number of reprogramming factors. In addition, non-integrative methods have proven to be inefficient and labor intensive, requiring additional reprogramming factors or not being effective in reprogramming all somatic cells (Lee *et al.*, 2013).

Several challenges associated with the culture of pluripotent stem cells have yet to be addressed to allow the cells to become suitable for future industrial and clinical applications. In the most commonly used conventional culture system, hESCs and hiPSCs are maintained on feeder cells while passaged as clumps to prevent extensive cell death and genomic aberrations (Thomson *et al.*, 1998). The inability to single cell culture hiPSCs in a feeder-free (FF) environment severely limits potential industrial scale screening or cell therapy applications (Skottman *et al.*, 2007; Valamehr *et al.*, 2011). In addition, recent efforts on improving hiPSCs have focused on lentiviral derived hiPSC that were not transgene-free, limiting the therapeutic relevance of such efforts.

Another challenge yet to be successfully addressed, short of genome modification, is the propensity for spontaneous differentiation of human pluripotent stem cells in culture (Pera and Trounson, 2004; Sathananthan and Trounson, 2005; Valamehr *et al.*, 2011).

Studies in hESCs and hiPSCs have been described, but continuous ectopic expression of pluripotency genes were necessary to maintain the ground state resulting in genome modified human pluripotent stem cells (Hanna *et al.*, 2010a), which are unsuitable for industrial- and clinical-grade pluripotent cells.

Accordingly, the absence of compositions and methods for high-throughput, transgene or footprint free generation of human pluripotent cell products has thus far proven to be a substantial hurdle in the development and commercialization of future pluripotent stem cell therapies.

BRIEF SUMMARY

The ~~invention~~disclosure generally provides improved cell culture platforms.

In one aspect, the present disclosure provides a method of culturing induced pluripotent cells, reducing spontaneous differentiation of induced pluripotent cells, or manufacturing induced pluripotent cells, comprising culturing said cells in a culture medium comprising: (a) a Wnt pathway agonist; (b) a MEK inhibitor; and (c) a ROCK inhibitor,

thereby maintaining pluripotency of the cultured cells, and wherein the culture medium does not comprise a TGFβ receptor (TGFβR) inhibitor, and is feeder-free.

In another aspect, the present disclosure provides a composition comprising: (a) a Wnt pathway agonist; (b) a MEK inhibitor; (c) a ROCK inhibitor; and (d) a population of induced pluripotent stem cells (iPSCs), wherein the composition does not comprise a TGFβR inhibitor.

In yet another aspect, the present disclosure provides a use of a culture medium in culturing induced pluripotent cells, wherein said culture medium comprises: (a) a Wnt pathway agonist; (b) a MEK inhibitor; and (c) a ROCK inhibitor, wherein the culture medium does not comprise a TGFβR inhibitor.

In various ~~embodiment~~examples, the ~~invention~~disclosure contemplates, in part, a composition comprising: (a) a Wnt pathway agonist; (b) a MEK inhibitor; and (c) a ROCK inhibitor, wherein the composition does not comprise a TGFβR inhibitor.

In particular ~~embodiment~~examples, the Wnt pathway agonist is a GSK3 inhibitor.

In certain ~~embodiment~~examples, the GSK3 inhibitor is CHIR99021 or BIO.

In additional ~~embodiment~~examples, the MEK inhibitor is PD98059 or PD032901.

In further ~~embodiment~~examples, the ROCK inhibitor is thiazovivin or Y27632.

In some ~~embodiment~~examples, the GSK3 inhibitor is CHIR99021; the MEK inhibitor is PD032901; and the ROCK inhibitor is thiazovivin.

In certain ~~embodiment~~examples, any of the foregoing compositions further comprises bFGF or LIF.

In further ~~embodiment~~examples, any of the foregoing compositions further comprises bFGF and LIF.

In various ~~embodiment~~examples, a culture medium is provided comprising any of the foregoing compositions, wherein the medium does not comprise a TGFβR inhibitor.

In some ~~embodiment~~examples, a method of culturing one or more pluripotent cells comprising culturing the one or more pluripotent cells in a cell culture medium according to any of the foregoing culture media.

In additional ~~embodiment~~examples, the one or more pluripotent cells are embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs).

In particular ~~embodiment~~examples, the one or more pluripotent cells are iPSCs.

In certain ~~embodiment~~examples, the composition comprises a population of pluripotent cells.

In further ~~embodiment~~examples, the population of pluripotent cells is a homogenous population of pluripotent cells.

In particular ~~embodiment~~examples, at least 95% of the population of pluripotent cells expresses SSEA4-FITC and TRA1-81 or TRA1-60.

In some ~~embodiment~~examples, at most 5% of the population of pluripotent cells expresses α-smooth muscle actin (SMA), TUJ1, or FoxA2.

In particular ~~embodiment~~examples, the pluripotent cells were previously cultured in a cell culture medium comprising a TGFβR inhibitor.

In additional ~~embodiment~~examples, culturing the pluripotent cells in the cell culture medium reduces spontaneous differentiation of the cultured cells.

In one ~~embodiment~~example, expression of one or more differentiation marker genes in the cultured cells is decreased by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the one or more differentiation marker genes in a pluripotent cell cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.

In another ~~embodiment~~example, the one or more differentiation marker genes is selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.

In yet another ~~embodiment~~example, expression of two or more differentiation marker genes is decreased in the cultured cells by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the two or more differentiation marker genes in a pluripotent cell cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.

In still yet another ~~embodiment~~example, the two or more differentiation marker genes are selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.

In a particular ~~embodiment~~example, expression of three or more differentiation marker genes is decreased in the cultured cells by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the three or more differentiation marker genes in a pluripotent cell cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.

In a certain ~~embodiment~~example, the three or more differentiation marker genes are selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.

In an additional ~~embodiment~~example, expression of five or more differentiation marker genes is decreased in the cultured cells by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the five or more differentiation marker genes in a pluripotent cell cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.

In a further ~~embodiment~~example, the five or more differentiation marker genes are selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.

In a certain ~~embodiment~~example, culturing the pluripotent cells in the cell culture medium maintains or induces a ground state of pluripotency.

In particular ~~embodiment~~examples, the ground state of pluripotency of the one or more pluripotent cells is maintained for at least 5 passages.

In certain particular ~~embodiment~~examples, the ground state of pluripotency of the one or more pluripotent cells is maintained for at least 10 passages.

In further particular ~~embodiment~~examples, the ground state of pluripotency of the one or more pluripotent cells is maintained for at least 50 passages.

In additional particular ~~embodiment~~examples, the ground state of pluripotency of the one or more pluripotent cells is maintained for at least 100 passages.

In various ~~embodiment~~examples, the foregoing methods further comprise dissociating the one or more pluripotent cells during passaging.

In certain ~~embodiment~~examples, the viability of the one or more pluripotent cells is maintained during passaging.

In certain particular ~~embodiment~~examples, the one or more pluripotent cells comprise a normal karyotype.

In certain additional ~~embodiment~~examples, the one or more pluripotent cells are cultured in a feeder free environment.

In certain further ~~embodiment~~examples, the genomic stability of the one or more pluripotent cells is maintained for at least 10 passages.

In certain related ~~embodiment~~examples, the genomic stability of the one or more pluripotent cells is maintained for at least 50 passages.

In certain other ~~embodiment~~examples, the genomic stability of the one or more pluripotent cells is maintained for at least 100 passages.

In various ~~embodiment~~examples, the present ~~invention~~disclosure contemplates, in part, a method of adapting pluripotent cells to a feeder-free culture comprising: (a) isolating one or more pluripotent cells that are cultured in the presence of feeder cells; (b) culturing the one or more pluripotent cell in a chemically defined cell culture medium comprising: a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a ROCK inhibitor, wherein the medium does not comprise a TGFβR inhibitor.

In various particular ~~embodiment~~examples, the present ~~invention~~disclosure contemplates, in part, a method of culturing pluripotent cells enzymatically passaged as single cells comprising: (a) enzymatically treating one or more pluripotent cells to passage a single pluripotent cell; (b) culturing the single pluripotent cell in a feeder free environment;(c) culturing the single pluripotent cell in a chemically defined cell culture medium comprising: a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a ROCK inhibitor, wherein the medium does not comprise a TGFβR inhibitor.

