EPISOMAL REPROGRAMMING OF ISOLATED CELL FRACTION FROM CORD BLOOD UNITS AND GENERATION OF INDUCED PLURIPOTENT STEM CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application includes a claim of priority under 35 U.S.C. §119(e) to U.S. provisional patent application No. 63/551,637, filed February 9, 2024, the entirety of which is hereby incorporated by reference.

FIELD OF INVENTION

[0002] This invention relates to episomal reprogramming of isolated cell fraction from cord blood units and generation of induced pluripotent stem cells.

BACKGROUND

[0003] All publications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0004] Cord blood provides a source for cells that can be utilized for reprogramming into induced pluripotent stem cells (iPSCs) among other things. However, development of successful and reliable processes for episomal reprogramming of isolated cell fraction from cord blood units and generation of induced pluripotent stem cells, and at the same time conform to current good manufacturing practices (cGMP) are difficult. Thus, there remains a need in the art for these processes.

BRIEF DESCRIPTION OF THE FIGURES

[0005] Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

[0006] Figure 1 depicts an exemplary schematic showing the location of wells to be coated in partially coated plates.

[0007] Figure 2 depicts exemplary schematics for clone isolation.

[0008] Figures 3A-3C depict representative pictures of clones to be picked.

[0009] Figures 4A-4B show UMAP clustering of single cell RNA-sequencing data separates distinct sample sets into defined clusters.

[0010] Figure 5 shows distinct subclusters and separation across all samples.

[0011] Figure 6 shows dot plot of specific marker genes across a matrix of clusters as defined in Figure 5 and categorizing them as specific cell type.

[0012] Figures 7-15 show unique CD genes, or cluster of differentiation genes, encode proteins that are cell surface markers expressed in iPSCs. These marker genes trace origin from cord blood isolated cell fraction and demonstrate a novel composition of iPSCs generated by our process.

[0013] Figures 16-25 show novel genes expressed uniquely in cord blood derived iPSC sample set.

[0014] Figures 26-30 show that known pluripotency genes are also expressed in the cord blood derived iPSC sample set confirming that these are *bona fide* pluripotent stem cells

[0015] Figure 31 shows Reprogrammed Mix (Partially reprogrammed) Violin Plot and iPSCs Violin Plot.

SUMMARY OF THE INVENTION

[0016] The following embodiments and aspects thereof are described and illustrated in conjunction with compositions and methods which are meant to be exemplary and illustrative, not limiting in scope.

[0017] Various embodiments of the invention provide for a method for reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs), comprising: (a) providing the ICF; (b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising: one or more plasmids encoding MYCL, LIN28, POU5F1, p53 shRNA, SOX2, LTAg, and KLF4, and optionally, EBNA1; (c) adding the cell suspension having the ICF to (i) each well of a multi-well plate, wherein the multi-well plate was previously coated with CT521 laminin, or (ii) a plate, wherein the plate was previously coated with CT521 laminin, or (iii) a flask, wherein the flask was previously coated with CT521 laminin; (d) incubate the multi-well plate, the plate, or the flask at about 32°C-42°C and about 3-7% CO_2; and (e) feeing the cells beginning on day 2-4.

[0018] In various embodiments, reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs), can comprise: (a) providing the ICF; (b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising: a first plasmid encoding MYCL-LIN28A, EBNA1, a second plasmid encoding POU5F1, p53 shRNA, EBNA1, a third plasmid encoding EBNA1, a fourth plasmid encoding SOX2-KLF4, EBNA1, and a fifth plasmid encoding POU5F1-SOX2-LTAg-KLF4, EBNA1; (c) adding the cell suspension to each well of a multi-well plate, wherein the multi-well plate was previously coated with CT521 laminin; (d) incubate the multi-well plate at about 32°C-42°C and about 3-7% CO_2; (e) feeing the cells beginning on day 2-4.

[0019] In various embodiments, reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs), can comprise: (a) providing the ICF; (b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising: a first plasmid encoding MYCL-LIN28A, EBNA1, a second plasmid encoding POU5F1, p53 shRNA, EBNA1, a third plasmid encoding EBNA1, a fourth plasmid

encoding SOX2-KLF4, EBNA1, and a fifth plasmid encoding POU5F1-SOX2-LTAg-KLF4, EBNA1; (c) adding the cell suspension to each well of a 12 well plate, wherein the 12 well plate was previously coated with CT521 laminin; (d) incubate the 12 well plate at about 37°C and about 5% CO_2; (e) feeing the cells beginning on day 3.

[0020] In various embodiments, the CT521 laminin coated multi-well plate, plate, or flask can be made by applying CT521 laminin to the plate, and allowing the plates to coat for at least 8 hours at 2°C-8°C. In various embodiments, CT521 laminin coated multi-well plate, plate, or flask can be prepared at least 24 hours before adding the cell suspension and up to 30 days before adding the cell suspension.

[0021] Various embodiments provide for a method of feeding isolated cell fraction undergoing episomal reprogramming, comprising: (i) providing isolated cell fraction (ICF) undergoing episomal reprogramming ("cells"), or performing the reprogramming methods of the present invention; (ii) feeding the cells day 2-4 after nucleofection, by adding mTeSR Plus media or GMP-grade reprogramming media to each well having the ICF undergoing episomal reprogramming; (iii) feeding the cells on day 3 - day 10 after nucleofection, by removing spent media from each well and adding mTeSR Plus media or GMP-grade reprogramming media to each well; (iv) feeding the cells on day 9 - day 18 after nucleofection, by removing spent media from each well and adding mTeSR Plus media or GMP-grade reprogramming media to each well; (v) feeding the cells on day 14 – day 40 after nucleofection, by removing spent media from each well and adding mTeSR Plus media or GMP-grade reprogramming media to each well , wherein in step (v) the cells are cultured for 14-40 days.

[0022] In various embodiments, feeding isolated cell fraction undergoing episomal reprogramming, can comprise: (i) providing isolated cell fraction (ICF) undergoing episomal reprogramming ("cells"), or performing the reprogramming methods of the present invention; (ii) feeding the cells day 2-4 after nucleofection, by adding 0.25-0.75 mL of mTeSR Plus media or GMP-grade reprogramming media to each well having the ICF undergoing episomal reprogramming; (iii) feeding the cells on day 3 - day 10 after nucleofection, by removing spent media from each well and adding 0.5-1.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well; (iv) feeding the cells on day 9 - day 18 after nucleofection, by removing spent media from each well and adding 0.5-1.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well or adding 1.5-2.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well; (v) feeding the cells on day 14 – day 40 after nucleofection, by removing spent media from each well and adding 0.5-1.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well or adding 1.5-2.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well, wherein in step (v) the cells are cultured for 14-40 days.

[0023] In various embodiments, feeding isolated cell fraction undergoing episomal reprogramming can comprise: (i) providing isolated cell fraction (ICF) undergoing episomal reprogramming ("cells"), or performing the reprogramming methods of the present invention; (ii) feeding

the cells day 3 after nucleofection, by adding 0.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well having the ICF undergoing episomal reprogramming; (iii) feeding the cells on day 4-day 9 after nucleofection, by removing spent media from each well and adding 1 mL of mTeSR Plus media or GMP-grade reprogramming media to each well; (iv) feeding the cells on day 11- day 16 after nucleofection, by removing spent media from each well and adding 1 mL of mTeSR Plus mediaor GMP-grade reprogramming media to each well or adding 2 mL of mTeSR Plus media or GMP-grade reprogramming media to each well; (v) feeding the cells on day 17-day 40 after nucleofection, by removing spent media from each well and adding 1 mL of mTeSR Plus media to each well or adding 2 mL of mTeSR Plus media to each well, wherein in step (v) the cells are cultured for 17-40 days.

[0024] In various embodiments, in step (iv) or step (v), when adding 1.5-2.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well or when adding 2 mL of mTesR Plus media or GMP-grade reprogramming media to each well, feeding the cells can skip one or two days until the next feeding.

[0025] In various embodiments, in step (ii), adding the mTesR Plus media or GMP-grade reprogramming media can comprise adding the mTesR Plus media or GMP-grade reprogramming media at a rate of 1-4 minutes per 6 wells of a 12 well plate; in step (iii), removing spent media and adding the mTesR Plus media or GMP-grade reprogramming media can be performed at a rate of up to 9 minutes per 6 wells of a 12 well plate; in step (iv), removing spent media and adding the mTesR Plus media or GMP-grade reprogramming media can be performed at a rate of up to 7 minutes per 6 wells of a 12 well plate; in step (v), adding the mTesR Plus media can comprise adding the mTesR Plus media at a rate of 1-4 minutes per 6 wells of a 12 well plate.

[0026] In various embodiments, in step (ii), adding the mTesR Plus media or GMP-grade reprogramming media can comprise adding the mTesR Plus media or GMP-grade reprogramming media at a rate of 2-3 minutes per 6 wells of a 12 well plate; in step (iii), removing spent media and adding the mTesR Plus media or GMP-grade reprogramming media can be performed at a rate of up to 7 minutes per 6 wells of a 12 well plate; in step (iv), removing spent media and adding the mTesR Plus media or GMP-grade reprogramming media can be performed at a rate of up to 5 minutes per 6 wells of a 12 well plate; in step (v), removing spent media and adding the mTesR Plus media can be performed at a rate of 2-3 minutes per 6 wells of a 12 well plate.

[0027] Various embodiments provide for a method of isolating iPSC clones from a reprogramming plate, comprising: (i) feeding the iPSC clones from the reprogramming plate; and (ii) manually isolating the iPSC clones from the reprogramming plate.

[0028] Various embodiments provide for a method of isolating iPSC clones from a reprogramming plate, comprising: (i) feeding the iPSC clones from the reprogramming plate; (ii) enzymatically isolating the iPSC clones.

[0029] In various embodiments, feeding the iPSC clones from the reprogramming plate can comprise: changing media in all wells of a reprogramming plate having one or more colonies, or low-density seeded plate having one or more colonies by removing spent median and replacing with mTeSR Plus per well; selecting a colony that is not physically in contact with another colony, preferably the selected colony is compact and have morphology similar to iPSC colonies.

[0030] In various embodiments, replacing with mTeSR Plus per well can comprise replacing with 1 mL of mTeSR Plus per well.

[0031] In various embodiments, manually isolating the iPSC clones from the reprogramming plate can comprise: cutting and transferring the selected colony to a new Laminin CT521 coated plate, wherein the laminin has been removed from the coated plate and replaced with mTeSR Plus media; transferring the plate to 35-39°C CO_2 incubator until the next feeding; rocking the new plate back and forth and side to side to distribute the clumps.

[0032] In various embodiments, manually isolating the iPSC clones from the reprogramming plate can comprise: cutting and transferring the selected colony to a new Laminin CT521 coated plate, wherein the laminin has been removed from the coated plate and replaced with 1 mL of mTeSR Plus media per well; transferring the plate to 37°C CO_2 incubator until the next feeding; rocking the new plate back and forth and side to side to distribute the clumps.

[0033] In various embodiments, cutting and transferring the selected colony can comprise: cutting the selected colony into small pieces; nudging clump pieces off the bottom of the well; transferring the floating pieces to the well containing mTeSR Plus media in the new laminin coated plate.

[0034] In various embodiments, cutting and transferring the selected colony can comprise: using an insulin syringe needed to cut the selected colony into small pieces; using a P1000 tip inside a P20 tip to nudge clump pieces off the bottom of the well; using a P200 micropipette to transfer the floating pieces to the well containing mTeSR Plus media in the new laminin coated plate.

[0035] In various embodiments, enzymatically isolating the iPSC clones can comprise: (a) removing spent media from each well from the reprogramming plate; (b) adding DPBS-/- to each well; (c) aspirating the DPBS-/-; repeating step (c); (d) adding TrypLE CTS and transferring the reprogramming plate to the incubator and incubate at 37°C for about 5-10 minutes; (e) gently pipette up and down to break up the clumps; (f) adding mTeSR plus to the well to neutralize the TrypLE CTS; (g) transferring cell suspensions to a tube; repeating steps (d)-(g) for each well and pool into the same tube; (h) centrifuge the tube at 200xG-400xG for about 5 minutes at room temperature; (i) removing supernatant from the tube without disturbing the cells pellet; (j) dislodging the pellet by flicking the tub; (k) resuspending the pellet in mTeSR Plus with Rock inhibitor; (l) adding mTeSR Plus with Rock inhibitor; (m) transferring well mixed cell suspension to a tube.

[0036] In various embodiments, the method can further comprise performing a cell count.

[0037] In various embodiments, the method can further comprise comprising transferring a volume of cells comprising about 10,000-30,000 cells to each well of a multi-well plate and rocking the 6 well plate in an incubator at about 37°C and 5% CO_2 to distribute the cells evenly.

[0038] In various embodiments, the method can further comprise (n) adding MoxiCyte viability reagent to cell suspension, mix and incubate in the dark for about 2-7 minutes; (o) mixing the cell suspension in the tube; (p) transferring a volume of cells comprising about 10,000-30,000 cells to each well of a multi-well plate; (q) rocking the 6 well plate in an incubator at about 37°C and 5% CO_2 to distribute the cells evenly.

[0039] In various embodiments, enzymatically isolating the iPSC clones can comprise: (a) removing spent media from each well from the reprogramming plate; (b) adding about 1 mL of DPBS-/- to each well; (c) aspirating the DPBS-/-; repeating step (c); (d) adding about 1 mL of TrypLE CTS and transferring the reprogramming plate to the incubator and incubate at about 37°C for about 7 minutes; (e) gently pipette up and down using a P1000 micropipette to break up the clumps; (f) adding about 1 mL of mTeSR plus to the well to neutralize the TrypLE CTS; (g) transferring cell suspensions to a 15 mL conical tube; repeating steps (d)-(g) for each well and pool into the same 15 mL conical tub; (h) centrifuge the conical tube at about 300x g for about about 5 minutes at room temperature; (i) removing supernatant from the conical tube without disturbing the cells pellet; (j) dislodging the pellet by flicking the tub; (k) resuspending the pellet in about 1 mL of mTeSR Plus with Rock inhibitor; (l) adding mTeSR Plus with Rock inhibitor to bring the volume to about 4 mL per well dissociated; and (m) transferring about 20 uL of well mixed cell suspension to a 1.5 mL microcentrifuge tube.

