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 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 examples, the 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 examples, the Wnt pathway agonist is a GSK3 inhibitor.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

In various examples, the present 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 examples, the present 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 examples, the present 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 examples, the present 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 examples, the one or more non-pluripotent cells comprise a somatic cell.

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

In certain 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 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 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 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 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 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 examples, the one or more polynucleotides are a lentiviral vector.

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

In related particular 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 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 examples, the iPSCs comprise a population of iPSCs.

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

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

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

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

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

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

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

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

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

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

In certain examples, the iPSCs comprise a normal karyotype.

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

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

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

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

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

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

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

In other 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 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 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 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 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 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 examples, a lentiviral vector comprises the one or more polynucleotides.

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

In other 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 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 examples, the iPSCs comprise a population of iPSCs.

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

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

In particular 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 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 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 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 examples, the reduced spontaneous differentiation is maintained for at least 5 passages.

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

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

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

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

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

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

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

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

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

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

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

In various examples, the 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 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 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 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 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 example, the retrovirus is a lentivirus.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

In one example, the retrovirus is a lentivirus.

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

In another 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 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 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 example, the retrovirus is a lentivirus.

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

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

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

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

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

In yet another 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.