

CLAIMS

What is claimed is:

- 5 1. A nucleic acid construct comprising at least two coding regions, wherein the coding regions are linked to each by a nucleic acid that encodes a self-cleaving peptide so as to form a single open reading frame, and wherein the coding regions encode first and second reprogramming factors capable, either alone or in combination with one or more additional reprogramming factors, of reprogramming a
10 mammalian somatic cell to pluripotency.
- 15 2. The nucleic acid construct of claim 1, further comprising a third coding regions that encodes a third reprogramming factor, wherein the three coding regions are linked to each other by nucleic acids that encode self-cleaving peptides so as to form a single open reading frame.
- 20 3. The nucleic acid construct of claim 1, further comprising third and fourth genes that encode third and fourth reprogramming factors, wherein the four coding regions are linked to each other by nucleic acids that encode self-cleaving peptides so as to form a single open reading frame.
- 25 4. The nucleic acid construct of claim 1, wherein the self-cleaving peptide is a viral 2A peptide.
- 30 5. The nucleic acid construct of claim 1, wherein the self-cleaving peptide is an aphthovirus 2A peptide.
6. The nucleic acid construct of claim 1, wherein the reprogramming factors are selected from Oct4, Nanog, Sox2, Klf4, Lin28, and c-Myc.
7. The nucleic acid construct of claim 1, wherein the coding regions encode first and second reprogramming factors that require at least one additional reprogramming factor in order to reprogram a mammalian somatic cell to pluripotency.

8. The nucleic acid construct of claim 1, wherein the construct does not encode Oct4.

5 9. The nucleic acid construct of claim 1, wherein the construct does not encode Klf4.

10. The nucleic acid construct of claim 1, wherein the construct does not encode Sox2.

10 11. The nucleic acid construct of claim 1, wherein the construct does not encode c-Myc.

12. The nucleic acid construct of claim 1, wherein the construct does not encode Lin28.

15 13. The nucleic acid construct of claim 1, wherein the construct does not encode Nanog.

20 14. The nucleic acid construct of claim 1, wherein the coding regions encode a set of reprogramming factors selected from: (i) the set consisting of Oct4 and Sox2; (ii) the set consisting of Oct4 and Klf4; (iii) the set consisting of Oct4 and Nanog; (iv) the set consisting of Oct4, Klf4, and Sox2; (v) the set consisting of Oct4, Nanog, and Lin28; (vi) the set consisting of Oct4, Nanog, and Sox2; and (vii) the set consisting of any of (i) – (vi) and further including c-Myc.

25 15. An expression cassette comprising the nucleic acid construct of claim 1 operably linked to a promoter, wherein the promoter drives transcription of a polycistronic message that encodes the reprogramming factors, each reprogramming factor being linked to at least one other reprogramming factor by a self-cleaving peptide.

30 16. The expression cassette of claim 15, further comprising one or more sites that mediate integration into the genome of a mammalian cell.

17. An expression vector comprising the expression cassette of claim 15.
18. The expression vector of claim 17, wherein the vector is retroviral.
19. The expression vector of claim 18, wherein the promoter is inducible.
20. A mammalian cell comprising the expression cassette of claim 15.
21. The mammalian cell of claim 20, wherein the cell is a somatic cell.
22. The mammalian cell of claim 20, wherein the cell is a terminally differentiated somatic cell.
23. The mammalian cell of claim 20, wherein the cell is an iPS cell.
24. A chimeric mouse generated at least in part from the mammalian iPS cell of claim 23.
25. The chimeric mouse of claim 24, wherein the mouse is generated by injecting the mammalian iPS cell into a mouse blastocyst and allowing the blastocyst to develop into a mouse in vivo.
26. A cell obtained from the mouse of claim 24, wherein the cell is derived from the iPS cell.
27. The mammalian cell of claim 20, wherein the cell is a human cell.
28. The mammalian cell of claim 20, wherein the expression cassette is integrated into the genome at a locus whose disruption has minimal or no effect on the cell.
29. The mammalian cell of claim 20, wherein the cell expresses two reprogramming factors from the expression cassette.