In various certain ~~embodiment~~examples, the present ~~invention~~disclosure contemplates, in part, a method of reducing spontaneous differentiation of one or more pluripotent cells comprising: (a) culturing the one or more pluripotent cells in a feeder free environment; (b) culturing the one or more pluripotent cells in a chemically defined cell culture medium comprising: a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a ROCK inhibitor, wherein the medium does not comprise a TGFβR inhibitor.

In various additional ~~embodiment~~examples, the present ~~invention~~disclosure contemplates, in part, a method of manufacturing induced pluripotent stem cells (iPSCs) comprising: (a) obtaining one or more non-pluripotent cells; (b) reprogramming the one or more non-pluripotent cells to a pluripotent state; (c) culturing the pluripotent cells in a cell culture medium that does not comprise a TGFβR inhibitor thereby producing iPSCs.

In particular ~~embodiment~~examples, the one or more non-pluripotent cells comprise a somatic cell.

In some ~~embodiment~~examples, the one or more non-pluripotent cells comprise an adult stem cell.

In certain ~~embodiment~~examples, the one or more non-pluripotent cells are reprogrammed to a pluripotent state by increasing the expression of endogenous OCT4 in the cell.

In further ~~embodiment~~examples, reprogramming the one or more non-pluripotent cells to the pluripotent state comprises introducing one or more polynucleotides encoding one or more reprogramming factors selected from the group consisting of: OCT4, SOX2,

NANOG, KLF4, LIN28, C-MYC, ECAT1, UTF1, ESRRB, and SV40LT into the one or more non-pluripotent cells.

In additional ~~embodiment~~examples, reprogramming the one or more non-pluripotent cells to the pluripotent state comprises introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, SOX2, NANOG, KLF4, LIN28, C-MYC, ECAT1, UTF1, ESRRB, and SV40LT into the one or more non-pluripotent cells.

In certain ~~embodiment~~examples, reprogramming the one or more non-pluripotent cells to the pluripotent state comprises introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, SOX2, and NANOG into the one or more non-pluripotent cells.

In certain ~~embodiment~~examples, reprogramming the one or more non-pluripotent cells to the pluripotent state comprises introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, NANOG, ECAT1, UTF1, and ESRRB into the one or more non-pluripotent cells.

In certain ~~embodiment~~examples, reprogramming the one or more non-pluripotent cells to the pluripotent state comprises introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, ECAT1, and UTF1 into the one or more non-pluripotent cells.

In particular ~~embodiment~~examples, the one or more polynucleotides are a lentiviral vector.

In some ~~embodiment~~examples, the one or more polynucleotides are an episomal vector.

In related particular ~~embodiment~~examples, the cell culture medium comprises a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a ROCK inhibitor.

In further particular ~~embodiment~~examples, reprogramming the one or more non-pluripotent cells comprises contacting the one or more non-pluripotent cells with a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a TGFβR inhibitor, and optionally a ROCK inhibitor.

In additional ~~embodiment~~examples, the iPSCs comprise a population of iPSCs.

In particular ~~embodiment~~examples, the population of iPSCs is a homogenous population of iPSCs.

In particular ~~embodiment~~examples, at least 95% of the population of iPSCs expresses SSEA4 and TRA1-81 or TRA1-60.

In certain ~~embodiment~~examples, at most 5% of the population of pluripotent cells expresses α-smooth muscle actin (SMA), TUJ1, or FoxA2.

In particular ~~embodiment~~examples, culturing the pluripotent cells in the cell culture medium reduce spontaneous differentiation, or maintain or induce a ground state of pluripotency.

In additional ~~embodiment~~examples, expression of one or more, two or more, three or more, four or more, or five or more differentiation marker genes is decreased in the iPSCs by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the one or more differentiation marker genes in iPSCs cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.

In particular ~~embodiment~~examples, the one or more differentiation marker genes is selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.

In certain ~~embodiment~~examples, the reduction in spontaneous differentiation is maintained for at least 5 passages.

In other ~~embodiment~~examples, the reduction in spontaneous differentiation is maintained for at least 10 passages.

In particular ~~embodiment~~examples, the reduction in spontaneous differentiation is maintained for at least 50 passages.

In additional ~~embodiment~~examples, the reduction in spontaneous differentiation is maintained for at least 100 passages.

In further ~~embodiment~~examples, the foregoing methods comprise dissociating the iPSCs during passaging.

In additional ~~embodiment~~examples, the viability of the iPSCs is maintained during passaging.

In certain ~~embodiment~~examples, the iPSCs comprise a normal karyotype.

In other ~~embodiment~~examples, the iPSCs are cultured in a feeder free environment.

In particular ~~embodiment~~examples, the genomic stability of the iPSCs is maintained for at least 10 passages.

In additional ~~embodiment~~examples, the genomic stability of the iPSCs is maintained for at least 50 passages.

In particular additional ~~embodiment~~examples, the genomic stability of the iPSCs is maintained for at least 100 passages.

In various ~~embodiment~~examples, the ~~invention~~disclosure provides, in part, an induced pluripotent stem cell (iPSC) comprising ground state pluripotency produced according to any one of foregoing ~~embodiment~~examples.

In various certain ~~embodiment~~examples, the ~~invention~~disclosure provides, in part, an induced pluripotent stem cell (iPSC) comprising ground state pluripotency, wherein the iPSC does not comprise an exogenously introduced polynucleotide encoding a reprogramming factor polypeptide.

In various particular ~~embodiment~~examples, the ~~invention~~disclosure provides, in part, an method of manufacturing induced pluripotent stem cells (iPSCs) comprising: a) obtaining one or more pluripotent stem cells; (b) culturing the one or more pluripotent stem cells in a cell culture medium that does not comprise a TGFβR inhibitor thereby producing ground state iPSCs.

In certain ~~embodiment~~examples, the one or more iPSCs comprises a reprogrammed somatic cell.

In additional ~~embodiment~~examples, the one or more iPSCs comprises a reprogrammed adult stem cell.

In other ~~embodiment~~examples, the one or more iPSCs were reprogrammed to a pluripotent state by increasing the expression of endogenous OCT4 in the one or more iPSCs.

In particular ~~embodiment~~examples, the one or more iPSCs were reprogrammed by introducing one or more polynucleotides encoding one or more reprogramming factors selected from the group consisting of: OCT4, SOX2, NANOG, KLF4, LIN28, C-MYC, ECAT1, UTF1, ESRRB, and SV40LT into the one or more non-pluripotent cells.

In certain particular ~~embodiment~~examples, the one or more iPSCs were reprogrammed by introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, SOX2, NANOG, KLF4, LIN28, C-MYC, ECAT1, UTF1, ESRRB, and SV40LT.

In additional ~~embodiment~~examples, the one or more iPSCs were reprogrammed by introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, SOX2, and NANOG into the one or more non-pluripotent cells.

In additional ~~embodiment~~examples, the one or more iPSCs were reprogrammed by introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, NANOG, ECAT1, UTF1, and ESRRB into the one or more non-pluripotent cells.

In another ~~embodiment~~example, the one or more iPSCs were reprogrammed by introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, ECAT1, and UTF1 into the one or more non-pluripotent cells.

In various ~~embodiment~~examples, a lentiviral vector comprises the one or more polynucleotides.

In particular ~~embodiment~~examples, an episomal vector comprises the one or more polynucleotides.

In other ~~embodiment~~examples, the cell culture medium comprises a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a ROCK inhibitor.

In certain ~~embodiment~~examples, obtaining the one or more iPSCs comprises contacting the one or more non-pluripotent cells or partially pluripotent cells with a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a TGFβR inhibitor, and optionally a ROCK inhibitor to produce the one or more iPSCs.

In additional ~~embodiment~~examples, the iPSCs comprise a population of iPSCs.

In additional ~~embodiment~~examples, the population of iPSCs is a homogenous population of iPSCs.

In particular ~~embodiment~~examples, at least 95% of the population of iPSCs expresses SSEA4 and TRA1-81 or TRA1-60.