[0040] In various embodiments, the method can further comprise performing a cell count.

[0041] In various embodiments, the method can further comprise transferring a volume of cells comprising about 10,000-30,000 cells to each well of a multi-well plate and rocking the 6 well plate in an incubator at about 37°C and 5% CO_2 to distribute the cells evenly.

[0042] In various embodiments, the method can further comprise (n) adding 180 uL of MoxiCyte viability reagent to cell suspension, mix and incubate in the dark for about 5 minutes; (o) mixing the cell suspension in the 15 mL conical tube; (p) transferring a volume of cells comprising about 20,000 cells to each well of a 6 well plate; (q) rocking the 6 well plate in an incubator at about 37°C and about 5% CO_2 to distribute the cells evenly.

[0043] In various embodiments, the method can further comprise passaging the cells.

[0044] In various embodiments, after 3 passages, iPSC can appear.

[0045] In various embodiments, iPSCs can appear in less than 10 passages.

[0046] In various embodiments, the volume of cells can be calculated by using the following formula: $cell\ suspension\ volume\ (ml) = \frac{20{,}000\ cells}{average\ viable\ cell\ concentration}$

[0047] In various embodiments, mTeSR Plus with 10 μM ROCK can comprise:

$$volume\ of\ 10\ mM\ ROCK\ Inhibitor\ stock\ (uL) = \frac{10\ uM\ x\ volume\ of\ mTeSR\ Plus\ (mL)}{10\ mM}.$$

[0048] Various embodiments provide for induced pluripotent stem cells (iPSCs) reprogrammed from isolated cell fraction (ICF) from cord blood units (CBUs), wherein the iPSCs express one or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326, or wherein the iPSCs express one or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL, or wherein the iPSCs express one or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin).

[0049] In various embodiments, the iPSCs are generated by any one of the methods of the present invention.

[0050] Various embodiments provide for a composition comprising iPSCs of the present invention; and cell media.

[0051] Other features and advantages of the invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, various features of embodiments of the invention.

DESCRIPTION OF THE INVENTION

[0052] All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0053] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

[0054] As used herein the term "about" when used in connection with a referenced numeric indication means the referenced numeric indication plus or minus up to 5% of that referenced numeric indication, unless otherwise specifically provided for herein. In various embodiments, the term "about" when used in connection with a referenced numeric indication can mean the referenced numeric indication plus or minus up to 4%, 3%, 2%, 1%, 0.5%, or 0.25% of that referenced numeric indication, *if* specifically provided for in the claims.

[0055] "Room temperature" as used herein refers to a temperature between 15°C to 25°C. In various embodiments, "room temperature" refers to a temperature between 18°C to 22°C, or between 19°C to 21°C, or about 20°C, *if* specifically provided for in the claims.

[0056] StemSpan AOF referenced herein is an animal origin free media. It is a cGMP medium, for culture and expansion of human hematopoietic cells. It contains only recombinant proteins and synthetic components, and does not contain serum or other human- or animal-derived components.

[0057] StemSpan CD34+ expansion supplement is a serum-free culture supplement for expansion of human CD34+ hematopoietic cells. It contains a combination of recombinant human cytokines and other additives formulated to selectively promote the expansion of CD34+ cells isolated from human cord blood (CB) or bone marrow (BM) samples.

[0058] Table below provides the definitions of various acronyms used in the present application.

Acronym/Term	Definition
Addition	Only adding cell culture medium to the well, not removing any medium volume from the well.
Areas of Interest	Areas on the plate where cells are attached initially as single cells that proliferate over time to form loose groups of cells and eventually form tightly compact iPSC colonies.
BPR	Batch Production Record
BSC	Biosafety Cabinet
CBC	Cedars-Sinai Biomanufacturing Center
CBU	Cord Blood Unit
CSS	Complete StemSpan Media. StemSpan-AOF medium that has the CD34+ expansion supplement and rock inhibitor added to it.
Clone Isolation	Clone isolation is when a perceived iPSC colony is physically cut (manually) out of the reprogramming plate and moved to a new plate for expansion or enzymatically harvested and replated into a new plate at a low cell dilution and grown until an iPSC clone emerges.
cGMP(s)	current Good Manufacturing Practice(s)
GMP RM	GMP Reprogramming Medium
ICF	Isolated Cell Fraction. Population of cells isolated from Umbilical Cord Blood Units
EM	Environmental Monitoring
Feeding	The addition or full replacement of cell culture medium.
Full Feed	The total replacement of cell culture medium.
MFG	Manufacturing
MPR	Master Production Record

Nucleofection PM	Nucleofection Plasmid Mix
mTeSR Plus	Complete mTeSR Plus Medium
NS	Nucleofection Solution
NS+S	Nucleofection Solution + Supplement
NS+S+Cells	Nucleofection Solution + Supplement + Cell Suspension
PD	Process Development
Partial Feed	Replacement of approximately 75% of cell culture medium.
PBS +/+	DPBS with Ca++ and Mg++
PBS -/-	DPBS without Ca++ and Mg++
QA	Quality Assurance
QC	Quality Control
ROCKi	Y-27632 (ROCK Inhibitor).
SME	Subject Matter Expert
UCB	Umbilical Cord Blood

[0059] Described herein are procedures for reprogramming the isolated cell fraction (ICF) from Cord Blood Units (CBUs) under current Good Manufacturing Practices (cGMPs). Also described herein is the procedure for isolating induced pluripotent stem cell (iPSC) clones after reprogramming of human somatic primary donor cells under current Good Manufacturing Practice (cGMP).

[0060] Various embodiments provide for a method for reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs), comprising:

(a) providing the ICF;

(b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising: one or more plasmids encoding MYCL, LIN28, POU5F1, p53 shRNA, SOX2, LTAg, and KLF4, and optionally EBNA1;

(c) adding the cell suspension to (i) each well of a multi-well plate, wherein the multi-well plate was previously coated with CT521 laminin, or (ii) a plate, wherein the plate was previously coated with CT521 laminin, or (iii) a flask, wherein the flask was previously coated with CT521 laminin;

(d) incubate the multi-well plate, the plate, or the flask at about 32°C-42°C and about 3-7% CO_2; and

(e) feeing the cells beginning on day 2-4.

[0061] In various embodiments, the method for reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs), comprises:

(a) providing the ICF;

(b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising: one or more plasmids encoding MYCL, LIN28, POU5F1, p53 shRNA, SOX2, LTAg, and KLF4, and optionally EBNA1;

(c) adding the cell suspension to (i) each well of a multi-well plate, wherein the multi-well plate was previously coated with CT521 laminin, or (ii) a plate, wherein the plate was previously coated with CT521 laminin, or (iii) a flask, wherein the flask was previously coated with CT521 laminin;

(d) incubate the multi-well plate, the plate, or the flask at about 37°C and about 5% CO_2; and

(e) feeing the cells beginning on day 3.

[0062] In various embodiments, the method for reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs), comprises:

(a) providing the ICF;

(b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising:

a first plasmid encoding MYCL-LIN28A, EBNA1,

a second plasmid encoding POU5F1, p53 shRNA, EBNA1,

a third plasmid encoding EBNA1,

a fourth plasmid encoding SOX2-KLF4, EBNA1, and

a fifth plasmid encoding POU5F1-SOX2-LTAg-KLF4, EBNA1;

(c) adding the cell suspension to (i) each well of a multi-well plate, wherein the multi-well plate was previously coated with CT521 laminin, or (ii) a plate, wherein the plate was previously coated with CT521 laminin, or (iii) a flask, wherein the flask was previously coated with CT521 laminin;

(d) incubate the multi-well plate, the plate, or the flask at about 32°C-42°C and about 3-7% CO_2; and

(e) feeing the cells beginning on day 2-4.

[0063] While the plasmids herein are referred to as "first", "second", "third", "fourth" and "fifth", these reference are only for convenience and to provide antecedent basis if needed, and do not imply that these plasmids are in such order.

[0064] Various embodiments provide for a method for reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs), comprising:

(a) providing the ICF;

(b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising:

a first plasmid encoding MYCL-LIN28A, EBNA1,

a second plasmid encoding POU5F1, p53 shRNA, EBNA1,

a third plasmid encoding EBNA1,

a fourth plasmid encoding SOX2-KLF4, EBNA1, and

a fifth plasmid encoding POU5F1-SOX2-LTAg-KLF4, EBNA1;

(c) adding the cell suspension to (i) each well of a multi-well plate, wherein the multi-well plate was previously coated with CT521 laminin, or (ii) a plate, wherein the plate was previously coated with CT521 laminin, or (iii) a flask, wherein the flask was previously coated with CT521 laminin;

(d) incubate the multi-well plate, the plate, or the flask at about 33°C-41°C and about 4-6% CO_2; and

(e) feeing the cells beginning on day 2.5-3.5.

[0065] In various embodiments, the method comprises

(a) providing the ICF;

(b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising:

a first plasmid encoding MYCL-LIN28A, EBNA1,

a second plasmid encoding POU5F1, p53 shRNA, EBNA1,

a third plasmid encoding EBNA1,

a fourth plasmid encoding SOX2-KLF4, EBNA1, and

a fifth plasmid encoding POU5F1-SOX2-LTAg-KLF4, EBNA1;

(c) adding the cell suspension to (i) each well of a multi-well plate, wherein the multi-well plate was previously coated with CT521 laminin, or (ii) a plate, wherein the plate was previously coated with CT521 laminin, or (iii) a flask, wherein the flask was previously coated with CT521 laminin;

(d) incubate the multi-well plate, the plate, or the flask at about 35°C-39°C and about 5% CO_2; and

(e) feeing the cells beginning on day 3.

[0066] In various embodiments, the method comprises

(a) providing the ICF;

(b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising:

a first plasmid encoding MYCL-LIN28A, EBNA1,

a second plasmid encoding POU5F1, p53 shRNA, EBNA1,

a third plasmid encoding EBNA1,

a fourth plasmid encoding SOX2-KLF4, EBNA1, and

a fifth plasmid encoding POU5F1-SOX2-LTAg-KLF4, EBNA1;

(c) adding the cell suspension to each well of a 12 well plate, wherein the 12 well plate was previously coated with CT521 laminin;

(d) incubate the 12 well plate at about 35°C-39°C and about 5% CO_2; and

(e) feeing the cells beginning on day 3.

[0067] In various embodiments, the method comprises

(a) providing the ICF;

(b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising:

a first plasmid encoding MYCL-LIN28A, EBNA1,

a second plasmid encoding POU5F1, p53 shRNA, EBNA1,

a third plasmid encoding EBNA1,

a fourth plasmid encoding SOX2-KLF4, EBNA1, and

a fifth plasmid encoding POU5F1-SOX2-LTAg-KLF4, EBNA1;

(c) adding the cell suspension to each well of a 12 well plate, wherein the 12 well plate was previously coated with CT521 laminin;

(d) incubate the 12 well plate at about 37°C and about 5% CO_2; and

(e) feeing the cells beginning on day 3.

[0068] In various embodiments, the CT521 laminin coated multi-well plates, plates or flasks are made by applying CT521 laminin to the multi-well plates, plates or flasks, and allowing the multi-well plates, plates or flasks to coat for at least 8 hours at 2°C-8°C. In various embodiments, the multi-well plates, plates or flasks are allowed to coat at about 4°C.

[0069] In various embodiments, the CT521 laminin coated multi-well plates, plates or flasks are prepared at least 24 hours before adding the cell suspension and up to 30 days before adding the cell suspension.

[0070] In various embodiments, each plasmid is in a concentration as shown in the following table:

Plasmid Name	Factors	Final Concentration Needed	Formula to calculate volume needed
pCBC1	MYCL-LIN28A, EBNA1	0.2-2.0 µg	$\frac{0.2 \text{ to } 2.0\ \mu g}{pCBC1\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC2	POU5F1, p53 shRNA, EBNA1	0.2-2.0µg	$\frac{0.2 \text{ to } 2.0\ \mu g}{pCBC2\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC3	EBNA1	0.2-1.0 µg	$\frac{0.2 \text{ to } 1.0\ \mu g}{pCBC3\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC4	SOX2-KLF4, EBNA1	0.2-2.0µg	$\frac{0.2 \text{ to } 2.0\ \mu g}{pCBC4\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC5	POU5F1-SOX2-LTAg-KLF4, EBNA1	0.2-2.0 µg	$\frac{0.2 \text{ to } 2.0\ \mu g}{pCBC5\ concentration\ \frac{\mu g}{\mu L}} x\ 5$

[0071] In various embodiments, each plasmid is in a concentration as shown in the following table:

Plasmid Name	Factors	Final Concentration Needed	Formula to calculate volume needed
pCBC1	MYCL-LIN28A, EBNA1	0.4-1.8 µg	$\frac{0.4 \text{ to } 1.8\ \mu g}{pCBC1\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC2	POU5F1, p53 shRNA, EBNA1	0.4-1.8 µg	$\frac{0.4 \text{ to } 1.8\ \mu g}{pCBC2\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC3	EBNA1	0.4-0.8 µg	$\frac{0.4 \text{ to } 0.8\ \mu g}{pCBC3\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC4	SOX2-KLF4, EBNA1	0.4-1.8µg	$\frac{0.4 \text{ to } 1.8\ \mu g}{pCBC4\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC5	POU5F1-SOX2-LTAg-KLF4, EBNA1	0.4-1.8 µg	$\frac{0.4 \text{ to } 1.8\ \mu g}{pCBC5\ concentration\ \frac{\mu g}{\mu L}} x\ 5$

[0072] In various embodiments, each plasmid is in a concentration as shown in the following table:

Plasmid Name	Factors	Final Concentration Needed	Formula to calculate volume needed
pCBC1	MYCL-LIN28A, EBNA1	0.83 µg	$\frac{0.83\mu g}{pCBC1\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC2	POU5F1, p53 shRNA, EBNA1	0.83 µg	$\frac{0.83\mu g}{pCBC2\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC3	EBNA1	0.5 µg	$\frac{0.5\mu g}{pCBC3\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC4	SOX2-KLF4, EBNA1	0.83 µg	$\frac{0.83\mu g}{pCBC4\ concentration\ \frac{\mu g}{\mu L}} x\ 5$
pCBC5	POU5F1-SOX2-LTAg-KLF4, EBNA1	0.83 µg	$\frac{0.83\mu g}{pCBC5\ concentration\ \frac{\mu g}{\mu L}} x\ 5$

[0073] In various embodiments, the reprogramming vectors do not remain in the cell lines after reprogramming. That is, after the reprogramming process, or after 3-10 passages once the cells are reprogrammed, the reprogramming vectors are no longer present in the cells.