30. The mammalian cell of claim 20, wherein the cell expresses three reprogramming factors from the expression cassette.

5 31. The mammalian cell of claim 20, wherein the cell expresses three reprogramming factors from the expression cassette, wherein the three reprogramming factors are not sufficient to reprogram mammalian fibroblasts with an efficiency of at least 0.05%.

10 32. The mammalian cell of claim 20, wherein the cell expresses three reprogramming factors from the expression cassette, wherein the three reprogramming factors are sufficient to reprogram mammalian fibroblasts with an efficiency of at least 0.05%.

15 33. The mammalian cell of claim 20, wherein the cell further comprises a reporter gene integrated at a locus whose activation serves as a marker of reprogramming to pluripotency.

20 34. The mammalian cell of claim 31, wherein the locus is selected from Nanog and Oct4.

35. A composition comprising multiple essentially genetically identical cells of claim 20.

25 36. A composition for identifying a reprogramming agent, the composition comprising one or more mammalian cells of claim 20 and a test agent.

30 37. The composition of claim 34, further comprising an agent that induces expression of the reprogramming factors from the expression cassette.

38. A method of identifying a reprogramming agent comprising:

(i) maintaining the composition of claim 34 for a time period under conditions in which the reprogramming factors are expressed from the expression cassette and cell proliferation occurs; and

(ii) assessing the extent to which cells become reprogrammed, wherein the test agent is identified as a reprogramming agent or enhancer of reprogramming if reprogramming occurs at a significantly greater frequency than would be the case had the composition lacked the test agent.

39. The method of claim 36, wherein the composition is maintained for at least X days.

40. The method of claim 36, wherein the test agent is present for at least X days.

41. The method of claim 36, wherein the test agent is identified as a reprogramming agent if cells do not become reprogrammed at a detectable frequency if maintained for the time period in the absence of the test agent but do become reprogrammed at a detectable frequency if maintained in the presence of the test agent for at least a portion of the time period.

42. The method of claim 36, wherein the test agent is identified as an enhancer of reprogramming agent if cells become reprogrammed at a detectable frequency if maintained for the time period in the absence of the test agent and become reprogrammed at a significantly greater frequency if maintained in the presence of the test agent for at least a portion of the time period.

43. The method of claim 36, wherein the cells express a set of reprogramming factors from the expression cassette, wherein the set consists of: (i) Oct4 and Sox2; (ii) Oct4 and Klf4; (iii) Oct4 and Nanog; (iv) Oct4, Klf4, and Sox2; (v) Oct4, Nanog, and Lin28; (vi) Oct4, Nanog, and Sox2; and (vii) any of (i) – (vi) further including c-Myc.

44. A method of reprogramming a differentiated somatic cell to a pluripotent state, comprising the steps of:

- (a) contacting a differentiated somatic cell with at least one reprogramming agent that contributes to reprogramming of said cell to a pluripotent state;
 - (b) maintaining said cell under conditions appropriate for proliferation of said cell and for activity of said at least one reprogramming agent for a period of time sufficient to begin reprogramming of said cell; and
 - (c) functionally inactivating said at least one reprogramming agent.
45. A method of reprogramming a differentiated somatic cell to a pluripotent state, comprising the steps of:
- (a) providing a differentiated somatic cell that contains at least one exogenously introduced factor that contributes to reprogramming of said cell to a pluripotent state;
 - (b) maintaining said cell under conditions appropriate for proliferation of said cell and for activity of said at least one exogenously introduced factor for a period of time sufficient to activate at least one endogenous pluripotency gene; and
 - (c) functionally inactivating said at least one exogenously introduced factor.
46. A method of selecting a differentiated somatic cell that has been reprogrammed to a pluripotent state, comprising the steps of:
- (a) providing a differentiated somatic cell that contains at least one exogenously introduced factor that contributes to reprogramming of said cell to a pluripotent state;
 - (b) maintaining said cell under conditions appropriate for proliferation of said cell and for activity of said at least one exogenously introduced factor for a period of time sufficient to activate at least one endogenous pluripotency gene;
 - (c) functionally inactivating said at least one exogenously introduced factor; and
 - (d) differentiating cells which display one or more markers of pluripotency and cells which do not.