In particular ~~embodiment~~examples, the one or more iPSCs are obtained by reprogramming a population of pluripotent cells, wherein at most 5% of the population of pluripotent cells expresses α-smooth muscle actin (SMA), TUJ1, or FoxA2.

In further ~~embodiment~~examples, the foregoing methods comprise culturing the one or more iPSCs in the cell culture medium reduces spontaneous differentiation or maintains or induces a ground state of pluripotency.

In certain ~~embodiment~~examples, the iPSCs with reduced spontaneous differentiation comprise a gene expression wherein expression of one or more, two or more, three or more, four or more, or five or more differentiation marker genes is decreased in the iPSCs by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the one or more differentiation marker genes in iPSCs cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.

In additional ~~embodiment~~examples, the one or more differentiation marker genes is selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.

In other ~~embodiment~~examples, the reduced spontaneous differentiation is maintained for at least 5 passages.

In certain ~~embodiment~~examples, the reduced spontaneous differentiation is maintained for at least 10 passages.

In additional ~~embodiment~~examples, the reduced spontaneous differentiation is maintained for at least 50 passages.

In particular ~~embodiment~~examples, the reduced spontaneous differentiation is maintained for at least 100 passages.

In further ~~embodiment~~examples, the foregoing methods comprise dissociating the one or more iPSCs during passaging.

In particular ~~embodiment~~examples, the viability of the one or more iPSCs are maintained during passaging.

In other ~~embodiment~~examples, the one or more iPSCs comprise a normal karyotype.

In additional ~~embodiment~~examples, the one or more iPSCs are cultured in a feeder free environment.

In certain ~~embodiment~~examples, the genomic stability of the one or more iPSCs is maintained for at least 10 passages.

In additional ~~embodiment~~examples, the genomic stability of the one or more iPSCs is maintained for at least 50 passages.

In particular ~~embodiment~~examples, the genomic stability of the one or more iPSCs is maintained for at least 100 passages.

In various particular ~~embodiment~~examples, the ~~invention~~disclosure provides, in part, an induced pluripotent stem cell (iPSC) comprising ground state pluripotency produced according to any one of the foregoing ~~embodiment~~examples.

In various ~~embodiment~~examples, the ~~invention~~disclosure provides, in part, an induced pluripotent stem cell (iPSC) comprising ground state pluripotency, wherein the iPSC does not comprise an exogenously introduced polynucleotide encoding a reprogramming factor polypeptide.

In some ~~embodiment~~examples, a method for reprogramming a non-pluripotent cell to a pluripotent cell is provided comprising introducing into the nonpluripotent cell (i) one or more polynucleotides that encode at least one polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) at least one polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, thereby reprogramming the non-pluripotent cell to a pluripotent cell.

In a particular ~~embodiment~~example, introducing comprises (i) introducing one or more polynucleotides that encode an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) introducing a OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.

In another ~~embodiment~~example, introducing comprises (i) introducing one or more polynucleotides that encode an OCT4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide, or (ii) introducing an OCT-4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide.

In some ~~embodiment~~examples, the one or more polynucleotides are introduced by a retrovirus, Sendai virus, an adenovirus, an episome, mini-circle, vector system with expression cassette, or mRNA.

In a particular ~~embodiment~~example, the retrovirus is a lentivirus.

In another ~~embodiment~~example, the pluripotent cell is free of exogenous polynucleotides.

In another ~~embodiment~~example, the one or more polynucleotides is excised by CRE-mediated excision.

In some ~~embodiment~~examples, the method further comprises introducing into the non-pluripotent cell (i) a polynucleotide that encodes a SV40LT polypeptide, or (ii) a SV40LT polypeptide.

In one ~~embodiment~~example, the method comprises contacting the nonpluripotent cell with at least one of a TGFβR inhibitor, a Wnt pathway agonist, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway agonist is optionally a GSK3 inhibitor.

In another ~~embodiment~~example, the nonpluripotent cell is contacted with a TGFβR inhibitor, a Wnt pathway agonist, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway agonist is optionally a GSK3 inhibitor.

In a particular ~~embodiment~~example, the method further comprises culturing the pluripotent cell in a culture medium comprising a Wnt pathway agonist, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway agonist is optionally a GSK3 inhibitor, and wherein the culture medium does not contain TGFβR inhibitor.

In some ~~embodiment~~examples, the Rock inhibitor is thiazovivin or Y27632, the TGFβR inhibitor is A-83-01 or SB431542, the GSK3 inhibitor is CHIR99021 or BIO, or the MEK inhibitor is PD98059 or PD032901.

In another ~~embodiment~~example, pluripotency of the pluripotent cell is maintained for at least 5 cell divisions or at least 10 cell divisions.

In a particular ~~embodiment~~example, the one or more polynucleotides is introduced as a polycistronic vector comprising a plurality of polynucleotides that are separated by at least one 2A peptide.

In one ~~embodiment~~example, the polycistronic vector comprises a plurality of polynucleotides that encode an OCT4 polypeptide.

In some ~~embodiment~~examples, the method comprises identifying the pluripotent cell by selecting for OCT4 expression in the pluripotent cell.

In one ~~embodiment~~example, selecting for OCT4 expression comprises selecting for ectopic Oct-4 expression.

In another ~~embodiment~~example, culturing produces a population of pluripotent stem cells.

In another ~~embodiment~~example, the population of pluripotent stem cells are at least 70% homogenous, at least 80% homogenous, or at least 90% homogenous.

In yet another ~~embodiment~~example, at least 70%, at least 80%, or at least 90% of the population of pluripotent cells expresses SSEA and Tra-181.

In an ~~embodiment~~example, the pluripotent cell or population of pluripotent cells are capable of single cell passaging.

In one ~~embodiment~~example, the cells produced by single cell passaging have a normal karyotype.

In one ~~embodiment~~example, the ~~invention~~disclosure provides a pluripotent cell produced according to the method of any one of the methods above.

In another ~~embodiment~~example, the ~~invention~~disclosure provides a composition comprising an isolated non-pluripotent cell that comprises (i) one or more exogenous polynucleotides that encode at least one polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) at least one exogenous polypeptide selected from an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.

In one ~~embodiment~~example, the cell comprises (i) one or more exogenous polynucleotides that encode at least two of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) at least two exogenous polypeptides selected from an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.

In another ~~embodiment~~example, the cell comprises (i) one or more exogenous polynucleotides that encode at least three of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) at least three exogenous polypeptides selected from an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.

In one particular ~~embodiment~~example, the cell comprises (i) one or more exogenous polynucleotides that encode an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) an exogenous OCT4 polypeptide, an exogenous ECAT1 polypeptide, an exogenous UTF1 polypeptide, an exogenous NANOG polypeptide, and an exogenous ESRRB polypeptide.

In another ~~embodiment~~example, the cell comprises one or more exogenous polynucleotides that encode an OCT4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide, or an exogenous OCT4 polypeptide, an exogenous ECAT1 polypeptide, and an exogenous UTF1 polypeptide.

In one ~~embodiment~~example, the cell has been contacted with at least one of a TGFβR inhibitor, a GSK3 inhibitor, a MEK inhibitor and a ROCK inhibitor.

In another ~~embodiment~~example, the cell has been contacted with a TGFβR inhibitor, a Wnt pathway activator, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway activator is optionally a GSK3 inhibitor.

In a particular ~~embodiment~~example, the Rock inhibitor is thiazovivin or Y27632, the TGFβR inhibitor is A-83-01 or SB431542, the GSK3 inhibitor is CHIR99021 or BIO, or the MEK inhibitor is PD98059 or PD032901.

In another ~~embodiment~~example, the one or more exogenous polynucleotides are introduced to the non-pluripotent cell by a retrovirus, Sendai virus, an adenovirus, an episome, mini-circle, vector system with expression cassette, or mRNA.

In another ~~embodiment~~example, the retrovirus is a lentivirus.

In a particular ~~embodiment~~example, the cell is free of exogenous polynucleotides.

In an ~~embodiment~~example, the one or more exogenous polynucleotides are removed by CRE-mediated excision.

In another ~~embodiment~~example, the cell comprises an exogenous polynucleotide that encodes a SV40LT antigen polypeptide, or an exogenous a SV40LT antigen polypeptide.

