culture medium of any of claims 31-34, further comprising ascorbic acid.

36. The culture medium of claim 35, wherein said ascorbic acid is provided at a concentration of 25-100 μg/ml.

37. The culture medium of claim 27, or 28, wherein said bFGF is provided at a concentration of 100 ng/ml and said IL6RIL6 is provided at a concentration of 100 ng/ml.

38. The culture medium of claim 27, or 28, wherein said bFGF is provided at a concentration of 100 ng/ml and said IL6RIL6 is provided at a concentration of 100 pg/ml.

39. The culture medium of any of claims 31-34 further comprising TGFβ.

40. The culture medium of claim 39, wherein said TGFβ comprises TGFβ1.

41. The culture medium of claim 39, wherein said TGFβ comprises TGFβ3.

42. A cell culture comprising pluripotent stem cells and the culture medium of any of claims 24-41.

43. A culture system comprising a matrix and the culture medium of any of claims 24-41.

44. A cell culture comprising pluripotent stem cells and a serum-free culture medium, said culture medium comprising 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.

45. A cell culture comprising pluripotent stem cells and a culture medium which comprises interleukin 11 (IL11) and oncostatin.

46. A method of expanding and maintaining pluripotent stem cells in an undifferentiated state, the method comprising culturing the pluripotent stem cells in the culture medium of any of claims 24-41, thereby expanding and maintaining the pluripotent stem cells in the undifferentiated state.

47. The method of claim 46, wherein said expanding and maintaining said pluripotent stem cells in said undifferentiated state is effected in a suspension culture.

48. The method of any of claims 46 and 47, wherein said culturing is effected under conditions which enable expansion of said pluripotent stem cells as single cells.

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

(a) culturing the pluripotent stem cells according to the method of any of claims 46-48, to thereby obtain expanded, undifferentiated stem cells;

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

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

50. A cell culture comprising a population of pluripotent stem cells generated according to the method of any of claims 46-48, said population comprises at least 1000 pluripotent stem cells per milliliter of culture medium.

51. 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).

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 $OCT4^{+}$/TRA1-60^{-}/TRA1-81^{-} /$SSEA1^{+}$/$SSEA4^{-}$ 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.