47. An isolated pluripotent cell derived from a reprogrammed differentiated somatic cell.
48. A purified population of somatic cells comprising at least 70% pluripotent cells derived from reprogrammed differentiated somatic cells.
49. An isolated pluripotent cell produced by a method comprising:
 - (a) providing a differentiated somatic cell that contains at least one exogenously introduced factor that contributes to reprogramming of said cell to a pluripotent state;
 - (b) maintaining said cell under conditions appropriate for proliferation of said cell and for activity of said at least one exogenously introduced factor for a period of time sufficient to activate at least one endogenous pluripotency gene;
 - (c) functionally inactivating said at least one exogenously introduced factor; and
 - (d) differentiating cells which display one or more markers of pluripotency from cells which do not.
50. A purified population of somatic cells comprising at least 70% pluripotent cells derived from reprogrammed differentiated somatic cells produced by a method comprising:
 - (a) providing a differentiated somatic cell that contains at least one exogenously introduced factor that contributes to reprogramming of said cell to a pluripotent state;
 - (b) maintaining said cell under conditions appropriate for proliferation of said cell and for activity of said at least one exogenously introduced factor for a period of time sufficient to activate at least one endogenous pluripotency gene;
 - (c) functionally inactivating said at least one exogenously introduced factor; and

- (d) differentiating cells which display one or more markers of pluripotency and cells which do not.

51. A method of producing a pluripotent cell from a somatic cell, comprising the steps of:

- (a) providing one or more somatic cells that each contain at least one exogenously introduced factor that contributes to reprogramming of said cell to a pluripotent state, wherein said exogenously introduced factor is introduced using an inducible vector which is not subject to methylation-induced silencing;
- (b) maintaining said one or more cells under conditions appropriate for proliferation of said cells and for activity of said at least one exogenously introduced factor for a period of time sufficient to activate at least one endogenous pluripotency gene;
- (c) functionally inactivating said at least one exogenously introduced factor;
- (d) selecting one or more cells which display a marker of pluripotency;
- (e) generating a chimeric embryo utilizing said one or more cells which display a marker of pluripotency;
- (f) obtaining one or more somatic cells from said chimeric embryo;
- (g) maintaining said one or more somatic cells under conditions appropriate for proliferation of said cells and for activity of said at least one exogenously introduced factor for a period of time sufficient to activate at least one endogenous pluripotency gene; and
- (h) differentiating between cells which display one or more markers of pluripotency and cells which do not.

52. An isolated pluripotent cell produced by a method comprising:

- (a) providing one or more somatic cells that each contain at least one exogenously introduced factor that contributes to reprogramming of said cell to a pluripotent state, wherein said exogenously introduced factor is introduced using an inducible vector which is not subject to methylation-induced silencing;

- (b) maintaining said one or more cells under conditions appropriate for proliferation of said cells and for activity of said at least one exogenously introduced factor for a period of time sufficient to activate at least one endogenous pluripotency gene;
- (c) functionally inactivating said at least one exogenously introduced factor;
- (d) selecting one or more cells which display a marker of pluripotency;
- (e) generating a chimeric embryo utilizing said one or more cells which display a marker of pluripotency;
- (f) obtaining one or more differentiated somatic cells from said chimeric embryo;
- (g) maintaining said one or more differentiated somatic cells under conditions appropriate for proliferation of said cells and for activity of said at least one exogenously introduced factor for a period of time sufficient to activate at least one endogenous pluripotency gene; and
- (h) differentiating cells which display one or more markers of pluripotency and cells which do not.

53. A purified population of somatic cells comprising at least 70% pluripotent cells derived from reprogrammed differentiated somatic cells produced by a method comprising:

- (a) providing one or more somatic cells that each contain at least one exogenously introduced factor that contributes to reprogramming of said cell to a pluripotent state, wherein said exogenously introduced factor is introduced using an inducible vector which is not subject to methylation-induced silencing;
- (b) maintaining said one or more cells under conditions appropriate for proliferation of said cells and for activity of said at least one exogenously introduced factor for a period of time sufficient to activate at least one endogenous pluripotency gene;
- (c) functionally inactivating said at least one exogenously introduced factor;
- (d) selecting one or more cells which display a marker of pluripotency;