[0074] CT521 laminin is a cell therapy grade laminin and it is well known in the field that it is challenging to successfully use it in reprogramming methods. CT521 has never been used for reprogramming cord blood derived iPSCs from previous literature. Described herein, the inventors have determined the process for successfully and consistently cultivating iPSCs using CT521 laminin.

[0075] The innovative method for reprogramming cord blood-derived iPSCs using cell therapy grade laminin 521 (CT521) per cGMPs has several unexpected aspects and benefits including but are not limited to the following.

[0076] Enhanced efficiency: CT521 supports significantly increased proliferation compared to fragmented laminins, yielding 5-20 times more pluripotent stem cells from cord blood cells as a starting population compared to PBMCs and other starting cell types. CT521 is critical to achieving superior results during reprogramming iPSCs like higher efficiency, faster colony formation, better cell viability. This leads to a more efficient reprogramming process for cord blood-derived iPSCs.

[0077] Our data shows that cord blood–derived iPSCs reprogram more quickly and yield higher-quality colonies this CT521-based method compared to standard fibroblast or peripheral blood methods. Given that cord blood cells are considered superior for reprogramming due to their young age and high proliferation rate, our method specifically optimized for cord blood-derived cells using CT521 yields higher efficiency and quality iPSCs compared to other cell sources.

[0078] Improved cell signaling: Unlike fragmented laminin products, full-length laminin CT521 can bind with all relevant cell surface receptors, including integrins, dystroglycans, and syndecans. This results in more authentic cell signaling during the reprogramming process, potentially improving the quality and stability of the resulting iPSCs.

[0079] Clinically relevant conditions: CT521 is designed for clinical research and complies with USP Chapter 1043, making it suitable for potential therapeutic applications. This allows for a seamless transition from research to clinical development.

[0080] Animal component-free culture: CT521 is animal component-free to the secondary level, reducing the risk of xenogenic contamination and improving the safety profile of the reprogramming process.

[0081] Biologically relevant microenvironment: CT521 recreates a more authentic culture environment, mimicking the natural stem cell niche leading to more stable and functionally superior iPSCs.

[0082] Compatibility with single-cell approaches: CT521 supports reliable single-cell expansion of human pluripotent stem cells, which is advantageous for developing more controlled and scalable reprogramming protocols.

[0083] Enhanced maturation and organization: CT521 has been shown to support efficient differentiation, maturation of differentiated cell types. This can be beneficial for downstream applications of the reprogrammed iPSCs.

[0084] Synergy with episomal reprogramming to create a fully integration-free and a cGMP clinically relevant reprogramming system.

[0085] In various embodiments, after 3 passages, iPSCs appear. In various embodiments, iPSCs appear in less than 6 passages. In various embodiments, iPSCs appear in less than 10 passages.

[0086] These cord blood–derived cells differ from adult somatic cells (e.g., fibroblasts) and peripheral blood mononuclear cells. About 10-50 times fewer starting cells are needed to reprogram into iPSCs with a lower starting cell count and faster proliferation kinetics when iPSCs are generated.

[0087] In various embodiments, the iPSCs express one or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express five or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express 10 or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express 15 or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express all markers selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, and CD326.

[0088] In various embodiments, the iPSCs express one or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 5 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 10 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 15 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17,

APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express all markers selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, and COBL.

[0089] In various embodiments, the iPSCs express one or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express 5 or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express 8 or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express all markers selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, and PODXL (CD34 sialomucin).

[0090] Various embodiments of the invention provide for a method of feeding isolated cell fraction undergoing episomal reprogramming, comprising:

(i) providing isolated cell fraction (ICF) undergoing episomal reprogramming ("cells"), OR reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs) by the methods of the present invention described herein;

(ii) feeding the cells on day 2 to day 4 after nucleofection, by adding mTeSR Plus media or GMP-grade reprogramming media to each well having the ICF undergoing episomal reprogramming;

(iii) feeding the cells on day 3 to day 10 after nucleofection, by removing spent media from each well and adding mTeSR Plus media or GMP-grade reprogramming media to each well;

(iv) feeding the cells on day 9 to day 18 after nucleofection, by removing spent media from each well and adding mTeSR Plus media or GMP-grade reprogramming media to each well;

(v) feeding the cells on day 14 to day 40 after nucleofection, by removing spent media from each well and mTeSR Plus media to each well, wherein in step (v), the cells are cultured for 14-40 days. As such, in step (v) the cells can be cultured for 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 29 or 40 days. In various embodiments in step (v), the cells are cultured for 14-16 days, 17-22 days, 23-27 days, 28-30 days, 29-37 days or 38-40 days.

[0091] In various embodiments, wherein in step (iv) or step (v), when adding mTesR Plus or GMP-grade reprogramming media to each well, feeding the cells can skip one or two days until the next feeding if about double the amount of mTesR Plus or GMP-grade reprogramming media is added to each well as compared to daily feeding.

[0092] Various embodiments of the invention provide for a method of feeding isolated cell fraction undergoing episomal reprogramming, comprising:

(i) providing isolated cell fraction (ICF) undergoing episomal reprogramming ("cells"), OR reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs) by the methods of the present invention described herein;

(ii) feeding the cells on day 2 to day 4 after nucleofection, by adding 0.25-0.75 mL of mTeSR Plus media or GMP-grade reprogramming media to each well having the ICF undergoing episomal reprogramming;

(iii) feeding the cells on day 3 to day 10 after nucleofection, by removing spent media from each well and adding 0.5-1.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well;

(iv) feeding the cells on day 9 to day 18 after nucleofection, by removing spent media from each well and adding 0.5-1.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well or adding 1.5-2.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well;

(v) feeding the cells on day 14 to day 40 after nucleofection, by removing spent media from each well and adding 0.5-1.5 mL of mTeSR Plus media to each well or adding 1.5-2.5 mL of mTeSR Plus media to each well, wherein in step (v), the cells are cultured for 14-40 days. As such, in step (v) the cells can be cultured for 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 29 or 40 days. In various embodiments in step (v), the cells are cultured for 14-16 days, 17-22 days, 23-27 days, 28-30 days, 29-37 days or 38-40 days.

[0093] In various embodiments, wherein in step (iv) or step (v), when adding 1.5-2.5 mL of mTesR Plus or GMP-grade reprogramming media to each well, feeding the cells can skip one or two days until the next feeding.

[0094] In various embodiments, feeding isolated cell fraction undergoing episomal reprogramming, comprises:

(i) providing isolated cell fraction (ICF) undergoing episomal reprogramming ("cells"), OR reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs) by the methods of the present invention described herein;

(ii) feeding the cells on day 2.5 to day 3.5 after nucleofection, by adding 0.375-0.625 mL of mTeSR Plus media or GMP-grade reprogramming media to each well having the ICF undergoing episomal reprogramming;

(iii) feeding the cells on day 3.5 to day 9.5 after nucleofection, by removing spent media from each well and adding 0.75-1.25 mL of mTeSR Plus media or GMP-grade reprogramming media to each well;

(iv) feeding the cells on day 10 to day 17 after nucleofection, by removing spent media from each well and adding 0.75-1.25 mL of mTeSR Plus media or GMP-grade reprogramming media to each well or adding 1.75-2.25 mL of mTeSR Plus media or GMP-grade reprogramming media to each well;

(v) feeding the cells on day 15 to day 40 after nucleofection, by removing spent media from each well and adding 0.5-1.5 mL of mTeSR Plus media to each well or adding 1.75-2.25 mL of mTeSR Plus

media to each well, wherein in step (v), the cells are cultured for 15-40 days. As such, in step (v) the cells can be cultured for 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 29 or 40 days. In various embodiments in step (v), the cells are cultured for 15-17 days, 18-22 days, 23-27 days, 28-30 days, 29-37 days or 38-40 days.

[0095] In various embodiments, wherein in step (iv) or step (v), when adding 1.75-2.25 mL of mTesR Plus or GMP-grade reprogramming media to each well, feeding the cells can skip one or two days until the next feeding.

[0096] In various embodiments, feeding isolated cell fraction undergoing episomal reprogramming, comprises:

(i) providing isolated cell fraction (ICF) undergoing episomal reprogramming ("cells"), OR reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs) by the methods of the present invention described herein;

(ii) feeding the cells on day 3 after nucleofection, by adding 0.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well having the ICF undergoing episomal reprogramming;

(iii) feeding the cells on day 4 to day 9 after nucleofection, by removing spent media from each well and adding 1 mL of mTeSR Plus media or GMP-grade reprogramming media to each well;

(iv) feeding the cells on day 11 to day 16 after nucleofection, by removing spent media from each well and adding 1 mL of mTeSR Plus media or GMP-grade reprogramming media to each well or adding 2 mL of mTeSR Plus media or GMP-grade reprogramming media to each well;

(v) feeding the cells on day 17 to day 40 after nucleofection, by removing spent media from each well and adding 1 mL of mTeSR Plus media to each well or adding 2 mL of mTeSR Plus media to each well, wherein in step (v), the cells are cultured for 17-40 days. As such, in step (v) the cells can be cultured for 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 29 or 40 days. In various embodiments in step (v), the cells are cultured for 17-22 days, 23-27 days, 28-30 days, 29-37 days or 38-40 days.

[0097] In various embodiments, wherein in step (iv) or step (v), when adding 2 mL of mTesR Plus or GMP-grade reprogramming media to each well, feeding the cells can skip one or two days until the next feeding.

[0098] In various embodiments, wherein in step (ii), adding the mTesR Plus media or GMP-grade reprogramming media comprises adding the mTesR Plus media or GMP-grade reprogramming media at a rate of 1-4 minutes per 6 wells of a 12 well plate; in step (iii), removing spent media and adding the mTesR Plus media or GMP-grade reprogramming media is performed at a rate of up to 9 minutes per 6 wells of a 12 well plate; in step (iv), removing spent media and adding the mTesR Plus media or GMP-grade reprogramming media is performed at a rate of up to 7 minutes per 6 wells of a 12 well plate; in step (v),

adding the mTesR Plus media comprises adding the mTesR Plus media at a rate of 1-4 minutes per 6 wells of a 12 well plate.

[0099] In various embodiments, wherein in step (ii), adding the mTesR Plus media or GMP-grade reprogramming media comprises adding the mTesR Plus media at a rate of 2-3 minutes per 6 wells of a 12 well plate; in step (iii), removing spent media and adding the mTesR Plus media is performed at a rate of up to 7 minutes per 6 wells of a 12 well plate; in step (iv), removing spent media and adding the mTesR Plus media is performed at a rate of up to 5 minutes per 6 wells of a 12 well plate; in step (v), removing spent media and adding the mTesR Plus media is performed at a rate of 2-3 minutes per 6 wells of a 12 well plate.

[0100] In various embodiments, the GMP-grade reprogramming media comprises DMEM/F12, MEM NEAA, GlutaMAX, N2, B27, bFGF, hLIF, HA-100, PD0325901, CHIR99021, A-83-01, β-mercaptoethanol.

[0101] In various embodiments, the GMP-grade reprogramming media comprises a final concentration of 0.5-1.5% DMEM/F12, 0.5-1.5% MEM NEAA, 0.5-1.5% GlutaMAX, 0.5-1.5% N2, 1-3% B27, 75-125 ng/mL bFGF, 7.5-12.5 ng/mL hLIF, 7.5-12.5 μM HA-100, 0.25-0.75 μM PD0325901, 2-4 μM CHIR99021, 0.25-0.75 μM A-83-01, 0.05-0.15 μM β-mercaptoethanol.

[0102] In various embodiments, the GMP-grade reprogramming media comprises a final concentration of 0.75-1.25% DMEM/F12, 0.75-1.25% MEM NEAA, 0.75-1.25% GlutaMAX, 0.75-1.25% N2, 1.5-2.5% B27, 90-110 ng/mL bFGF, 9-11 ng/mL hLIF, 9-11 μM HA-100, 0.4-0.6 μM PD0325901, 2.5-3.5 μM CHIR99021, 0.4-0.6 μM A-83-01, 0.08-0.12 μM β-mercaptoethanol.

[0103] In various embodiments, the GMP-grade reprogramming media comprises a final concentration of 1% DMEM/F12, 1% MEM NEAA, 1% GlutaMAX, 1% N2, 2% B27, 100 ng/mL bFGF, 10 ng/mL hLIF, 10 μM HA-100, 0.5 μM PD0325901, 3μM CHIR99021, 0.5 μM A-83-01, 0.1 μM β-mercaptoethanol.

[0104] In various embodiments, the iPSCs express one or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express five or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express 10 or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express 15 or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express all markers selected from

CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, and CD326.

[0105] In various embodiments, the iPSCs express one or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 5 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 10 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 15 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express all markers selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, and COBL.

[0106] In various embodiments, the iPSCs express one or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express 5 or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express 8 or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express all markers selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, and PODXL (CD34 sialomucin).

[0107] Various embodiments provide for a method of isolating iPSC clones from a reprogramming plate, comprising:

(i) feeding the iPSC clones from the reprogramming plate; and

(ii) manually isolating the iPSC clones from the reprogramming plate.

[0108] Various embodiments provide for a method of isolating iPSC clones from a reprogramming plate, comprising:

(i) feeding the iPSC clones from the reprogramming plate;

(ii) enzymatically isolating the iPSC clones.