In one ~~embodiment~~example, the one or more exogenous polynucleotides is introduced as a polycistronic vector comprising a plurality of polynucleotides that are separated by at least one 2A peptide.

In another ~~embodiment~~example, the polycistronic vector comprises a plurality of polynucleotides that encode an OCT4 polypeptide.

In an ~~embodiment~~example, the exogenous polynucleotide encoding an OCT4 polypeptide is linked to a selectable marker.

In one particular ~~embodiment~~example, the ~~invention~~disclosure provides a composition consisting of at least one of, at least two of, or at least three of (i) a cDNA encoding an OCT4 polypeptide, (ii) a cDNA encoding an ECAT1 polypeptide, (iii) a cDNA encoding a UTF1 polypeptide, (iv) a cDNA encoding a NANOG polypeptide, and (v) a cDNA encoding an ESRRB polypeptide.

In an ~~embodiment~~example, the composition consists of a cDNA encoding an OCT4 polypeptide, a cDNA encoding an ECAT1 polypeptide, a cDNA encoding a UTF1 polypeptide, a cDNA encoding a NANOG polypeptide, and a cDNA encoding an ESRRB polypeptide.

In another ~~embodiment~~example, the composition consists of a cDNA encoding an OCT4 polypeptide, a cDNA encoding an ECAT1 polypeptide, and a cDNA encoding a UTF1 polypeptide.

In a particular ~~embodiment~~example, each cDNA is encoded in a retrovirus, Sendai virus, an adenovirus, an episome, a mini-circle, a vector system with expression cassette, or mRNA.

In one ~~embodiment~~example, the ~~invention~~disclosure provides a vector comprising of one or more polynucleotides that encode at least one reprogramming factor polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.

In an ~~embodiment~~example, the one or more polynucleotides encode an OCT polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.

In another ~~embodiment~~example, the one or more polynucleotides encode an OCT polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide.

In another particular ~~embodiment~~example, the vector further comprises a polynucleotide that encodes an SV40LT antigen polypeptide.

In one ~~embodiment~~example, the vector is a retrovirus, Sendai virus, an adenovirus, an episome, mini-circle, vector system with expression cassette, or mRNA.

In one ~~embodiment~~example, the retrovirus is a lentivirus.

In one particular ~~embodiment~~example, the ~~invention~~disclosure provides a kit for reprogramming a non-pluripotent cell to a pluripotent cell, the kit comprising: one or more polynucleotides that encode at least one polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, or an ESRRB polypeptide; or at least one polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, or an ESRRB polypeptide; and at least one of a TGFβR

inhibitor, a Wnt pathway activator, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway activator is optionally a GSK3 inhibitor.

In an ~~embodiment~~example, the one or more polynucleotides encode an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or the at least one polypeptide comprises an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.

In another ~~embodiment~~example, the one or more one or more polynucleotides encode an OCT4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide, or the at least one polypeptide comprises an OCT4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide.

In one ~~embodiment~~example, the kit comprises a polynucleotide that encodes a SV40LT antigen polypeptide, or a SV40LT antigen polypeptide.

In another ~~embodiment~~example, the kit comprises a TGFβR inhibitor, a Wnt pathway activator, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway activator is optionally a GSK3 inhibitor.

In one ~~embodiment~~example, the Rock inhibitor is thiazovivin or Y27632, the TGFβR inhibitor is A-83-01 or SB431542, the GSK3 inhibitor is CHIR99021 or BIO, or the MEK inhibitor is PD98059 or PD032901.

In another ~~embodiment~~example, the at least one polynucleotide is encoded in a retrovirus, Sendai virus, an adenovirus, an episome, a mini-circle, a vector system with expression cassette, or mRNA.

In another ~~embodiment~~example, the retrovirus is a lentivirus.

In one ~~embodiment~~example, the at least one polynucleotide is encoded in a polycistronic vector with each polynucleotide being separated by a 2A peptide.

In yet another ~~embodiment~~example, the polycistronic vector comprises two or more polynucleotides that encode an OCT4 polypeptide.

In a particular ~~embodiment~~example, the at least one polynucleotide that encodes an OCT4 polypeptide is linked to a selectable marker.

In another particular ~~embodiment~~example, the ~~invention~~disclosure provides a method for producing a population of pluripotent stem cells comprising: providing a population of

non-pluripotent cells; introducing into the population of non-pluripotent cells a polynucleotide that encodes an OCT4 polypeptide that is linked to a selectable marker; incubating the population of non-pluripotent cells with the polynucleotide under conditions sufficient to reprogram at least a portion of the population of non-pluripotent cells to pluripotent cells; selecting cells that express the selectable marker thereby providing a population of pluripotent stem cells.

In another ~~embodiment~~example, the polynucleotide is introduced as a polycistronic vector comprising a plurality of polynucleotides that encode an OCT4 polypeptide.

In one ~~embodiment~~example, the plurality of polynucleotides are separated by at least one 2A peptide.

In yet another ~~embodiment~~example, at least 10 % of the cells in the population of cells express SSEA and TRA-181.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figures 1A-1E show results from a multistage culture platform for enhanced reprogramming and hiPSC maintenance. (1A) Lentiviral generated hiPSC clone FTi088 maintained a homogeneous population of undifferentiated cells in SMC4 while spontaneous differentiation was seen in the lentiviral generated hiPSC line FTi096 cultured in SMC4. Spontaneous differentiation was minimized when FTi096 was transitioned to FMM for 3 passages as shown by morphology (upper panels) and flow cytometry for SSEA4 and TRA1-81 (lower panels). (1B) qRT-PCR for transgene expression of viral element WPRE. Expression was normalized to GAPDH and relative to WPRE expression of parental fibroblast line four days post lentiviral infection (Day 4 P.I.). Uninfected fibroblast line (fibroblast) and human ESC line HUES9 were used as negative controls. Value of each set is indicated above the bar. (1C) Screen of various medium components' effect on SSEA4 and TRA1-81population of transgene free lentiviral-induced hiPSC after 10 passages; removal of SB431542 (-TGFβRi), increase from 10 to 100 ng/mL bFGF, addition of 10 ng/mL LIF. (1D) Fibroblast cell line was transfected with lentiviral construct containing gene set OCT4/KLF4/SOX2 and split into various media (conventional medium; Conv.), cultured for 17 days and sorted for SSEA4 and TRA1-81 double positive population at day 17. Sort gate is highlighted in

CLAIMS

1. A method of culturing induced pluripotent cells, reducing spontaneous differentiation of induced pluripotent cells, or manufacturing induced pluripotent cells, comprising culturing said cells in a culture medium comprising:

    (a) a Wnt pathway agonist;

    (b) a MEK inhibitor; and

    (c) a ROCK inhibitor,

thereby maintaining pluripotency of the cultured cells, and

wherein the culture medium does not comprise a TGFβ receptor (TGFβR) inhibitor, and is feeder-free.

2. The method of claim 1, wherein the induced pluripotent cells comprise a homogenous population of pluripotent cells, optionally wherein

(i) at least 95% of the population of pluripotent cells express SSEA4-FITC and TRA1-81 or TRA1-60; or

(ii) at most 5% of the population of pluripotent cells expresses α-smooth muscle actin (SMA), TUJ1, or FoxA2.

3. The method of claim 1 or 2, further comprising passaging the cultured induced pluripotent cells; and optionally dissociating the induced pluripotent cells into single cells before or during passaging, in a culture medium comprising a Wnt pathway agonist, a MEK inhibitor and a ROCK inhibitor.

4. The method of claim 1, wherein the cultured induced pluripotent cells comprise decreased expression of one, two, three, four, five or more differentiation marker genes by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the differentiation marker genes in an induced pluripotent cell cultured in a medium comprising a TGFβR inhibitor; wherein the one, two, three, four, five or more differentiation marker genes are (i) selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10,

CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1; or (ii) selected from the group consisting of: T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.

5. The method of any one of claims 1-4, further comprising, prior to the step of culturing the induced pluripotent cell in said culture medium, isolating induced pluripotent cells that are cultured in the presence of feeder cells and/or a TGFβR inhibitor.