- (e) generating a chimeric embryo utilizing said one or more cells which display a marker of pluripotency;
 - (f) obtaining one or more differentiated somatic cells from said chimeric embryo;
 - (g) maintaining said one or more differentiated somatic cells under conditions appropriate for proliferation of said cells and for activity of said at least one exogenously introduced factor for a period of time sufficient to activate at least one endogenous pluripotency gene; and
 - (h) differentiating cells which display one or more markers of pluripotency and cells which do not.
54. A method of reprogramming a differentiated immune cell to a pluripotent state, comprising the steps of:
- (a) providing a differentiated immune cell that contains exogenously introduced Oct4, Sox2, Klf-4 and c-Myc, each under the control of an inducible vector, and further contains exogenously introduced C/EBP α ;
 - (b) maintaining said cell under conditions appropriate for proliferation of said cell and for activity of Oct4, Sox2, Klf-4, c-Myc and C/EBP α for a period of time sufficient to activate endogenous *Nanog* and/or *Oct4*; and
 - (c) functionally inactivating exogenously introduced Oct4, Sox2, Klf-4 and c-Myc.
55. A purified population of immune cells comprising at least 70% pluripotent cells derived from reprogrammed differentiated immune cells produced by a method comprising the steps of:
- (a) providing a differentiated immune cell that contains exogenously introduced Oct4, Sox2, Klf-4 and c-Myc, each under the control of an inducible vector, and further contains exogenously introduced C/EBP α ;
 - (b) maintaining said cell under conditions appropriate for proliferation of said cell and for activity of Oct4, Sox2, Klf-4, c-Myc and C/EBP α for

a period of time sufficient to activate endogenous *Nanog* and/or *Oct4*;
and

- (c) functionally inactivating exogenously introduced *Oct4*, *Sox2*, *Klf-4* and *c-Myc*.

56. A method of identifying a reprogramming agent comprising:

- (a) providing one or more somatic cells that each contain at least one exogenously introduced factor that contributes to reprogramming of said cell to a pluripotent state, wherein each of said exogenously introduced factors is introduced using an inducible vector which is not subject to methylation-induced silencing and the expression of which is controlled by regulatory elements induced by distinct inducers;
- (b) maintaining said one or more cells under conditions appropriate for proliferation of said cells and for activity of said at least one exogenously introduced factor for a period of time sufficient to reprogram said cell or to activate at least one endogenous pluripotency gene;
- (c) functionally inactivating said at least one exogenously introduced factor;
- (d) selecting one or more cells which display a marker of pluripotency;
- (e) generating a chimeric embryo utilizing said one or more cells which display a marker of pluripotency;
- (f) obtaining one or more somatic cells from said chimeric embryo;
- (g) maintaining said one or more somatic cells under conditions appropriate for proliferation of said cells and for activity of said at least one exogenously introduced factor wherein activity of said at least one exogenously introduced factor is insufficient by itself to activate at least one endogenous pluripotency gene;
- (h) contacting the somatic cell of (g) with one or more candidate reprogramming agents; and
- (i) identifying cells contacted with said one or more candidate reprogramming agents which display one or more markers of pluripotency, wherein candidate reprogramming agents which induce

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the somatic cell of (g) to display one or more markers of pluripotency
are identified as reprogramming agents.

CLAIMS

1. A nucleic acid construct comprising at least two coding regions, wherein the coding regions are linked to each by a nucleic acid that encodes a self-cleaving peptide so as to form a single open reading frame, and wherein the coding regions encode first and second reprogramming factors capable, either alone or in combination with one or more additional reprogramming factors, of reprogramming a mammalian somatic cell to pluripotency, wherein the nucleic acid construct is not a plasmid that comprises two or more reprogramming factors ligated across the 2A sequence of foot-and-mouth disease virus.
2. The nucleic acid construct of claim 1, further comprising:
 - (a) a third coding region that encodes a third reprogramming factor, wherein the three coding regions are linked to each other by nucleic acids that encode self-cleaving peptides; or
 - (b) third and fourth coding regions that encode third and fourth reprogramming factors, wherein the four coding regions are linked to each other by nucleic acids that encode self-cleaving peptides.
3. The nucleic acid construct of claim 1, wherein:
 - (a) the self-cleaving peptide is a viral 2A peptide or an aphthovirus 2 A peptide; or
 - (b) the reprogramming factors are selected from Oct4, Nanog, Sox2, Klf4, Lin28, and c-Myc; or
 - (c) the coding regions encode first and second reprogramming factors that require at least one additional reprogramming factor in order to reprogram a mammalian somatic cell to pluripotency; or
 - (d) the construct does not encode one of the reprogramming factors selected from the group consisting of: Oct4, Klf4, Sox2, c-Myc, Lin28, and Nanog; or