[0109] In various embodiments, feeding the iPSC clones from the reprogramming plate comprises: changing media in all wells of a reprogramming plate having one or more colonies, or low-density seeded

plate having one or more colonies by removing spent median and replacing with mTeSR Plus per well; selecting a colony that is not physically in contact with another colony, preferably the selected colony is compact and have morphology similar to iPSC colonies. In various embodiments, replacing with mTeSR Plus per well comprises replacing with 0.5-2 mL of mTeSR Plus per well. In various embodiments, replacing with mTeSR Plus per well comprises replacing with 0.5-1.5 mL of mTeSR Plus per well. In various embodiments, replacing with mTeSR Plus per well comprises replacing with about 1 mL of mTeSR Plus per well.

[0110] In various embodiments, feeding the iPSC clones from the reprogramming plate comprises the feeding iPSC clones in the same manner as feeding the isolated cell fraction undergoing episomal reprogramming described herein.

[0111] In various embodiments manually isolating the iPSC clones from the reprogramming plate comprises: cutting and transferring the selected colony to a new Laminin CT521 coated plate, wherein the laminin has been removed from the coated plate and replaced with mTeSR Plus media; transferring the plate to 35-39°C CO_2 incubator until the next feeding; rocking the new plate back and forth and side to side to distribute the clumps.

[0112] In various embodiments, manually isolating the iPSC clones from the reprogramming plate comprises: cutting and transferring the selected colony to a new Laminin CT521 coated plate, wherein the laminin has been removed from the coated plate and replaced with 1 mL of mTeSR Plus media per well; transferring the plate to an about 37°C CO_2 incubator until the next feeding; rocking the new plate back and forth and side to side to distribute the clumps.

[0113] In various embodiments, cutting and transferring the selected colony comprises: cutting the selected colony into small pieces; nudging clump pieces off the bottom of the well; transferring the floating pieces to the well containing mTeSR Plus media in the new laminin coated plate.

[0114] In various embodiments, cutting and transferring the selected colony comprises: using an insulin syringe needed to cut the selected colony into small pieces; using a P1000 tip inside a P20 tip to nudge clump pieces off the bottom of the well; using a P200 micropipette to transfer the floating pieces to the well containing mTeSR Plus media in the new laminin coated plate.

[0115] In various embodiments, enzymatically isolating the iPSC clones comprises:

(a) removing spent media from each well from the reprogramming plate;

(b) adding DPBS-/- to each well;

(c) aspirating the DPBS-/-;

repeating step (c);

(d) adding TrypLE CTS and transferring the reprogramming plate to the incubator and incubate at about 35-39°C for about 5-10 minutes;

(e) gently pipette up and down to break up the clumps;

(f) adding mTeSR plus to the well to neutralize the TrypLE CTS;

(g) transferring cell suspensions to a tube;

repeating steps (d)-(g) for each well and pool into the same tube;

(h) centrifuge the tube at about 200xG-400xG for about 3-7 minutes at about room temperature;

(i) removing supernatant from the tube without disturbing the cells pellet;

(j) dislodging the pellet by flicking the tub;

(k) resuspending the pellet in mTeSR Plus with Rock inhibitor;

(l) adding mTeSR Plus with Rock inhibitor;

(m) transferring well mixed cell suspension to a tube.

In various embodiments, the method further comprises:

(n) adding MoxiCyte viability reagent to cell suspension, mix and incubate in the dark for about 2-7 minutes;

(o) mixing the cell suspension in the tube;

(p) transferring a volume of cells comprising about 10,000-30,000 cells to each well of a multi-well plate;

(q) rocking the 6 well plate in an incubator at about 35-39°C and about 3-7% CO_2 to distribute the cells evenly.

[0116] In various embodiments, the method further comprising performing a cell count after step (m).

[0117] In various embodiments, after step (m) the method further comprising transferring a volume of cells comprising about 10,000-30,000 cells to each well of a multi-well plate and rocking the 6 well plate in an incubator at about 35-39°C and 3-7% CO_2 to distribute the cells evenly.

[0118] In various embodiments, enzymatically isolating the iPSC clones comprises:

(a) removing spent media from each well from the reprogramming plate;

(b) adding DPBS-/- to each well;

(c) aspirating the DPBS-/-;

repeating step (c);

(d) adding TrypLE CTS and transferring the reprogramming plate to the incubator and incubate at 37°C for about 5-10 minutes;

(e) gently pipette up and down to break up the clumps;

(f) adding mTeSR plus to the well to neutralize the TrypLE CTS;

(g) transferring cell suspensions to a tube;

repeating steps (d)-(g) for each well and pool into the same tube;

(h) centrifuge the tube at 200xG-400xG for about 5 minutes at room temperature;

(i) removing supernatant from the tube without disturbing the cells pellet;

(j) dislodging the pellet by flicking the tub;

(k) resuspending the pellet in mTeSR Plus with Rock inhibitor;

(l) adding mTeSR Plus with Rock inhibitor;

(m) transferring well mixed cell suspension to a tube.

In various embodiments, the method further comprises:

(n) adding MoxiCyte viability reagent to cell suspension, mix and incubate in the dark for about 2-7 minutes;

(o) mixing the cell suspension in the tube;

(p) transferring a volume of cells comprising about 10,000-30,000 cells to each well of a multi-well plate;

(q) rocking the 6 well plate in an incubator at about 37°C and 5% CO_2 to distribute the cells evenly.

[0119] In various embodiments, enzymatically isolating the iPSC clones comprises:

(a) removing spent media from each well from the reprogramming plate;

(b) adding about 0.5-1.5 mL of DPBS-/- to each well;

(c) aspirating the DPBS-/-;

repeating step (c);

(d) adding 0.5-1.5 mL of TrypLE CTS and transferring the reprogramming plate to the incubator and incubate at about 36-38°C for about 8-9 minutes;

(e) gently pipette up and down using a micropipette to break up the clumps;

(f) adding about 0.5-1.5 mL of mTeSR plus to the well to neutralize the TrypLE CTS;

(g) transferring cell suspensions to a 10-20 mL conical tube;

repeating steps (d)-(g) for each well and pool into the same 10-20 mL conical tub;

(h) centrifuge the conical tube at about 250-350x g for about 5 minutes at room temperature;

(i) removing supernatant from the conical tube without disturbing the cells pellet;

(j) dislodging the pellet by flicking the tub;

(k) resuspending the pellet in 0.5-1.5 mL of mTeSR Plus with Rock inhibitor;

(l) adding mTeSR Plus with Rock inhibitor to bring the volume to about 3-5 mL per well dissociated;

(m) transferring about 10-30 uL of well mixed cell suspension to a 1-3 mL microcentrifuge tube.

In various embodiments, the method further comprises

(n) adding about 160-200 uL of MoxiCyte viability reagent to cell suspension, mix and incubate in the dark for about 4-6 minutes;

(o) mixing the cell suspension in the 10-20 mL conical tube;

(p) transferring a volume of cells comprising about 15,000-25,000 cells to each well of a 6 well plate;

(q) rocking the 6 well plate in an incubator at about 36-38°C and 4-6% CO_2 to distribute the cells evenly.

[0120] In various embodiments, the method further comprising performing a cell count after step (m).

[0121] In various embodiments, after step (m) the method further comprising transferring a volume of cells comprising about 15,000-25,000 cells to each well of a 6 well plate and rocking the 6 well plate in an incubator at about 36-38°C and 4-6% CO_2 to distribute the cells evenly.

[0122] In various embodiments, enzymatically isolating the iPSC clones comprises:

(a) removing spent media from each well from the reprogramming plate;

(b) adding about 1 mL of DPBS-/- to each well;

(c) aspirating the DPBS-/-;

repeating step (c);

(d) adding 1 mL of TrypLE CTS and transferring the reprogramming plate to the incubator and incubate at about 37°C for about about 7 minutes;

(e) gently pipette up and down using a P1000 micropipette to break up the clumps;

(f) adding about 1 mL of mTeSR plus to the well to neutralize the TrypLE CTS;

(g) transferring cell suspensions to a 15 mL conical tube;

repeating steps (d)-(g) for each well and pool into the same 15 mL conical tub;

(h) centrifuge the conical tube at about 300x g for about aout 5 minutes at room temperature;

(i) removing supernatant from the conical tube without disturbing the cells pellet;

(j) dislodging the pellet by flicking the tub;

(k) resuspending the pellet in about 1 mL of mTeSR Plus with Rock inhibitor;

(l) adding mTeSR Plus with Rock inhibitor to bring the volume to about 4 mL per well dissociated;

(m) transferring about 20 μL of well mixed cell suspension to a 1.5 mL microcentrifuge tube.

In various embodiments, the method further comprises

(n) adding about 180 uL of MoxiCyte viability reagent to cell suspension, mix and incubate in the dark for about 5 minutes;

(o) mixing the cell suspension in the about 15 mL conical tube;

(p) transferring a volume of cells comprising about 20,000 cells to each well of a 6 well plate;

(q) rocking the 6 well plate in an incubator at about 37°C and 5% CO_2 to distribute the cells evenly.

[0123] In various embodiments, the method further comprising performing a cell count after step (m).

[0124] In various embodiments, after step (m) the method further comprising transferring a volume of cells comprising about 20,000 cells to each well of a multi-well plate and rocking the 6 well plate in an incubator at about 37°C and about 5% CO_2 to distribute the cells evenly.

[0125] In various embodiments, the method further comprises comprising passaging the cells.

[0126] In various embodiments, after 3 passages, iPSC appear. In various embodiments, iPSCs appear in less than 6 passages. In various embodiments, iPSCs appear in less than 10 passages.

[0127] In various embodiments, the volume of cells is calculated by using the following formula

$$cell\ suspension\ volume\ (ml) = \frac{20{,}000\ cells}{average\ viable\ cell\ concentration}$$

[0128] In various embodiments, mTeSR Plus with 10 μM ROCK comprises $volume\ of\ 10\ mM\ ROCK\ Inhibitor\ stock\ (uL) = \frac{10\ uM\ x\ volume\ of\ mTeSR\ Plus\ (mL)}{10\ mM}$.

[0129] In various embodiments, the iPSCs express one or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express five or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express 10 or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express 15 or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express all markers selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, and CD326.

[0130] In various embodiments, the iPSCs express one or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 5 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 10 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 15 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express all markers selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, and COBL.

[0131] In various embodiments, the iPSCs express one or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express 5 or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express 8 or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express all markers selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, and PODXL (CD34 sialomucin).

[0132] Various embodiments provide for iPSCs reprogrammed from isolated cell fraction (ICF) from cord blood units (CBUs). In various embodiments, the iPSCs generated from the reprogramming methods of the present invention.

[0133] Various embodiments provide a composition comprising iPSCs reprogrammed from isolated cell fraction (ICF) from cord blood units (CBUs); and cell media. In various embodiments, the iPSCs generated from the reprogramming methods of the present invention. In various embodiments, the composition is a cell culture comprising the iPSCs.

[0134] In various embodiments, the iPSCs express one or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express five or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express 10 or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express 15 or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326. In various embodiments, the iPSCs express all markers selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, and CD326.

[0135] In various embodiments, the iPSCs express one or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 5 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 10 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1,

TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express 15 or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL. In various embodiments, the iPSCs express all markers selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, and COBL.

[0136] In various embodiments, the iPSCs express one or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express 5 or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express 8 or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin). In various embodiments, the iPSCs express all markers selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, and PODXL (CD34 sialomucin).

KITS

[0137] The present invention is also directed to kits for episomal reprogramming of isolated cell fraction from cord blood units and isolation of induced pluripotent stem cell clones. The kit is useful for practicing the inventive method of episomal reprogramming of isolated cell fraction from cord blood units and isolation of induced pluripotent stem cell clones. The kit is an assemblage of materials or components. Thus, in some embodiments the kit contains a composition including the components of the buffers as described herein.

[0138] The exact nature of the components configured in the inventive kit depends on its intended purpose. For example, some embodiments are configured for the purpose of episomal reprogramming of isolated cell fraction from cord blood units. In one embodiment, the kit is configured particularly for the purpose of isolation of induced pluripotent stem cell clones.

[0139] Instructions for use may be included in the kit. "Instructions for use" typically include a tangible expression describing the technique to be employed in using the components of the kit to effectuate a desired outcome. Optionally, the kit also contains other useful components, such as, diluents, pharmaceutically acceptable carriers, syringes, catheters, applicators, pipetting or measuring tools, or other useful paraphernalia as will be readily recognized by those of skill in the art.

[0140] The materials or components assembled in the kit can be provided to the practitioner stored in any convenient and suitable ways that preserve their operability and utility. For example, the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen

temperatures. The components are typically contained in suitable packaging material(s). As employed herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit, such as inventive compositions and the like. The packaging material is constructed by well-known methods, preferably to provide a sterile, contaminant-free environment. As used herein, the term "package" refers to a suitable solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding the individual kit components. The packaging material generally has an external label which indicates the contents and/or purpose of the kit and/or its components.

EXAMPLES

[0141] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

Example 1

Reprogramming

PREPARATION OF LAMININ CT521 COATED PLATES

[0142] Reprogramming will use 2 x 12 well-plates with all 12 wells coated in each. Multiple plates can be used for reprogramming at the same time from the same sample. Plate(s) will be coated with CT521 laminin as described herein.

[0143] Allow the CT521 coated plates to coat, at a minimum, overnight at 2°C - 8°C. CT521 Coated plates are to be prepared at least one day before they are needed but they can be prepared up to 30 days before needed and are stored at 4°C.

PREPARATION OF COMPLETE STEMSPAN MEDIUM

[0144] Remove the StemSpan-AOF medium aliquot from the 4°C storage. Remove the StemSpan CD34+ Expansion Supplement and Y27632 (ROCKi) 10 mM aliquots from the -20°C storage.

[0145] Let the aliquots thaw at room temperature. Once the aliquots have thawed, place in a mini microcentrifuge and spin down to ensure the liquid is pooled at the bottom of the tube. Add 36 mL of StemSpan-AOF to a 50 mL conical tube. Add 4 mL of StemSpan CD34+ Expansion Supplement to the conical tube containing StemSpan-AOF prepared in the previous step. Add 40 µL of ROCKi 10mM to the same conical tube containing StemSpan-AOF+ Supplement for a final concentration of 10 µM.