6. The method of claim 1, further comprising, prior to the step of culturing the induced pluripotent cells, a step of obtaining induced pluripotent cells comprising: reprogramming obtained non-pluripotent cells to pluripotent cells.

7. The method of claim 6, wherein the step (b) of reprogramming further comprises (i) increasing the expression of endogenous OCT4 in the non-pluripotent cells; or (ii) introducing into the non-pluripotent cells one or more of:

(a) a vector comprising at least two OCT4 encoding polynucleotides;

(b) a vector comprising at least one ECAT1 encoding polynucleotide, and at least one UTF1 encoding polynucleotide;

(c) a vector comprising at least one NANOG encoding polynucleotide, and at least one ESRRB encoding polynucleotide;

(d) a vector comprising at least one OCT4 encoding polynucleotide and at least one ESRRB encoding polynucleotide; and

(e) a vector comprising at least one OCT4 encoding polynucleotide and at least one DPPA2 encoding polynucleotide;

wherein the non-pluripotent cells comprise (i) a somatic cell; or (ii) an adult stem cell.

8. The method of claim 7, wherein the method further comprises contacting the non-pluripotent cells of step (b) with a mixture comprising a Wnt pathway agonist, a MEK inhibitor and a TGFβR inhibitor, and optionally a ROCK inhibitor.

9. The method of claim 1 comprising:

(a) treating induced pluripotent cells to obtain single cell dissociated pluripotent cells prior to culturing;

(b) culturing and passaging the single cell dissociated pluripotent cells in said culture medium.

10. A composition comprising:

(a) a Wnt pathway agonist;

(b) a MEK inhibitor;

(c) a ROCK inhibitor; and

(d) a population of induced pluripotent stem cells (iPSCs),

wherein the composition does not comprise a TGFβR inhibitor.

11. The composition of claim 10, wherein

(i) the Wnt pathway agonist is a GSK3 inhibitor; optionally wherein the GSK3 inhibitor is CHIR99021 or BIO;

(ii) the MEK inhibitor is PD98059 or PD032901;

(iii) the ROCK inhibitor is thiazovivin or Y27632;

(iv) the GSK3 inhibitor is CHIR99021; the MEK inhibitor is PD032901; and the ROCK inhibitor is thiazovivin; or

(v) the composition further comprises bFGF and/or LIF.

12. The method of claim 1, wherein the induced pluripotent cells comprise induced pluripotent stem cells (iPSCs) that are obtained by introducing to non-pluripotent cells:

(i) one or more exogenous polynucleotides that encode at least one, two, three, or all polypeptides selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide; or

(ii) at least one, two, three, or all exogenous polypeptides selected from an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.

13. The method of claim 12, wherein the iPSCs are obtained by introducing to the non-pluripotent cell:

(i) one or more exogenous polynucleotides that encode an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide;

(ii) one or more exogenous polynucleotides that encode an OCT4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide;

(iii) an exogenous polynucleotide that encodes a SV40LT antigen polypeptide; or

(iv) an exogenous SV40LT antigen polypeptide.

14. The method of claim 12, wherein the one or more exogenous polynucleotides comprise a polycistronic vector comprising a plurality of polynucleotides that are separated by at least one 2A peptide encoding sequence; optionally wherein the polycistronic vector comprises a plurality of polynucleotides each encoding an OCT4 polypeptide.

15. Use of a culture medium in culturing induced pluripotent cells, wherein said culture medium comprises:

(a) a Wnt pathway agonist;

(b) a MEK inhibitor; and

(c) a ROCK inhibitor,

wherein the culture medium does not comprise a TGFβR inhibitor.

~~1. A composition comprising:~~

~~(a) a Wnt pathway agonist;~~

~~(b) a MEK inhibitor; and~~

~~(c) a ROCK inhibitor,~~

~~wherein the composition does not comprise a TGFβR inhibitor.~~

~~2. The composition of claim 1, wherein the Wnt pathway agonist is a GSK3 inhibitor.~~

~~3. The composition of claim 2, wherein the GSK3 inhibitor is CHIR99021 or BIO.~~

~~4. The composition of claim 1, wherein the MEK inhibitor is PD98059 or PD032901.~~

~~5. The composition of claim 1, wherein the ROCK inhibitor is thiazovivin or Y27632.~~

6. The composition of claim 1, wherein the GSK3 inhibitor is CHIR99021; the MEK inhibitor is PD032901; and the ROCK inhibitor is thiazovivin.

7. The composition according to any one of claims 1 to 6, further comprising bFGF or LIF.

8. The composition according to any one of claims 1 to 6, further comprising bFGF and LIF.

9. A culture medium comprising the composition of claim 1,

wherein the medium does not comprise a TGFβR inhibitor.

10. A method of culturing one or more pluripotent cells comprising culturing the one or more pluripotent cells in a cell culture medium according to claim 9.

11. The method of claim 10, wherein the one or more pluripotent cells are embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs).

12. The method of claim 10, wherein the one or more pluripotent cells are iPSCs.

13. The method of claim 10, wherein the composition comprises a population of pluripotent cells.

14. The method of claim 13, wherein the population of pluripotent cells is a homogenous population of pluripotent cells.

15. The method of claim 13, wherein at least 95% of the population of pluripotent cells expresses SSEA4-FITC and TRA1-81 or TRA1-60.

16. The method of claim 13, wherein at most 5% of the population of pluripotent cells expresses α-smooth muscle actin (SMA), TUJ1, or FoxA2.

17. The method of claim 10, wherein the pluripotent cells were previously cultured in a cell culture medium comprising a TGFβR inhibitor.

18. The method of claim 13, wherein culturing the pluripotent cells in the cell culture medium reduces spontaneous differentiation of the cultured cells.

19. The method of claim 18, wherein expression of one or more differentiation marker genes in the cultured cells is decreased by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the one or more differentiation marker genes in a pluripotent cell cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2,

~~LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.~~

~~20. The method of claim 19, wherein the one or more differentiation marker genes is selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.~~

~~21. The method of claim 18, wherein expression of two or more differentiation marker genes is decreased in the cultured cells by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the two or more differentiation marker genes in a pluripotent cell cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.~~

~~22. The method of claim 21, wherein the two or more differentiation marker genes are selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.~~

~~23. The method of claim 18, wherein expression of three or more differentiation marker genes is decreased in the cultured cells by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the three or more differentiation marker genes in a pluripotent cell cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.~~

~~24. The method of claim 23, wherein the three or more differentiation marker genes are selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.~~

~~25. The method of claim 18, wherein expression of five or more differentiation marker genes is decreased in the cultured cells by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the five or more differentiation marker genes in a pluripotent cell cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.~~

~~26. The method of claim 25, wherein the five or more differentiation marker genes are selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.~~

~~27. The method of claim 18, wherein culturing the pluripotent cells in the cell culture medium maintains or induces a ground state of pluripotency.~~

~~28. The method of claim 27, wherein the ground state of pluripotency of the one or more pluripotent cells is maintained for at least 5 passages.~~

~~29. The method of claim 27, wherein the ground state of pluripotency of the one or more pluripotent cells is maintained for at least 10 passages.~~

~~30. The method of claim 27, wherein the ground state of pluripotency of the one or more pluripotent cells is maintained for at least 50 passages.~~

~~31. The method of claim 27, wherein the ground state of pluripotency of the one or more pluripotent cells is maintained for at least 100 passages.~~

~~32. The method of any one of claims 10 to 31, further comprising dissociating the one or more pluripotent cells during passaging.~~

~~33. The method of claim 32, wherein the viability of the one or more pluripotent cells is maintained during passaging.~~

~~34. The method of any one of claims 10 to 33, wherein the one or more pluripotent cells comprise a normal karyotype.~~

~~35. The method of any one of claims 10 to 34, wherein the one or more pluripotent cells are cultured in a feeder free environment.~~

~~36. The method of any one of claims 10 to 35, wherein the genomic stability of the one or more pluripotent cells is maintained for at least 10 passages.~~

~~37. The method of any one of claims 10 to 35, wherein the genomic stability of the one or more pluripotent cells is maintained for at least 50 passages.~~