(e) the coding regions encode a set of reprogramming factors selected from the group consisting of:

- (i) Oct4 and Sox2; (ii) Oct4 and Klf4; (iii) Oct4 and Nanog; (iv) Oct4, Klf4, and Sox2; (v) Oct4, Nanog, and Lin28; (vi) Oct4, Nanog, and Sox2; and
- (vii) any of (i) - (vi) and further including c-Myc.

4. An expression cassette comprising: the nucleic acid construct of claim 1 operably linked to a promoter, wherein the promoter drives transcription of a polycistronic message that encodes the reprogramming factors, each reprogramming factor being linked to at least one other reprogramming factor by a self-cleaving peptide; or
the nucleic acid construct of claim 1 operably linked to a promoter, wherein the promoter drives transcription of a polycistronic message that encodes the reprogramming factors, each reprogramming factor being linked to at least one other reprogramming factor by a self-cleaving peptide and further comprising one or more sites that mediate integration into the genome of a mammalian cell.
5. An expression vector comprising:
 - (a) the expression cassette of claim 4; or
 - (b) the expression cassette of claim 4, wherein the vector is retroviral; or
 - (c) the expression cassette of claim 4, wherein the promoter is inducible.
6. An isolated mammalian cell comprising:
 - (a) the expression cassette of claim 4;
 - (b) the expression cassette of claim 4, wherein the cell is selected from the group consisting of: a somatic cell, a terminally differentiated somatic cell, and an iPS cell; or
 - (c) the expression cassette of claim 4, wherein the cell is a human cell; or
 - (d) the expression cassette of claim 4, wherein the cell expresses two or three reprogramming factors from the expression cassette; or

- (e) the expression cassette of claim 4, wherein the cell expresses three reprogramming factors from the expression cassette, wherein the three reprogramming factors are not sufficient to reprogram mammalian fibroblasts with an efficiency of at least 0.05%; or
- (f) the expression cassette of claim 4, wherein the cell expresses three reprogramming factors from the expression cassette, wherein the three reprogramming factors are sufficient to reprogram mammalian fibroblasts with an efficiency of at least 0.05%; or
- (g) the expression cassette of claim 4, wherein the cell further comprises a reporter gene integrated at a locus whose activation serves as a marker of reprogramming to pluripotency.

7. A method of identifying a reprogramming agent comprising:
 - (A) maintaining a composition comprising the mammalian cell of claim 6 having a reporter gene integrated at the Nanog or Oct4 locus whose activation serves as a marker of reprogramming to pluripotency, and a test agent, for a time period under conditions in which the reprogramming factors are expressed from the expression cassette and cell proliferation occurs; and
 - (B) assessing the extent to which cells become reprogrammed, wherein the test agent is identified as a reprogramming agent or enhancer of reprogramming if reprogramming occurs at a significantly greater frequency than would be the case had the composition lacked the test agent.
8. The method of claim 7, wherein the test agent is identified as a reprogramming agent if cells do not become reprogrammed at a detectable frequency if maintained for the time period in the absence of the test agent but do become reprogrammed at a detectable frequency if maintained in the presence of the test agent for at least a portion of the time period; or wherein the test agent is identified as an enhancer of reprogramming agent if cells become reprogrammed at a detectable frequency if maintained for the time

period in the absence of the test agent and become reprogrammed at a significantly greater frequency if maintained in the presence of the test agent for at least a portion of the time period.

9. The method of claim 7, wherein the cells express a set of reprogramming factors from the expression cassette, wherein the set consists of: (i) Oct4 and Sox2; (ii) Oct4 and Klf4; (iii) Oct4 and Nanog; (iv) Oct4, Klf4, and Sox2; (v) Oct4, Nanog, and Lin28; (vi) Oct4, Nanog, and Sox2; and (vii) any of (i) - (vi) further including c-Myc.