[0146] Filter the solution using an appropriately sized 0.22 µm filtration system and the in-house vacuum or 60 mL syringe. Assign a 1-day expiration date. Record the date. Label the tube including, for

example, the following information: Complete StemSpan Medium, Date, Initials, Expiration Date: 1 day, Storage Conditions, 4°C

[0147] Keep the Complete StemSpan (CSS) Medium refrigerated in cool beads inside the BSC. Alternatively, use ice if working outside the clean room.

PREPARATION OF PLASMID MIXTURE

[0148] The plasmid mixture must be made fresh the day of reprogramming.

[0149] Remove the plasmid aliquots (pCBC1, pCBC2, pCBC3, pCBC4 and pCBC5) from the -20°C storage. Let the aliquots thaw at room temperature. Once the aliquots have thawed, place in the mini microcentrifuge and spin down to ensure the liquid is pooled at the bottom of the tube.

[0150] Label a 1.5 mL microcentrifuge tube as "Master Mix". Label another 1.5 mL microcentrifuge tube as "Nucleofection PM".

[0151] Record concentration for each plasmid. The table below shows the concentration needed for each plasmid and the formula to calculate volume of plasmid needed based on the concentration.

Table 1. Plasmid Preparation

Plasmid Name	Factors	Final Concentration Needed	Formula to calculate volume needed
pCBC1	MYCL-LIN28A, EBNA1	0.83 µg	$\frac{0.83\mu g}{pCBC1\ concentration\ \frac{\mu g}{\mu L}}\ x\ 5$
pCBC2	POU5F1, p53 shRNA, EBNA1	0.83 µg	$\frac{0.83\mu g}{pCBC2\ concentration\ \frac{\mu g}{\mu L}}\ x\ 5$
pCBC3	EBNA1	0.5 µg	$\frac{0.5\mu g}{pCBC3\ concentration\ \frac{\mu g}{\mu L}}\ x\ 5$
pCBC4	SOX2-KLF4, EBNA1	0.83 µg	$\frac{0.83\mu g}{pCBC4\ concentration\ \frac{\mu g}{\mu L}}\ x\ 5$
pCBC5	POU5F1-SOX2-LTAg-KLF4, EBNA1	0.83 µg	$\frac{0.83\mu g}{pCBC5\ concentration\ \frac{\mu g}{\mu L}}\ x\ 5$

[0152] Calculate the volume of plasmid needed based on Table 1 and record the volumes before proceeding.

[0153] **NOTE:** Plasmid mixture is prepared for 5 reactions. 1.0E+06 cells can be nucleofected per reaction. In various instances, 2.5E+05 to 5E+06 cells can be nucleofected per reaction.

[0154] Add the volumes from above for each plasmid to the tube labelled "Master Mix." Use a P-10, pipette mixture up and down 2-3 times to mix.

[0155] Use the following formula to determine the volume of plasmid mix to add to the tube labelled `Nucleofection PM'. Record the volume.

$$\frac{volume\ of\ pCBC\ 1\ +\ pCBC\ 2\ +\ pCBC\ 3\ +\ pCBC\ 4\ +\ pCBC\ 5}{5}$$

[0156] Use a P-10 to add the appropriate volume of plasmid mix to "Nucleofection PM' tube and record the volume. Assign a 1-day expiration date. Label tubes with expiration date. Keep media tubes with plasmid mixtures on ice if outside the clean room or cool beads if inside the clean room until ready to use. Tube `Nucleofection PM' will be used for reprogramming and tube `Master Mix' will be kept as back up and discarded after reprogramming.

PREPARATION OF NUCLEOFECTION SOLUTION

[0157] Remove the P3 Nucleofection Solution bottle and Supplement tube from the 4°C. Spin the Supplement tube in a microcentrifuge to pool the liquid to the bottom. Let the NS bottle and the Supplement tube equilibrate to room temperature inside the BSC, for at least 15-20 minutes. Record start and end time. Label a microcentrifuge tube with `NS+S' for Nucleofection Solution + Supplement.

[0158] Prepare Nucleofection Solution + Supplement (NS+S) per the manufacturer's instructions as described:

[0159] Use a P200, add 82 µL of P3 Nucleofection Solution to the tube labeled `NS+S'. Use a P20, add 18 µL of Supplement to the tube labeled `NS+S'. Use a P200, pipette the total volume up and down several times to mix.

[0160] **NOTE:** 1 volume of Nucleofection Solution + Supplement can be used for up to 1.0E+06 cells.

[0161] Once the mixture is prepared, keep in the BSC, at room temperature, until ready to use.

COUNTING OF FRESH ISOLATED CELL FRACTIONS

[0162] Label 2 new 50 mL conical tubes with “Cells.” Transfer flask(s) with cells to BSC. Use a 10 mL serological pipette, collect cells from flask(s) and move to tube(s) labelled “Cells.” Use a 10 mL serological pipette, rinse flask(s) with 10 mL StemSpan-AOF and collect remaining cells into ‘Cells’ tube.

[0163] Repeat rinse of flask(s) with StemSpan-AOF and collect remaining cells into ‘Cells’ tube.

[0164] Centrifuge the tube(s) at 600 x g for 10 minutes at room temperature. Record centrifuge RB, speed, time and temperature. Remove the supernatant from the tube(s), remove as much volume as possible while leaving the pellet(s) undisturbed. Replace the cap on the tube(s) and gently flick the bottom of the tube(s) to dislodge the pellet.

[0165] Use a P1000 to resuspend pellet using 1 mL of CSS for each tube. Record volume. Combine the cell suspension from all tubes into 1 tube. Measure total volume of cell suspension. Record total volume.

Use a P20, take 20 µL of cell suspension and transfer into a 1.5 mL microcentrifuge tube Label a 1.5 mL microcentrifuge tube with 'CC' for cell count. Add 180 µL of Moxi Viability reagent and incubate in the dark for 5 minutes.

[0166] Measure cell count and viability using the Moxi GO II cell counter**.** Attach form (MFG-FORM-009) and the PDF file with the raw data exported from Moxi GO II. If using BPR, only attach pdf file with the raw data exported from Moxi GO II.

[0167] **NOTE:** Cell viability must be ≥70% to proceed to next step. If viability is <70% notify MFG manager or designee (SME) to make further decision

[0168] **NOTE:** CVs for viability and live cell concentration must be ≤25%. If they are above, repeat cell count.

[0169] Record average cell viability, average live cell concentration and total live cell count.

[0170] Use the following formula to calculate the volume of cell suspension needed to obtain 5E+05 cells. If more cells are needed, adjust accordingly.

$$Volume\ Needed\ (ml) = \frac{5E + 05\ cells}{Average\ Live\ Cell\ Concentration\ (\frac{cells}{mL})}$$

[0171] Transfer volume from previous step to a 15 mL conical tube labeled "5E+05 cells." These cells will be used for nucleofection. Based on the cell count, adjust cell suspension volume to 2 mL by adding CSS, if necessary.

[0172] Centrifuge the conical tube at 600 x g for 10 minutes at room temperature. Record centrifugation speed, time and temperature.

SETTING UP THE 4-D NUCLEOFECTORTM X-UNIT

[0173] Turn on the system using the main power switch at the rear of the Core Unit. Identify equipment.

[0174] The blue LED at the front of the Core Unit will be lit. The system will boot - this process may take a few moments Once the start-up procedure is complete, the User Interface will display the software main screen.

[0175] If using equipment for the first time that day, perform Equipment inspection using 100 µL Single Nucleocuvette™ Vessels. Log activity.

[0176] From the main screen, "Choose a Device" will be displayed, select the "X-Unit."

[0177] After choosing X Unit, the screen will prompt to "Choose a vessel." Select the cuvette system. After selecting the cuvette system, the screen will prompt to "Choose Experiment or Position." Select the cuvette positions that will be used. Then select "OK." Record position selected.

[0178] Next the screen will prompt cell type, select "CD34+ cells, human" and click "OK." Record cell type selected.

[0179] After selecting the correct program, the screen will show the position of the cuvette, cell type and pulse code. The 4D-Nucleofector is now ready to use. Record program, position and pulse code.

NUCLEOFECTION

[0180] Label a new, sterile 50 mL conical tube with "Sample ID-N" for Sample – Nucleofected.

[0181] Add 24 mL of Complete StemSpan Medium (CSS) to conical tube. Record volume added. Set the tube labeled "Sample ID-N" to the side until after the cells have been nucleofected. Remove the supernatant from the conical tube containing the cell pellet that was centrifuged. Remove as much volume as possible while leaving the pellet undisturbed. Replace the cap on the conical and gently flick the bottom of the tube to dislodge pellet.

[0182] Use a P-200, add 100 µL of the Nucleofection Solution plus Supplement (NS+S) prepared in section 7.5 to the conical labelled "Cells" and pipette up and down no more than 5 times to break up the pellet.

[0183] **NOTE:** Leaving cells in Nucleofector™ Solution for extended periods of time may lead to reduced transfection efficiency and viability. It is important to work as quickly as possible

[0184] Pull up 100 µL of the NS+S+Cells suspension. With the pipet tip still submerged in the NS+S+Cells suspension, slowly adjust the volumeter on your P-200 to pull up the total volume of the suspension into the tip.

[0185] Adjust the volumeter so there is empty space at the end of the pipet tip to account for the volume that will be added by the plasmid mixture. Transfer the total volume of the NS+S+Cells suspension to the microcentrifuge tube labelled "Nucleofection PM" containing the plasmid mixture. Gently pipette up and down several times to mix. Avoid air bubbles while pipetting.

[0186] Transfer the total volume of NS+S+Cells+PLASMID Mixture to a cuvette provided by the P3 Primary Cell 4D-NucleofectorTM X Kit. Cap the cuvette and gently tap against the surface of the BSC to make sure the sample covers the bottom of the cuvette. Performer and Verifier to confirm program EO 100 is selected and press "OK."

[0187] Place cuvette with closed lid into the appropriate position in the retainer of the 4D-Nucleofector™ X Unit. Press 'Start'. Record position used.

[0188] After the program has finished, remove the cuvette from the unit.

[0189] Using the plastic pipette provided with the kit, transfer the entire liquid contents of the cuvette to 'Sample ID-N' conical.

[0190] Remove the CT521 laminin from both 12 well plates.

[0191] Gently mix the cell suspension in the 50 mL conical labeled Sample ID-N by pipetting up and down 2-3 times. Add 1 mL of cell suspension to each well of the 12 well plate. Record volume of cell suspension plated and number of wells seeded. Transfer the plate outside the BSC and label it with, for example, the following information: Project Name, BPR, Sample ID, ICF from CBU Episomal

Reprogramming, Batch Number, if applicable, Date of Nucleofection / Initials. Move the plate to the incubator set to 37°C ± 2°C and 5% CO_2. Record incubator RB and location.

[0192] **NOTE:** It is extremely important to not move or disturb the plate or incubator for 72 hours.

[0193] Perform label reconciliation, if necessary. Beginning on Day 3, plates will be fed following.

Example 2

Feeding of Isolated Cell Fraction Undergoing Episomal Reprogramming

GENERAL FEEDING INDICATIONS

[0194] Feeding with mTeSR starts on day 3 after nucleofection and will continue until clones have been isolated or the reprogramming has been designated a failure. Normally, the feeding process will not continue longer than 40 days. Feeding will occur daily from Monday through Friday. On Fridays a double feed is performed to skip feeding the cells on Saturday and Sunday.

[0195] Under specific circumstances, if the cells are ≤70% confluent, a double feed can be performed and the feeding the following day can be skipped with prior approval from the supervisor. If a double feed is performed, it must be documented on the respective step of the BPR. In such cases, perform a double feed the day before and resume the feeding schedule afterwards. Set Pipet-Aid speed on 'Slow' to remove and dispense media into wells. Use Table below as a guide to feed the cells as described in this procedure.

Table Post Nucleofection Feeding Guidelines

Day Post Nucleofection	Medium Volume	Type of Change	Speed of Feed
3	0.5 mL	Addition	Very Slowly(1)
4 – 9	1.0 mL or 2.0 mL (Double)	Partial Feed	Slowly(2)
10 – 16	mL or mL (Double)	Full Feed	Gently(3)
17 – 40	1.0 mL or 2.0 mL (Double)	Full Feed	iPSC-style(4)

(1) Add media to the wells so there is very little disturbance to the media already in the well. This can take as long as 2-3 mins per 6 wells of a 12 well plate.
(2) Slowly remove around 75% of the media in the well and replace 1 mL of fresh media. This can take as long as 7 mins per 6 wells of a 12 well plate.
(3) Remove the media in the well and replace with 1 mL of fresh media. This can take as long as 5 mins per 6 wells of a 12 well plate.
(4) Remove the media in the well and replace with the appropriate volume. This can take as long as 2-3 mins.

[0196] The cells will be assessed for cell attachment and cell quality before every feeding: Cell attachment; Lack of contamination; Areas of interest.

DAY 3 FEEDING

[0197] Obtain mTeSR Plus from 4 °C. On day 3 post nucleofection, carefully transfer the plates to the microscope for imaging. Check the cells for the following and record observations: Cell attachment, Lack of contamination, Areas of interest.

[0198] Take a picture after each feed with the image and include for example, the following: CBU ID, Days in culture, Magnification, Initials and date.

[0199] Very carefully move the plate from the microscope to the BSC. Prevent sloshing the media as much as possible. Very slowly, add 0.5 mL of mTeSR to each well. Record volume added.

[0200] **NOTE:** DO NOT ASPIRATE MEDIA FROM THE WELLS.

[0201] Very carefully move the plate back to the incubator. Prevent sloshing the media as much as possible. Do not move, bump, or disturb the plate or incubator again until the next feeding.