~~38. The method of any one of claims 10 to 35, wherein the genomic stability of the one or more pluripotent cells is maintained for at least 100 passages.~~

~~39. A method of adapting pluripotent cells to a feeder-free culture comprising:~~

~~(a) isolating one or more pluripotent cells that are cultured in the presence of feeder cells;~~

~~(b) culturing the one or more pluripotent cell in a chemically defined cell culture medium comprising: a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a ROCK inhibitor, wherein the medium does not comprise a TGFβR inhibitor.~~

~~40. A method of culturing pluripotent cells enzymatically passaged as single cells comprising:~~

~~(a) enzymatically treating one or more pluripotent cells to passage a single pluripotent cell;~~

~~(b) culturing the single pluripotent cell in a feeder free environment;~~

~~(c) culturing the single pluripotent cell in a chemically defined cell culture medium comprising: a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a ROCK inhibitor, wherein the medium does not comprise a TGFβR inhibitor.~~

~~41. A method of reducing spontaneous differentiation of one or more pluripotent cells comprising:~~

~~(a) culturing the one or more pluripotent cells in a feeder free environment;~~

~~(b) culturing the one or more pluripotent cells in a chemically defined cell culture medium comprising: a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a ROCK inhibitor, wherein the medium does not comprise a TGFβR inhibitor.~~

~~42. A method of manufacturing induced pluripotent stem cells (iPSCs) comprising:~~

~~(a) obtaining one or more non-pluripotent cells;~~

~~(b) reprogramming the one or more non-pluripotent cells to a pluripotent state;~~

~~(c) culturing the pluripotent cells in a cell culture medium that does not comprise a TGFβR inhibitor thereby producing iPSCs.~~

~~43. The method of claim 42, wherein the one or more non-pluripotent cells comprises a somatic cell.~~

~~44. The method of claim 42, wherein the one or more non-pluripotent cells comprises an adult stem cell.~~

~~45. The method of claim 42, wherein the one or more non-pluripotent cells is reprogrammed to a pluripotent state by increasing the expression of endogenous OCT4 in the cell.~~

~~46. The method of claim 42, wherein reprogramming the one or more non-pluripotent cells to the pluripotent state comprises introducing one or more polynucleotides encoding one or more reprogramming factors selected from the group consisting of: OCT4, SOX2, NANOG, KLF4, LIN28, C-MYC, ECAT1, UTF1, ESRRB, and SV40LT into the one or more non-pluripotent cells.~~

~~47. The method of claim 42, wherein reprogramming the one or more non-pluripotent cells to the pluripotent state comprises introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, SOX2, NANOG, KLF4, LIN28, C-MYC, ECAT1, UTF1, ESRRB, and SV40LT into the one or more non-pluripotent cells.~~

~~48. The method of claim 42, wherein reprogramming the one or more non-pluripotent cells to the pluripotent state comprises introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, SOX2, and NANOG into the one or more non-pluripotent cells.~~

~~49. The method of claim 42, wherein reprogramming the one or more non-pluripotent cells to the pluripotent state comprises introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, NANOG, ECAT1, UTF1, and ESRRB into the one or more non-pluripotent cells.~~

~~50. The method of claim 42, wherein reprogramming the one or more non-pluripotent cells to the pluripotent state comprises introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, ECAT1, and UTF1 into the one or more non-pluripotent cells.~~

~~51. The method of any one of claims 46 to 50, wherein the one or more polynucleotides is a lentiviral vector.~~

~~52. The method of any one of claims 46 to 50, wherein the one or more polynucleotides is an episomal vector.~~

53. The method of any one of claims 42-52, wherein the cell culture medium comprises a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a ROCK inhibitor.

54. The method of any one of claims 42 to 53, wherein reprogramming the one or more non-pluripotent cells comprises contacting the one or more non-pluripotent cells with a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a TGFβR inhibitor, and optionally a ROCK inhibitor.

55. The method of claim 42, wherein the iPSCs comprise a population of iPSCs.

56. The method of claim 55, wherein the population of iPSCs is a homogenous population of iPSCs.

57. The method of claim 55, wherein at least 95% of the population of iPSCs expresses SSEA4 and TRA1-81 or TRA1-60.

58. The method of claim 55, wherein at most 5% of the population of pluripotent cells expresses α-smooth muscle actin (SMA), TUJ1, or FoxA2.

59. The method of any one of claims 42-58, wherein culturing the pluripotent cells in the cell culture medium reduces spontaneous differentiation, or maintains or induces a ground state of pluripotency.

60. The method of claim 59, wherein expression of one or more, two or more, three or more, four or more, or five or more differentiation marker genes is decreased in the iPSCs by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the one or more differentiation marker genes in iPSCs cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.

61. The method of claim 60, wherein the one or more differentiation marker genes is selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.

62. The method of claim 60, wherein the reduction in spontaneous differentiation is maintained for at least 5 passages.

~~63. The method of claim 60, wherein the reduction in spontaneous differentiation is maintained for at least 10 passages.~~

~~64. The method of claim 60, wherein the reduction in spontaneous differentiation is maintained for at least 50 passages.~~

~~65. The method of claim 60, wherein the reduction in spontaneous differentiation is maintained for at least 100 passages.~~

~~66. The method of any one of claims 42 to 65, comprising dissociating the iPSCs during passaging.~~

~~67. The method of claim 66, wherein the viability of the iPSCs is maintained during passaging.~~

~~68. The method of any one of claims 42 to 67, wherein the iPSCs comprise a normal karyotype.~~

~~69. The method of any one of claims 42 to 68, wherein the iPSCs are cultured in a feeder free environment.~~

~~70. The method of any one of claims 42 to 69, wherein the genomic stability of the iPSCs is maintained for at least 10 passages.~~

~~71. The method of any one of claims 42 to 69, wherein the genomic stability of the iPSCs is maintained for at least 50 passages.~~

~~72. The method of any one of claims 42 to 69, wherein the genomic stability of the iPSCs is maintained for at least 100 passages.~~

~~73. An induced pluripotent stem cell (iPSC) comprising ground state pluripotency produced according to any one of claims 42 to 72.~~

~~74. An induced pluripotent stem cell (iPSC) comprising ground state pluripotency, wherein the iPSC does not comprise an exogenously introduced polynucleotide encoding a reprogramming factor polypeptide.~~

~~75. A method of manufacturing induced pluripotent stem cells (iPSCs) comprising:~~

~~a) obtaining one or more pluripotent stem cells;~~

~~(b) culturing the one or more pluripotent stem cells in a cell culture medium that does not comprise a TGFβR inhibitor thereby producing ground state iPSCs.~~

~~76. The method of claim 75, wherein the one or more iPSCs comprises a reprogrammed somatic cell.~~

~~77. The method of claim 75, wherein the one or more iPSCs comprises a reprogrammed adult stem cell.~~

78. The method of claim 75, wherein the one or more iPSCs were reprogrammed to a pluripotent state by increasing the expression of endogenous OCT4 in the one or more iPSCs.

79. The method of claim 75, wherein the one or more iPSCs were reprogrammed by introducing one or more polynucleotides encoding one or more reprogramming factors selected from the group consisting of: OCT4, SOX2, NANOG, KLF4, LIN28, C-MYC, ECAT1, UTF1, ESRRB, and SV40LT into the one or more non-pluripotent cells.

80. The method of claim 75, wherein the one or more iPSCs were reprogrammed by introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, SOX2, NANOG, KLF4, LIN28, C-MYC, ECAT1, UTF1, ESRRB, and SV40LT.

81. The method of claim 75, wherein the one or more iPSCs were reprogrammed by introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, SOX2, and NANOG into the one or more non-pluripotent cells.

82. The method of claim 75, wherein wherein the one or more iPSCs were reprogrammed by introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, NANOG, ECAT1, UTF1, and ESRRB into the one or more non-pluripotent cells.

83. The method of claim 75, wherein the one or more iPSCs were reprogrammed by introducing one or more polynucleotides encoding one or more copies of reprogramming factors selected from the group consisting of: OCT4, ECAT1, and UTF1 into the one or more non-pluripotent cells.

84. The method of any one of claims 79 to 83, wherein a lentiviral vector comprises the one or more polynucleotides.

85. The method of any one of claims 79 to 83, wherein an episomal vector comprises the one or more polynucleotides.

86. The method of claim 75, wherein the cell culture medium comprises a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3 inhibitor; a MEK inhibitor; and a ROCK inhibitor.

87. The method of any one of claims 75 to 86, wherein obtaining the one or more iPSCs comprises contacting the one or more non-pluripotent cells or partially pluripotent cells with a Wnt pathway agonist, optionally wherein the Wnt pathway agonist is a GSK3

~~inhibitor; a MEK inhibitor; and a TGFβR inhibitor, and optionally a ROCK inhibitor to produce the one or more iPSCs.~~

~~88. The method of claim 75, wherein the iPSCs comprise a population of iPSCs.~~

~~89. The method of claim 88, wherein the population of iPSCs is a homogenous population of iPSCs.~~

~~90. The method of claim 88, wherein at least 95% of the population of iPSCs expresses SSEA4 and TRA1-81 or TRA1-60.~~

~~91. The method of claim 88, wherein the one or more iPSCs are obtained by reprogramming a population of pluripotent cells, wherein at most 5% of the population of pluripotent cells expresses α-smooth muscle actin (SMA), TUJ1, or FoxA2.~~

~~92. The method of claim 75, wherein culturing the one or more iPSCs in the cell culture medium reduces spontaneous differentiation or maintains or induces a ground state of pluripotency.~~

~~93. The method of claim 92, wherein the iPSCs with reduced spontaneous differentiation comprise a gene expression wherein expression of one or more, two or more, three or more, four or more, or five or more differentiation marker genes is decreased in the iPSCs by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the expression of the one or more differentiation marker genes in iPSCs cultured in a medium comprising a TGFβR inhibitor, wherein the differentiation marker genes are selected from the group consisting of: FOXA2, FGF5, SOX17, XIST, NODAL, COL3A1, OTX2, DUSP6, EOMES, NR2F2, NR0B1, CXCR4, CYP2B6, GATA3, GATA4, ERBB4, GATA6, HOXC6, INHA, SMAD6, RORA, NIPBL, TNFSF11, CDH11, ZIC4, GAL, SOX3, PITX2, APOA2, CXCL5, CER1, FOXQ1, MLL5, DPP10, GSC, PCDH10, CTCFL, PCDH20, TSHZ1, MEGF10, MYC, DKK1, BMP2, LEFTY2, HES1, CDX2, GNAS, EGR1, COL3A1, TCF4, HEPH, KDR, TOX, FOXA1, LCK, PCDH7, CD1D FOXG1, LEFTY1, TUJ1, T gene (Brachyury) and ZIC1.~~

~~94. The method of claim 93, wherein the one or more differentiation marker genes is selected from the group consisting of T gene, CXCR4, NODAL, GATA4, SOX17, FOXA2, OTX2, and TUJ1.~~

~~95. The method of claim 93, wherein the reduced spontaneous differentiation is maintained for at least 5 passages.~~

~~96. The method of claim 93, wherein the reduced spontaneous differentiation is maintained for at least 10 passages.~~

97. The method of claim 93, wherein the reduced spontaneous differentiation is maintained for at least 50 passages.

98. The method of claim 93, wherein the reduced spontaneous differentiation is maintained for at least 100 passages.

99. The method of any one of claims 75 to 98, comprising dissociating the one or more iPSCs during passaging.

100. The method of claim 90, wherein the viability of the one or more iPSCs are maintained during passaging.

101. The method of any one of claims 75 to 100, wherein the one or more iPSCs comprise a normal karyotype.

102. The method of any one of claims 75 to 101, wherein the one or more iPSCs are cultured in a feeder-free environment.

103. The method of any one of claims 75 to 102, wherein the genomic stability of the one or more iPSCs is maintained for at least 10 passages.

104. The method of any one of claims 75 to 102, wherein the genomic stability of the one or more iPSCs is maintained for at least 50 passages.

105. The method of any one of claims 75 to 102, wherein the genomic stability of the one or more iPSCs is maintained for at least 100 passages.

106. An induced pluripotent stem cell (iPSC) comprising ground state pluripotency produced according to any one of claims 75 to 105.

107. An induced pluripotent stem cell (iPSC) comprising ground state pluripotency, wherein the iPSC does not comprise an exogenously introduced polynucleotide encoding a reprogramming factor polypeptide.

108. A method for reprogramming a non-pluripotent cell to a pluripotent cell comprising introducing into the nonpluripotent cell (i) one or more polynucleotides that encode at least one polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) at least one polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, thereby reprogramming the non-pluripotent cell to a pluripotent cell.

109. The method of claim 108, wherein introducing comprises (i) introducing one or more polynucleotides that encode an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) introducing a OCT4

~~polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.~~

~~110. The method of claim 108, wherein introducing comprises (i) introducing one or more polynucleotides that encode an OCT4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide, or (ii) introducing an OCT-4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide.~~

~~111. The method of any one of claims 108-110, wherein the one or more polynucleotides are introduced by a retrovirus, Sendai virus, an adenovirus, an episome, mini-circle, vector system with expression cassette, or mRNA.~~

~~112. The method of claim 111, wherein the retrovirus is a lentivirus.~~

~~113. The method of claim 111, wherein the pluripotent cell is free of exogenous polynucleotides.~~

~~114. The method of claim 113, wherein the one or more polynucleotides is excised by CRE-mediated excision.~~

~~115. The method of any one of claims 108-114, further comprising introducing into the non-pluripotent cell (i) a polynucleotide that encodes a SV40LT polypeptide, or (ii) a SV40LT polypeptide.~~

~~116. The method of any one of claims 108-115, further comprising contacting the nonpluripotent cell with at least one of a TGFβR inhibitor, a Wnt pathway agonist, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway agonist is optionally a GSK3 inhibitor.~~

~~117. The method of claim 116, wherein the nonpluripotent cell is contacted with a TGFβR inhibitor, a Wnt pathway agonist, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway agonist is optionally a GSK3 inhibitor.~~

~~118. The method of any one of claims 108-117, further comprising culturing the pluripotent cell in a culture medium comprising a Wnt pathway agonist, a MEK inhibitor and a ROCK inhibitor, , wherein the Wnt pathway agonist is optionally a GSK3 inhibitor, and wherein the culture medium does not contain TGFβR inhibitor.~~

~~119. The method of any one of claims 116-118, wherein (a) the Rock inhibitor is thiazovivin or Y27632, (b) the TGFβR inhibitor is A-83-01 or SB431542, (c) the GSK3 inhibitor is CHIR99021 or BIO, or (d) the MEK inhibitor is PD98059 or PD032901.~~

~~120. The method of claim 118 or 119, wherein pluripotency of the pluripotent cell is maintained for at least 5 cell divisions or at least 10 cell divisions.~~

~~121. The method of any one of claims 108-120, wherein the one or more polynucleotides is introduced as a polycistronic vector comprising a plurality of polynucleotides that are separated by at least one 2A peptide.~~

~~122. The method of claim 121, wherein the polycistronic vector comprises a plurality of polynucleotides that encode an Oct4 polypeptide.~~

~~123. The method of any one of claims 108-122, further comprising identifying the pluripotent cell by selecting for Oct34 expression in the pluripotent cell.~~

~~124. The method of claim 123, wherein selecting for Oct4 expression comprises selecting for ectopic Oct4 expression.~~

~~125. The method of any one of claims 118-124, wherein culturing produces a population of pluripotent stem cells.~~

~~126. The method of claim 125, wherein the population of pluripotent stem cells are at least 70% homogenous, at least 80% homogenous, or at least 90% homogenous.~~

~~127. The method of claim 125 or 126, wherein at least 70%, at least 80%, or at least 90% of the population of pluripotent cells expresses SSEA and Tra-181.~~

~~128. The method of any one of claims 108-127, wherein the pluripotent cell or population of pluripotent cells are capable of single cell passaging.~~

~~129. The method of claim 128, wherein the cells produced by single cell passaging have a normal karyotype.~~

~~130. A pluripotent cell produced according to the method of any one of claims 108-129.~~