DAY 4 – DAY 9 FEEDING

[0202] Obtain mTeSR Plus from the 4 °C. Beginning on Day 4, a partial feed will be performed. Carefully move the plate from the incubator. Prevent sloshing the media as much as possible. Slowly remove most of the media (~1 mL) from each well. Slowly, add 1 mL of mTeSR Plus to each well. Carefully transfer the plates to the microscope for imaging. Check the cells for the following and record observations: Cell attachment, Lack of contamination, Areas of interest, take a picture and record it. Name the image and include, for example, the following: CBU ID, Days in culture, Magnification, Initials and date.

[0203] Mark areas of interest using a colony marker installed on the microscope or make a circle around the area with lab marker. Carefully move the plate back to the incubator until the next feeding. Do not move, bump, or disturb the plate or incubator again until the next feeding.

DAY 11 – 16 FEEDING

[0204] Carefully transfer the plates to the microscope for imaging. Check the cells for the following and record observations: Cell attachment, Morphology, Lack of contamination, Areas of interest

[0205] Take a picture after each feed and record it. Name the image and include, for example, the following: CBU ID, Days in culture, Magnification, Initials and date

[0206] Mark areas of interest using a colony marker installed on the microscope and make a circle around the area with a lab marker. Move the plates back to the incubator.

[0207] Obtain mTeSR Plus aliquots from the 4°C. Move the plates from the incubator to the BSC. Use a P1000 micropipette to remove the spent mTeSR Plus from each well. Add mTeSR Plus to each well as described: Monday – Thursday: add 1 mL of mTeSR Plus to each well. Friday: add 2 mL of mTeSR Plus to each well. Move the plates back to the incubator until the next feeding.

DAY 17 – DAY 40 FEEDING

[0208] Continue feeding with mTeSR Plus until clones have been isolated or the reprogramming has been designated a failure. If clones are ready to be isolated, proceed per the isolation protocol described herein.

[0209] The same plate may undergo several rounds of clone picking. In that case, continue to feed the cells as described below. Feed the cells for as long as needed. If clones are successfully isolated before day 40, feeding can be stopped.

[0210] Obtain mTeSR Plus aliquots from 4°C. Move the plates from the incubator to the BSC. Remove the spent media from each well. Add mTeSR Plus to each well as described: Monday – Thursday: add 1 mL of mTeSR Plus to each well. Friday: add 2 mL of mTeSR Plus to each well. Saturday and Sunday: no feed required.

[0211] Transfer the plates to the microscope for imaging. Check the cells for the following and record observations: Cell attachment, Morphology, Lack of contamination, Areas of interest, Take a picture after each feed and record. Name the image and include, for example, the following: Sample ID, Days in culture, Magnification, Initials and date

[0212] Mark areas of interest using a colony marker installed on the microscope and make a circle around the area with a lab marker. Move the plate back to the incubator until the next feed or clone isolation.

Example 3

Isolation of iPSC clones from Reprogramming Plate

CLONE ISOLATION

[0213] When cells in the reprogramming plate have been fed with mTeSR Plus for at least 7 days (but not more than 40 days), clone isolation can performed following this procedure.

[0214] This procedure describes 3 primary methods to isolate clones from cells having undergone reprogramming. Figure 2 outlines the three methods described.

(1) Manual Clone Isolation (MCI): This procedure describes manually transferring a single area of interest from the reprogramming plate to one well of a 6/12W plate. This new well is assigned a unique clone-ID (as described below).

(2) Enzymatic Clone Isolation (ECI): This procedure describes enzymatically transferring cells from the reprogramming plate, to a 6 Well plate – plated at a low density (passage 1). From this plate, a single area of interest will be manually transferred to one well of a 6/12W plate. This new well (passage 2) is assigned a unique clone ID (as described below).

(3) Enzymatic Clone Isolation through Cryopreservation: This procedure is identical to ECI, except cells are first dissociated, cryopreserved, and at a later date, thawed and plated at a low density onto a new 6 well plate (passage 1).

[0215] Each designated iPSC clone will be cultured on its own plate. Clone ID are assigned as follows: PROJECT-DonorID-YY; Where YY describes the clone number. Consecutive numbers starting at 01

[0216] The Production Number for each clone isolation BPR will follow a similar convention described below: PROJECT-DonorID-R#_Z#; Where R"#" represents the number of times this starting material has undergone the nucleofection/reprogramming procedure. Z will be used to describe the procedure being performed: MCI: Manual Clone Isolation; ECI: Enzymatic Clone Isolation, DCP: Direct Cryopreservation. "#" represents the number of times this specific procedure is performed on this reprogramming run.

[0217] Following Clone Isolation, each iPSC clone will be assigned its own BPR (based on its clone ID as described above).

[0218] Isolated iPSC clones will be expanded for primary cell stock (PCS) or Seed Bank generation.

[0219] Clone isolation activities will be recorded on electronic batch production records (BPRs) corresponding to for manual clone isolation and enzymatic clone isolation.

FEEDING BEFORE CLONE SELECTION

[0220] Obtain required mTeSR Plus media aliquots prepared as noted herein. Transfer mTeSR Plus media and new Laminin coated plates for clone isolation into BSC. Remove the relevant plate from incubator (reprogramming plate or low-density seeded plate) and record incubator. Transfer the plate to BSC. Change media in all wells by removing spent media and replacing with 1 mL of mTeSR Plus per well.

[0221] After replacing media, check plate under the microscope and use a marker to select colonies that will be isolated (if performing manual clone selection). Follow instructions below:

[0222] Ensure colonies to be selected are not physically in contact with each other. Where possible, isolate colonies from individual wells. Record the number of wells to be picked on BPR. Selected colonies should be compact and have a morphology similar to iPSC colonies. Note that at this early stage, most colonies do not look exactly like iPSCs. Figure 3 provides for reference images on how colonies should look. Proceed to colony isolation as described herein.

MANUAL CLONE ISOLATION

[0223] This procedure will be performed in BSC with a microscope. Prior to clone isolation, project lead or SME will determine how many colonies to pick based on cell morphology and specific project requirements.

[0224] Transfer as many Laminin CT521 coated plates based on the number of colonies to pick. Record number of plates used on BPR. Remove laminin from the well on each plate that will be used to plate isolated colonies and add 1 mL of mTeSR Plus media per well.

[0225] Place one of the Laminin CT521 Coated plates near microscope. Place reprogramming or low-density plate on microscope with the objective set to 4X. Obtain image of first colony using microscope and record it.

[0226] Cut and transfer selected colony to the new laminin coated plate following these steps:

[0227] Use an insulin syringe needle to cut selected colony into small pieces. Try to cut the colony into at least quarters, but if possible, cut them into smaller pieces without shredding them.

[0228] **NOTE:** On use needles must be disposed of in a rigid, puncture proof container and should not be recapped.

[0229] Use a P1000 tip inside a P20 tip to nudge clump pieces off the bottom of the well.

[0230] Use a P200 micropipette to transfer the floating pieces to the well containing mTeSR Plus media in the new laminin coated plate.

[0231] Ensure that a single colony is not selected twice to avoid repeated clones.

[0232] Transfer the plate outside the hood and label it including, for example, the following information: - PROJECT-DonorID-YY_Px – Date; YY: consecutive numbers starting with 01 for the first isolated clone.

[0233] **NOTE:** If this is not the first time reprogramming cells from this starting material, be sure to continue numbering clones starting from the highest number used in the previous reprogramming run.

[0234] Px: passage number (if picking from the reprogramming plate, this will be P1, if picking from a low-density plate this will be P2).

[0235] Attach a picture of the label to the BPR.

[0236] Transfer plate to 37°C 5% CO2 incubator until the next feeding. Rock new plate back and forth and side to side to distribute clumps evenly in well. Identify and log incubator.

[0237] Repeat step the step until all colonies have been transferred to each corresponding new plate.

[0238] Record number of colonies isolated on BPR.

[0239] Place each plate in a separate location in the incubator. Avoid incubating more than 2 clones on a single shelf. Label each incubator shelf with the Clone ID, and Project Name.

[0240] After all colonies have been transferred to the new laminin coated plates, use a marker to mark the bottom of the plate with an "x" to designate where the colonies were cut from the reprogramming plate. Return the reprogramming plate to 37°C 5% CO_2 incubator until the next feeding. Record incubator RB number and location on the BPR.

[0241] Label the incubator shelf door with the reprogramming plate information.

[0242] If necessary, clone isolation can be performed again from the same reprogramming plate at a later date. Clone numbers will continue from previous clone isolation. It is recommended to isolate colonies more than necessary.

[0243] Continue to feed reprograming plate following respective feeding procedure until it is certain that enough clones have been successfully isolated. Isolated clones will be fed and expanded to generate a PCS or SB.

ENZYMATIC CLONE ISOLATION

[0244] Obtain representative images of reprogramming plate wells using microscope and record them. Identify it with Clone-ID, day post reprogramming, date.

[0245] Obtain Laminin CT521 coated 6 well plates. One well of a reprogramming plate (12 well plate) will be harvested into 2 wells of a 6 well plate at a seeding density of 20,000 cells/well.

[0246] Prepare mTeSR Plus media plus 10 µM ROCK inhibitor as described below: Obtain an aliquot of mTeSR Plus media prepared. Determine the volume of mTeSR Plus with 10 µM ROCK Inhibitor required and record the volume on BPR.

[0247] Calculate the volume of 10 mM ROCK Inhibitor needed for making mTeSR Plus with 10 µM ROCK Inhibitor by using the formula:

$$\frac{Volume\ of\ 10\ mM\ ROCK\ Inhibitor\ Stock\ (uL)\ x\ Volume\ of\ mTeSR\ Plus\ (mL)}{10\ mM}$$

[0248] Obtain required number of aliquots of 10 mM ROCK Inhibitor and record quantity used on BPR. Transfer the materials needed into the BSC.

[0249] In the BSC transfer the volume required of mTeSR Plus media to an appropriately sized conical tube.

[0250] Add the calculated volume of 10 mM ROCK Inhibitor to the mTeSR Plus media and mix using a serological pipette.

[0251] Label the tube as "mTeSR + 10 µM RI", initial and date. Leave the media in the BSC at room temperature for later use. Transfer new Laminin CT521 coated plate(s) for clone isolation into BSC.

[0252] Remove laminin from each well of a 6 well plate that will be passaged into and add 2 mL of complete mTeSR Plus with ROCK inhibitor to each well. Record number of plates and wells prepared. Transfer reprogramming plate to BSC.

[0253] Remove spent media from each well to be dissociated and discard. Add 1 mL of DPBS -/- to each well to be dissociated. Aspirate DPBS -/- and discard. Add another 1 mL of DPBS -/- to each well to be dissociated. Aspirate DPBS -/- and discard. Add 1 mL of TrypLE CTS and transfer the plate to the incubator. Incubate at 37°C for 7 minutes. Record incubation time on BPR.

[0254] Following incubation, transfer plate back to BSC.

[0255] For one well at a time: Using a P1000 micropipette, gently pipette up and down several times to break up the clumps. Gently add 1 mL of mTeSR plus to the well to neutralize TrypLE CTS. Transfer cell suspension to a 15 mL conical tube labelled as "Enzymatic Dissociation".

[0256] Repeat Steps for each well being dissociated and pool into the same 15mL conical tube.

[0257] Transfer tube outside the hood and centrifuge at 300 x g for 5 minutes at room temperature. Record centrifuge conditions on BPR. While centrifuging, transfer reprogramming plate to incubator. Record incubator information on BPR. After centrifugation is over, transfer tube back to BSC.

[0258] Remove supernatant from conical tube without disturbing the cells pellet. Dislodge the pellet flicking the tube. Use a P1000 micropipette to resuspend pellet in 1 mL of mTeSR Plus with Rock inhibitor. Add mTeSR Plus with Rock inhibitor to bring total volume to 4mL per well dissociated. For example, if dissociating 2 wells, add an additional 7mL of mTeSR Plus with Rock inhibitor to yield 8mL total. Pipette mixture twice to mix. Use a P20 micropipette to transfer 20 µL of well mixed cell suspension to 1.5 mL microcentrifuge tube.

[0259] Label tube with 'cell count' and add 180 µL of MoxiCyte Viability reagent to cell suspension and mix. Incubate in the dark for 5 minutes. Perform two cell counts, record cell count information on BPR. Include the average viability, average live cell concentration and the total live cells on BPR. Confirm CVs for viability and live cell concentration are ≤25%. Attach cell count data to the BPR.

[0260] Calculate the volume of cell suspension containing 20,000 cells using the formula:

$$Cell\ suspension\ volume(ml) = \frac{20{,}000\ cells}{Average\ viable\ cell\ concentration}$$

[0261] Mix cell suspension in 15 mL conical tube. Transfer the volume calculated above to each well of a 6 well plate to be plated (in general, seed into all 6 wells). Record number of wells plated on BPR. Label plate with, for example, the following information. PROJECT-DonorID-ECI#_Px ECI#: Enzymatic Clone Isolation, and a number corresponding to each consecutive time this procedure is performed on this reprogramming plate, Px: passage number will correspond to P1 20,000 cells/well, Date

[0262] Take a picture of the label and attach to the BPR.

[0263] Transfer plate to 37°C 5% CO_2 incubator. Rock new plate(s) back and forth and side to side to distribute cells evenly in well. Record incubator RB number and location in BPR.

[0264] Prepare a 15 mL conical tube as a balance using DPBS -/-, using the same volume as that in the tube labeled "Enzymatic Dissociation".

[0265] Transfer tube out of the BSC and centrifuge at 300 x g for 5 minutes at room temperature. Record centrifuge conditions on BPR. After centrifugation is over, transfer tube back to BSC.

[0266] Remove supernatant from conical tube without disturbing the cells pellet. Dislodge the pellet flicking the tube. Use a P1000 micropipette to resuspend pellet in 1 mL of CS10. Add additional CS10 to yield a final volume of 1mL per well dissociated. For example, if 3 wells were dissociated, add an additional 2 mL of CS10 to yield 3 mL of cell suspension in CS10. Using a serological pipette add 1mL of cell suspension into each cryovial. Record number of vials filled on BPR. Transfer vials outside the BSC and label them with, for example: PROJECT-DonorID-R#-DayX, where R# represents the number of times

this lot of PBMCs has undergone the nucleofection/reprogramming procedure, DayX represents the Day-since-reprogramming the cells were frozen, Date

[0267] Transfer the vials to a Mr. Frosty at room temperature and transfer to a -80°C freezer as soon as possible. Record freezer RB# and location on BPR.

[0268] At least 72 hours after placing Mr. Frosty in the -80°C freezer, transfer the vials to the LN2 tank. Record RB# and location on BPR.

[0269] Monitor the cells daily (in the low-density plate) and feed with mTeSR Plus following the routine schedule. When potential iPSC colonies start appearing, perform Manual Clone Isolation.

DIRECT CRYOPRESERVATION OF CELLS UNDERGOING REPROGRAMMING

[0270] This procedure will be performed if chosen to cryopreserve cells from the reprogramming plate to be used as a backup.

[0271] Obtain an aliquot of mTeSR Plus media prepared. Transfer reprogramming plate to BSC. Remove spent media from each well to be cryopreserved, and discard.

[0272] Add 1 mL of DPBS -/- to each well to be cryopreserved. Aspirate DPBS -/- and discard. Add another 1 mL of DPBS -/- to each well to be cryopreserved. Aspirate DPBS -/- and discard. Add 1 mL of TrypLE CTS to each well and transfer the plate to the incubator. Incubate at 37°C for 7 minutes. Record incubation time on BPR. Following incubation, transfer plate back to BSC.

[0273] For one well at a time: Using a P1000 micropipette, gently pipette up and down several times to break up the clumps. Gently add 1 mL of mTeSR plus to the well to neutralize TrypLE CTS. Transfer cell suspension to a 15 mL conical tube labelled as "Cells". Repeat Steps for each well that is dissociated and pool into the same 15mL conical tube.

[0274] Transfer tube outside the hood and centrifuge at 300 x g for 5 minutes at room temperature. Record centrifuge conditions on BPR. While centrifuging, transfer reprogramming plate to incubator. Record incubator information on BPR. After centrifugation is over, transfer tube back to BSC.

[0275] Remove supernatant from conical tube without disturbing the cells pellet. Dislodge the pellet flicking the tube. Use a P1000 micropipette to resuspend pellet in 1 mL of CS10. Add additional CS10 to yield a final volume of 1 mL per well dissociated. For example, if 3 wells were dissociated, add an additional 2 mL of CS10 to yield 3 mL of cell suspension in CS10. Using a serological pipette add 1 mL of cell suspension into each cryovial. Record number of vials filled on BPR. Transfer vials outside the BSC and label them with, for example: PROJECT-DonorID-R#-DayX, where R# represents the number of times this lot of PBMCs has undergone the nucleofection/reprogramming procedure, DayX represents the "Days-since-nucleofection" the cells were frozen, Date

[0276] Transfer the vials to a Mr. Frosty at room temperature and transfer to a -80°C freezer as soon as possible. Record freezer RB# and location on BPR. At least 72 hours after placing Mr. Frosty in the -80°C freezer, transfer the vials to the LN2 tank. Record RB# and location on BPR.

THAWING AND PLATING CELLS UNDERGOING REPROGRAMMING

[0277] Cells undergoing reprogramming which were frozen after enzymatic clone isolation, or directly from the reprogramming plate, can be thawed at a later date to continue culturing following this procedure.

[0278] Prepare mTeSR Plus media plus 10 pM ROCK inhibitor as described below:

[0279] Obtain an aliquot of mTeSR Plus media prepared. Obtain an aliquot of 10 mM ROCK Inhibitor and record quantity used on BPR. Transfer the materials needed into the BSC. In the BSC transfer 30 mL of mTeSR Plus media to 50ml conical tube.

[0280] Using a pipette, add 30 pL of 10 mM ROCK Inhibitor to the mTeSR Plus in the conical tube. Mix the solution using a serological pipette. Label the tube as "mTeSR + 10 pM RI" and date. Leave the media in the BSC at room temperature for later use.

[0281] Transfer new 6 Well Laminin CT521 coated into BSC.

[0282] Using a serological pipette, remove laminin from each well of a 6 well plate that will be seeded into and add 2 mL of mTeSR Plus with ROCK inhibitor to each well. Record number of plates and wells prepared.

[0283] Label a 15 mL conical tube with "CELLS". Add 9 mL of mTeSR Plus + 10 pM ROCK into the 15 mL conical tube.

[0284] Prepare a 15 mL conical tube with 10mL of DPBS -/- to be used as a balance. Label conical tube with "BALANCE"

[0285] Obtain vial of cells that were previously cryopreserved from a reprogramming plate following.

[0286] Transfer vial from LN2 tank into manufacturing suite. Record vial information on BPR and take a picture of vial and attach to the BPR.

[0287] Immerse the cryovial(s) in the beaker of water located in the 37°C bead bath, ensuring the O-ring stays above the water line and move the vial(s) in an "S" formation for 2-3 minutes until only a small visible ice crystal remains in the tube(s).Wipe down the outside of the cryovial(s) with 70% IPA and transfer to BSC.

[0288] Using a serological pipette, transfer the contents from the cryovial dropwise into the 15mL conical tube labeled as "CELLS". Pull up one mL of the cell suspension and transfer into the cryovial to rinse. Add the contents back into the 15 mL conical tube. Using a 10 mL serological pipette, gently pipette 2-3 times to ensure proper mixing of the cell suspension.

[0289] Transfer the conical tubes labeled as "CELLS" and "BALANCE" out of the BSC. Centrifuge tubes at 300 g for 5 minutes at room temperature. Once complete, transfer the tube labeled "CELLS" back into the BSC.

[0290] Remove supernatant from conical tube without disturbing the cells pellet. Dislodge the pellet flicking the tube. Use a P1000 micropipette to resuspend pellet in 1 mL of mTeSR Plus + Rock inhibitor. Add 3 mL of mTeSR Plus with Rock inhibitor to bring total volume to 4 mL. Pipette mixture twice to mix. Use a P20 micropipette to transfer 20 µL of well mixed cell suspension to 1.5 mL microcentrifuge tube. Label tube with 'cell count' and add 180 µL of MoxiCyte Viability reagent to cell suspension and mix. Incubate in the dark for 5 minutes.

[0291] Perform two cell counts, record cell count information on BPR. Include the average viability, average live cell concentration and the total live cells on BPR. Attach cell count data to the BPR.

[0292] Calculate the volume of cell suspension containing 20,000 cells using the formula below.

$$Cell\ suspension\ volume(ml) = \frac{20{,}000\ cells}{Average\ viable\ cell\ concentration}$$

[0293] Mix cell suspension in 15 mL conical tube.

[0294] Transfer the volume calculated above to each well of a 6 well plate to be plated (in general, seed into all 6 wells). Record number of wells plated on BPR.

[0295] Label plate with, for example, the following information: PROJECT-DonorID-ECI#_Px, ECI#: Enzymatic Clone Isolation, and a number corresponding to each consecutive time this procedure is performed on this reprogramming plate, Px: passage number will correspond to P1, 20,000 cells/well. Date

[0296] Take a picture of the label and attach to the BPR. Transfer plate to 37°C 5% CO2 incubator. Rock new plate(s) back and forth and side to side to distribute cells evenly in well. Record incubator RB number and location in form BPR.

[0297] Monitor the cells daily (in the low density plate) and feed with mTeSR Plus following the routine schedule. When potential iPSC colonies start appearing, perform Manual Clone Isolation.

Example 4

Media and Aliquot Preparation for GMP Manufacturing

MOXICYTE VIABILITY REAGENT ALIQUOT PREPARATION

Manufacturer	Catalog #	Aliquot Volume	Aliquot Tube	Storage	Expiration Date
Orflo	MAX055	300 µL	2 mL Amber Tube	4 °C	2 years from date of preparation

[0298] Obtain MoxiCyte Viability Reagent bottle. Carefully open the lid of MoxiCyte Viability Reagent bottle. Aliquot 300 µL of MoxiCyte Viability Reagent into amber 2 mL microcentrifuge tubes. Determine the number of 300 µL aliquots prepared and record on the BPR.

[0299] Assign an expiration date as indicated on the manufacturer's certificate or 2 years. Attach a picture of the label to the BPR.

[0300] Place labels onto the 2 mL amber microcentrifuge tubes. Perform label reconciliation. Store the aliquots in a box labelled with the same information and place in location at 4 °C. Record storage location and RB# of refrigerator in the BPR.

STEMSPAN AOF ALIQUOT PREPARATION

Manufacturer	**Catalog #**	**Aliquot Volume**	**Aliquot Tube**	**Storage**
STEMCELL Technologies	100-0130	36mL	50 mL conical tube	4°C

[0301] Obtain StemSpan AOF bottle(s).

[0302] Using 50 mL serological pipette Aliquot 36 mL of StemSpan AOF into 50 mL conical tubes. Determine the number of 36 mL aliquots prepared and record on BPR. Assign expiration date indicated on the manufacturer's CoA Attach a picture of the label to the BPR. Place labels onto the 50 mL conical tubes. Perform label reconciliation.

[0303] Store the aliquots in a rack and place in a 4°C refrigerator. Record the location of the aliquots on the BPR.

STEMSPAN CD34+ EXPANSION SUPPLEMENT ALIQUOT PREPARATION

Manufacturer	**Catalog #**	**Aliquot Volume**	**Aliquot Tube**	**Storage**
STEMCELL Technologies	02691	4mL	15 mL conical tube	-20°C

[0304] Obtain and thaw StemSpan CD34+ Expansion Supplement (10X) at room temperature until just thawed. Mix StemSpan CD34+ Expansion Supplement thoroughly. Aliquot 4 mL of StemSpan CD34+ Expansion Supplement into 15mL conical tubes. Determine the number of 4 mL aliquots prepared and record on BPR. Assign expiration date indicated on the manufacturer's CoA. Add note on label to not re-freeze aliquots. DO NOT RE-FREEZE

[0305] Attach a picture of the label to the BPR.

[0306] Place labels onto the 15 mL conical tubes. Perform label reconciliation. Store the aliquots in a rack and place in a -20°C refrigerator. Record the location of the aliquots on the BPR.

ROCK INHIBITOR (Y-27632) 10 mM STOCK SOLUTION AND ALIQUOT PREPARATION

Manufacturer	**Catalog #**	**Aliquot Volume**	**Aliquot Tube**	**Storage**	**Expiration Date**
Tocris	TB1254-GMP	150 µL	1.5 mL Microcentrifuge Screw-Cap Tube	-20 °C	30 days from date of aliquoting

[0307] Obtain one vial of ROCK Inhibitor (RI) stock. Calculate the volume of Water For Injection (WFI) to resuspend RI at concentration of 10 mM. Use the formula below:

$$Volume\ of\ WFI\ to\ add\ (\mathrm{m}L) = \left(\frac{Mass\ of\ ROCK\ inhibitor\ (mg)}{10\ mM \times Batch\ Molecular\ Weight\ from\ CoA\left(\frac{g}{mol}\right)}\right) \times 1000$$

[0308] **NOTE:** Rock Inhibitor Molecular Weight is 320.26 g/mol.

[0309] Carefully open the lid of RI stock vial and place the lid facing up in the BSC. Add the calculated volume of WFI (see Section 7.2.4) directly to the vial and close the lid. Shake the bottle or vortex vigorously for 1 minute to ensure the product has completely dissolved. Determine the number of 150 µL aliquots prepared and record on the BPR. Prepare an appropriate number of screw-cap microcentrifuge tubes required for the aliquoting. Aliquot RI at 150 µL to each screw-cap microcentrifuge tube. Place designated labels onto the microcentrifuge tubes. Perform label reconciliation (account for box label). Store the aliquots in a designated box in -20 °C freezer. Identify the box with a sample label. Record the location of the box and freezer RB# on the BPR.

CHIR99021 10 mM STOCK SOLUTION AND ALIQUOT PREPARATION

Manufacturer	**Catalog #**	**Aliquot Volume**	**Aliquot Tube**	**Storage**	**Expiration Date**
Tocris / Biotechne	TB4423-GMP	100 µL	1.5 mL Microcentrifuge Screw-Cap Tube	-80 °C	1 month from date of preparation

[0310] Obtain one bottle of CHIR99021 and one aliquot of 3 mL DMSO. Carefully open the lid of CHIR99021 bottle. Label two 1.5 mL microcentrifuge tubes with 'DMSO 1' and 'DMSO 2.' Using a micropipette, transfer 1 mL of DMSO to 'DMSO 1' tube. Using a micropipette, transfer 1150 µL (2 x 575 µL) of DMSO to 'DMSO 2' tube. Using a syringe, transfer the volume of DMSO previously aliquoted into CHIR99021 bottle (10 mg). Swirl CHIR99021 bottle to mix. Remove rubber stopper on CHIR99021 bottle.

[0311] Aliquot 100 µL of resuspended CHIR99021 into 1.5 mL microcentrifuge screw-cap tubes. Determine the number of 100 µL aliquots prepared and record on the BPR. Place designated labels onto the 1.5 mL microcentrifuge screw-cap tubes. Perform label reconciliation (account for box label). Store the aliquots in a designated box in -80 °C freezer. Identify the box with a sample label. Record the location of the box and freezer RB# on the BPR.

GLUTAMAX SUPPLEMENT ALIQUOT PREPARATION

Manufacturer	Catalog #	Aliquot Volume	Aliquot Tube	Storage
Gibco	35050-061	5 mL	15 mL Conical	RT

[0312] Obtain GlutaMAX™ supplement bottle(s). Carefully open the lid of GlutaMAX™ supplement bottle. Using serological pipette Aliquot 5 mL of GlutaMAX™ supplement into 15 mL conical tubes. Determine the number of 5 mL aliquots prepared and record on the BPR. Assign an expiration date as indicated on the manufacturer's certificate.

[0313] Verify label contents to include:, GlutaMAX™ Supplement Aliquot, Batch #, Aliquot Part #, Store at RT, Volume (5 mL), Expiration date (manufacturer expiration date), Preparation Date

[0314] Attach a picture of the label to the BPR. Place labels onto the 15 mL conical tubes. Perform label reconciliation.Store the aliquots in a rack and place in a bin at room temperature. Record the location of the aliquots on the BPR.