~~131. A composition comprising an isolated non-pluripotent cell that comprises (i) one or more exogenous polynucleotides that encode at least one polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) at least one exogenous polypeptide selected from an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.~~

~~132. The composition of claim 131, wherein the cell comprises (i) one or more exogenous polynucleotides that encode at least two of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) at least two exogenous polypeptides selected from an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.~~

~~133. The composition of claim 131, wherein the cell comprises (i) one or more exogenous polynucleotides that encode at least three of an OCT4 polypeptide, an ECAT1~~

~~polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) at least three exogenous polypeptides selected from an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.~~

~~134. The composition of claim 131, wherein the cell comprises (i) one or more exogenous polynucleotides that encode an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) an exogenous OCT4 polypeptide, an exogenous ECAT1 polypeptide, an exogenous UTF1 polypeptide, an exogenous NANOG polypeptide, and an exogenous ESRRB polypeptide.~~

~~135. The composition of claim 131, wherein the cell comprises (i) one or more exogenous polynucleotides that encode an OCT4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide, or (ii) an exogenous OCT4 polypeptide, an exogenous ECAT1 polypeptide, and an exogenous UTF1 polypeptide.~~

~~136. The composition of any one of claims 121-135, wherein the cell has been contacted with at least one of a TGFβR inhibitor, a GSK3 inhibitor, a MEK inhibitor and a ROCK inhibitor.~~

~~137. The composition of claim 136, wherein the cell has been contacted with a TGFβR inhibitor, a Wnt pathway activator, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway activator is optionally a GSK3 inhibitor.~~

~~138. The composition of claim 136 or 137, wherein (a) the Rock inhibitor is thiazovivin or Y27632, (b) the TGFβR inhibitor is A-83-01 or SB431542, (c) the GSK3 inhibitor is CHIR99021 or BIO, or (d) the MEK inhibitor is PD98059 or PD032901.~~

~~139. The composition of any one of claims 131-138, wherein the one or more exogenous polynucleotides are introduced to the non-pluripotent cell by a retrovirus, Sendai virus, an adenovirus, an episome, mini-circle, vector system with expression cassette, or mRNA.~~

~~140. The composition of claim 139, wherein the retrovirus is a lentivirus.~~

~~141. The composition of claim 139, wherein the cell is free of exogenous polynucleotides.~~

~~142. The method of claim 141, wherein the one or more exogenous polynucleotides are removed by CRE-mediated excision.~~

~~143. The composition of any one of claims 131-142, wherein the cell further comprises (i) an exogenous polynucleotide that encodes a SV40LT antigen polypeptide, or (ii) an exogenous a SV40LT antigen polypeptide.~~

~~144. The composition of any one of claims 131-143, wherein the one or more exogenous polynucleotides is introduced as a polycistronic vector comprising a plurality of polynucleotides that are separated by at least one 2A peptide.~~

~~145. The composition of claim 144, wherein the polycistronic vector comprises a plurality of polynucleotides that encode an OCT4 polypeptide.~~

~~146. The composition of any one of claims 131-145, wherein the exogenous polynucleotide encoding an OCT4 polypeptide is linked to a selectable marker.~~

~~147. A composition consisting of at least one of, at least two of, or at least three of (i) a cDNA encoding an OCT4 polypeptide, (ii) a cDNA encoding an ECAT1 polypeptide, (iii) a cDNA encoding a UTF1 polypeptide, (iv) a cDNA encoding a NANOG polypeptide, and (v) a cDNA encoding an ESRRB polypeptide.~~

~~148. The composition of claim 147, wherein the composition consists of a cDNA encoding an OCT4 polypeptide, a cDNA encoding an ECAT1 polypeptide, a cDNA encoding a UTF1 polypeptide, a cDNA encoding a NANOG polypeptide, and a cDNA encoding an ESRRB polypeptide.~~

~~149. The composition of claim 148, wherein the composition consists of a cDNA encoding an OCT4 polypeptide, a cDNA encoding an ECAT1 polypeptide, and a cDNA encoding a UTF1 polypeptide.~~

~~150. The composition of any one of claims 147-149, wherein each cDNA is encoded in a retrovirus, Sendai virus, an adenovirus, an episome, a mini-circle, a vector system with expression cassette, or mRNA.~~

~~151. A vector comprising of one or more polynucleotides that encode at least one reprogramming factor polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.~~

~~152. The vector of claim 151, wherein the one or more polynucleotides encode an OCT polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.~~

~~153. The vector of claim 152, wherein the one or more polynucleotides encode an OCT polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide.~~

~~154. The vector of any one of claims 151-153, wherein the vector further comprises a polynucleotide that encodes an SV40LT antigen polypeptide.~~

155. The vector of any one of claims 151-154, wherein the vector is a retrovirus, Sendai virus, an adenovirus, an episome, mini-circle, vector system with expression cassette, or mRNA.

156. The vector of claim 155, wherein the retrovirus is a lentivirus.

157. A kit for reprogramming a non-pluripotent cell to a pluripotent cell, the kit comprising:

(a)one or more polynucleotides that encode at least one polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, or an ESRRB polypeptide; or

(b) at least one polypeptide selected from the group consisting of an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, or an ESRRB polypeptide; and

(c)at least one of a TGFβR inhibitor, a Wnt pathway activator, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway activator is optionally a GSK3 inhibitor.

158. The kit of claim 157, wherein (i) the one or more polynucleotides encode an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide, or (ii) the at least one polypeptide comprises an OCT4 polypeptide, an ECAT1 polypeptide, a UTF1 polypeptide, a NANOG polypeptide, and an ESRRB polypeptide.

159. The kit of claim 157, wherein (i) the one or more one or more polynucleotides encode an OCT4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide, or (ii) the at least one polypeptide comprises an OCT4 polypeptide, an ECAT1 polypeptide, and a UTF1 polypeptide.

160. The kit of any one of claims 157-159, wherein the kit further comprises (i) a polynucleotide that encodes a SV40LT antigen polypeptide, or (ii) a SV40LT antigen polypeptide.

161. The kit of any one of claims 157-160, wherein the kit comprises a TGFβR inhibitor, a Wnt pathway activator, a MEK inhibitor and a ROCK inhibitor, wherein the Wnt pathway activator is optionally a GSK3 inhibitor.

162. The kit of any one of claims 157-161, wherein (a) the Rock inhibitor is thiazovivin or Y27632, (b) the TGFβR inhibitor is A-83-01 or SB431542, (c) the GSK3 inhibitor is CHIR99021 or BIO, or (d) the MEK inhibitor is PD98059 or PD032901.

~~163. The kit of any one of claims 157-162, wherein the at least one polynucleotide is encoded in a retrovirus, Sendai virus, an adenovirus, an episome, a mini-circle, a vector system with expression cassette, or mRNA.~~

~~164. The kit of claim 163, wherein the retrovirus is a lentivirus.~~

~~165. The kit of any one of claims 157-164, wherein the at least one polynucleotide is encoded in a polycistronic vector with each polynucleotide being separated by a 2A peptide.~~

~~166. The kit of claim 165, wherein the polycistronic vector comprises two or more polynucleotides that encode an OCT4 polypeptide.~~

~~167. The kit of any one of claims 157-166, wherein the at least one polynucleotide that encodes an OCT4 polypeptide is linked to a selectable marker.~~

~~168. A method for producing a population of pluripotent stem cells comprising:~~

~~a) providing a population of non-pluripotent cells;~~

~~b) introducing into the population of non-pluripotent cells a polynucleotide that encodes an OCT4 polypeptide that is linked to a selectable marker;~~

~~c) incubating the population of non-pluripotent cells with the polynucleotide under conditions sufficient to reprogram at least a portion of the population of non-pluripotent cells to pluripotent cells; and~~

~~d) selecting cells that express the selectable marker thereby providing a population of pluripotent stem cells.~~

~~169. The method of claim 168, wherein the polynucleotide is introduced as a polycistronic vector comprising a plurality of polynucleotides that encode an OCT4 polypeptide.~~

~~170. The method of claim 169, wherein the plurality of polynucleotides are separated by a 2A peptide.~~

~~171. The method of claim 169, wherein at least 10 % of the cells in the population of cells express SSEA and TRA-181.~~