β-MERCAPTOETHANOL 5 mM STOCK SOLUTION AND ALIQUOT PREPARATION

Manufacturer	Catalog #	Aliquot Volume	Aliquot Tube	Storage	Expiration Date
Gibco	21985	100 µL	1.5 mL Microcentrifuge Screw-Cap Tube	4°C	1 year from date of preparation

[0315] Obtain 1 β-Mercaptoethanol 55 mM bottle and one 25 mL DPBS -/- aliquot. Transfer reagents and materials to BSC. Label a 15 mL conical tube with '5 mM β-Mercaptoethanol.' Using a micropipette, transfer 1800 µL of DPBS -/- into the microcentrifuge tube labeled '5 mM β-Mercaptoethanol.' Using a micropipette, transfer 180 µL of 55 mM β-Mercaptoethanol into a microcentrifuge tube labeled '5mM β-Mercaptoethanol.' Using a P1000 micropipette, mix by pipetting up and down 2-3 times. Aliquot 100 µL of resuspended 5 mM β-Mercaptoethanol into microcentrifuge tubes. Determine the number of 100 µL aliquots prepared and record on the BPR. Attach a picture of the label to the BPR. Place labels onto the 1.5 mL microcentrifuge tubes. Perform label reconciliation.

[0316] Store the aliquots in a designated box with a sample label attached in 4 °C refrigerator. Record the location of the aliquots and refrigerator RB# on the BPR.

PD0325901 0.5 mM STOCK SOLUTION AND ALIQUOT PREPARATION

Manufacturer	Catalog #	Aliquot Volume	Aliquot Tube	Storage	Expiration Date
Cayman Chemical	13034	275 µL	1.5 mL Microcentrifuge Screw-Cap Tube	-80 °C	1 year from date of preparation

[0317] Obtain one vial of PD0325901 and two aliquots of 3 mL DMSO. Using a micropipette, transfer 4.15 mL of DMSO into a 15 mL conical tube. Carefully open the lid of PD0325901 vial. Reconstitute PD0325901 (1 mg) with 1000 µL of DMSO from the 15 mL conical by pipetting up and down several times in the vial. Close vial and invert to resuspend any PD0325901 that might be on the cap. Rinse sides of vial with DMSO 2 times using P1000 micropipette. Transfer PD0325901 solution to the 15 mL conical tube with 3.15 mL of DMSO and homogenize. Aliquot 275 µL of resuspended PD0325901 into 1.5 mL microcentrifuge screw-cap tubes. Determine the number of 275 µL aliquots prepared and record on the BPR. Attach a picture of the label to the BPR. Place labels onto the 1.5 mL microcentrifuge screw-cap tubes. Perform label reconciliation (account for box label).

[0318] Store the aliquots in a designated box with a sample label attached in -80°C freezer. Record the location of the box and freezer RB# on the BPR.

A-83-01 10 mM STOCK SOLUTION ALIQUOT PREPARATION

Manufacturer	Catalog #	Aliquot Volume	Aliquot Tube	Storage	Expiration Date
Tocris	2939	25 µL	1.5 mL Microcentrifuge Screw-Cap Tube	-80°C	1 month from date of preparation

[0319] Obtain one vial of A-83-01 (10 mg) and one aliquot of 3 mL DMSO. If necessary, pulse spin A-83-01 vial in centrifuge for 20 seconds. Transfer reagents and materials to BCS. Using a micropipette, transfer 2.37 mL of DMSO into a 15 mL conical tube. Carefully open the lid of A-83-01 vial. Reconstitute A-83-01 with the full volume of DMSO from the 15 mL conical by pipetting up and down several times in the A-83-01 vial. Rinse sides of vial with DMSO 2-3 times using a P1000 micropipette. Transfer A-83-01 solution to the 15 mL conical tube. Aliquot 25 µL of resuspended A-83-01 into 1.5 mL microcentrifuge screw-cap tubes. Determine the number of 25 µL aliquots prepared and record on the BPR.

[0320] Attach a picture of the label to the BPR. Place labels onto the 1.5 mL microcentrifuge screw-cap tubes. Perform label reconciliation (account for box label). Store the aliquots in a designated box with a sample label attached in -80 °C freezer. Record the location of the box and freezer RB# on the BPR.

HA-100 10 mM ALIQUOT PREPARATION

Manufacturer	Catalog #	Aliquot Volume	Aliquot Tube	Storage	Expiration Date
Santa Cruz Biotech	203072	300 µL	1.5 mL Microcentrifuge Screw-Cap Tube	-80 °C	1 year from date of preparation

[0321] Obtain one bottle of HA-100 and one aliquot of 3 mL DMSO. Transfer reagents and materials to BCS. Using a micropipette, transfer 1.43 mL of DMSO into a 1.5 mL microcentrifuge screw-cap tube. Carefully open the lid of HA-100 bottle.

[0322] Reconstitute HA-100 bottle with 1000 µL of DMSO. Rinse sides of bottle with the remaining DMSO 2-3 times using a P1000 micropipette. Transfer solution to the microcentrifuge tube. Aliquot 300 µL of resuspended HA-100 into 1.5 mL microcentrifuge screw-cap tubes. Determine the number of 300 µL aliquots prepared and record on the BPR.

[0323] Attach a picture of the label to the BPR. Place labels onto the 1.5 mL microcentrifuge screw-cap tubes. Perform label reconciliation (account for box label). Store the aliquots in a designated box with a sample label attached in -80 °C freezer. Record the location of the box and freezer RB# on the BPR.

Example 5

Preparation of GMP-grade Reprogramming Medium (RM)

[0324] Remove the following items from the 4°C or Room Temperature storage:

- DMEM/F12
- MEM NEAA aliquot
- GlutaMAX aliquot
- β-mercaptoethanol aliquot

[0325] Remove the following items from the -20°C storage:

- N2 Supplement
- B-27 Supplement

bFGF 25 µg

[0326] Remove the following items/aliquots from the -80°C storage:

- PD0325901 0.5 mM aliquot
- CHIR99021 10 mM aliquot
- A-83-01 10 mM aliquot
- HA-100 10 mM aliquot

hLIF 10 µg/mL aliquot

[0327] Once all the aliquots have thawed, place in the mini microcentrifuge and spin down for 10 seconds to ensure all liquid is pooled at the bottom of the tube. Transfer reagents and materials into the BSC .

Preparation of 100 µg/mL bFGF stock solution

[0328] Get a vial with 25 µg bFGF. Lift tab cap and disinfect surface of rubber stopper. Use sterile syringe to get 250µL of sterile water. Puncture the rubber stopper using the needle and add the sterile water

to the vial. Mix gently avoiding foam formation. Transfer the resuspended bFGF to a 1.5 mL microcentrifuge tube labeled 100 µg/mL bFGF, initials and date.

Preparation of GMP RM

[0329] GMP RM is prepared in volumes of 250 mL. In the BSC, use the table below, which shows the volumes to add of the media components to the filter top of a 0.22µm 250mL filter system.

Component Name	**Stock Concentration**	**Final Concentration**	**Volume required for 250mL GMP RM**
DMEM/F12	N/A	N/A	236 mL
MEM NEAA	100X	1%	2.5 mL
GlutaMAX	100X	1%	2.5 mL
N2	100X	1%	2.5 mL
B27	50X	2%	5 mL
bFGF	100 µg/mL	100 ng/mL	250 µL
hLIF	10 µg/mL	10 ng/mL	250 µL
HA-100	10 mM	10 µM	250 µL
PD0325901	0.5 mM	0.5 µM	250 µL
CHIR99021	10 mM	3 µM	75 µL
A-83-01	10 mM	0.5 µM	12.5 µL
β-mercaptoethanol	5 mM	0.1 µM	5 µL

[0330] Verify the volumes of each component added on MFG-FORM-082 or corresponding BPR. Filter the solution using the in-house vacuum system. Aliquot 10 mL of filtered media into light sensitive 15 mL conical tubes and record the number of tubes aliquoted and volume. Alternatively, aliquots of bigger volumes may be prepared. Record volume and number of aliquots.

[0331] Aliquot 5 mL of filtered media into light sensitive 15 mL conical tube as a retain sample. Clearly label the tube with the label information as well as a designation of "RETAIN SAMPLE".

[0332] Assign a 3-week expiration date. Record the date.

[0333] Label aliquot tubes with: GMP RM, Volume of aliquot, Lot Number, if applicable, Preparation Date, Operator Initials, Expiration Date (3 weeks from preparation date), Storage Conditions, 4°C

[0334] Perform label reconciliation. Transfer to 4°C for storage and record storage location in MFG-FORM-082 or BPR. Transfer retain sample to Quality Control Laboratory.

[0335] Discard all remaining media and reagent components from manufacturing suite.

Example 6

Coating of Plates and Flasks with Laminin CT521

PREPARATION OF REAGENTS AND MATERIALS

[0336] Determine the number of plates/vessels and wells/plate to be coated. Record type of plates/vessels and number on BPR.

[0337] The ratios between Laminin CT521 and DPBS 1X +/+ appear in Table 2 and Table 2, depending on the desired Laminin concentration. Use these as a reference when calculating required volumes.

Table 2. Volumes Needed for Multiple Plate/Flasks Sizes Coated with 1x Laminin Concentration

Plate/Flask Size	Coating Solution		Total Coating Solution Volume
	Laminin Stock (100 µg/mL)	DPBS 1X +/+	
6-well	200 µL/well	1800 µL/well	2000 µL/well
12-well	100 µL/well	900 µL/well	1000 µL/well
T75	1.6 mL	14.4 mL	16 mL
T175	3.8 mL	34.2 mL	38 mL
T225	4.9 mL	44.1 mL	49 mL

Table 3. Volumes Needed for Multiple Plates/Flasks Coated with 2x Laminin Concentration

Plate/Flask Size	Coating Solution		Total Coating Solution Volume
	Laminin Stock (100 µg/mL)	DPBS 1X+/+	
12-well	200 µL/well	800 µL/well	1000 µL/well

[0338] Calculate the volumes needed of Laminin CT521 and DPBS 1X +/+ using Table 4 or Table 5.

[0339] Note: Table 4 and Table 5 preserve the required ratio between the two necessary stock solutions and factors in a pipetting error margin thereby accounting for the natural volume loss that occurs when working with viscous solutions.

Table 4. Volumes Needed for Plate/Flasks Sizes Coated with 1 x Laminin Concentration

	# of Well or Vessels [a]	Error Margin for Vessel [b]	Volume of Laminin stock (100 µg/mL) /Vessel [c]	Total Volume Laminin stock (100 µg/mL) Required Per Vessel [a] x [b] x [c]	DPBS 1X +/+ Volume Per Vessel [d]	Total Volume DPBS, 1X +/+ Required [a] x [b] x [d]
2/12 plate	(1)	1.1	100 µL/well	(2)	900 µL/well	(3)
6/12 plate		1.1	100 µL/well		900 µL/well	

12/12 plate		1.1	100 µL/well		900 µL/well	
2/6 plate		1.1	200 µL/well		1800 µL/well	
3/6 plate		1.1	200 µL/well		1800 µL/well	
4/6 plate		1.1	200 µL/well		1800 µL/well	
6/6 plate		1.1	200 µL/well		1800 µL/well	
T-75 Flask		1.1	1.6 mL		14.4 mL	
T-175 Flask		1.1	3.8 mL		34.2 mL	
T-225 Flask		1.1	4.9 mL		44.1 mL	
Total Volume Laminin C521 stock (100 µg /mL) [mL]				(4)	Total Volume DPBS 1X +/+ [mL]	(5)

(1) Use this column to enter the number of vessels [a] of each type to be coated.
(2) Use this column to record the results of [a] x [b] x [c] = total volume of Laminin CT521 Stock Solution (100 µg/mL) required to coat the number of wells/vessels of each type.
(3) Use this column to record the results of [a] x [b] x [d] = total volume of DPBS, 1X +/+ required to coat the number of wells/vessels of each type.
(4) Use this field to record total volume of Laminin CT521 Stock Solution (100 µg/mL) required resulting from the sum of the volumes listed in this column.
(5) Use this field to record total volume of DPBS 1X +/+ required resulting from the sum of the volumes listed in this column.

Table 5. Volumes Needed for Multiple Plate/Flasks Sizes Coated with 2x Laminin Concentration

	# of Well or Vessels **[a]**	Error Margin for Vessel **[b]**	Volume of Laminin stock (100 µg/mL) /Vessel **[c]**	Total Volume Laminin stock (100 µg/mL) Required Per Vessel **[a] x [b] x [c]**	DPBS 1X +/+ Volume Per Vessel **[d]**	Total Volume DPBS 1X +/+ Required **[a] x [b] x [d]**
6/12 plate – 2X	(1)	1.1	200 µL/well	(2)	800 µL/well	(3)
Total Volume Laminin C521 stock (100ug/mL) [mL]				(4)	Total Volume DPBS 1X +/+ [mL]	(5)

(1) Use this column to enter the number of vessels **[a]** of each type to be coated.
(2) Use this column to record the results of **[a] x [b] x [c]** = total volume of Laminin CT521 Stock Solution (100 µg/mL) required to coat the number of wells/vessels of each type.
(3) Use this column to record the results of **[a] x [b] x [d]** = total volume of DPBS, 1X +/+ required to coat the number of wells/vessels of each type.

(4) Use this field to record total volume of Laminin CT521 Stock Solution (100 µg/mL) required resulting from the sum of the volumes listed in this column. (5) Use this field to record total volume of DPBS 1X +/+ required resulting from the sum of the volumes listed in this column.

[0340] Remove the Laminin CT521 from the -80 °C storage and let it thaw at room temperature for 30 minutes. Record start and end time on BPR.

LAMININ CT521 COATING PROCESS

[0341] The following procedure can be performed to coat either 1x Laminin Plates (using the values from Tables 1 and 3) or 2x Laminin Plates (using the values from Tables 2 and 4) at one time. 1x and 2x Laminin Plates cannot be prepared at the same time by preparing a bulk solution as the ratios will not remain consistent. 1x Laminin Plates will be prepared following MPR 84. 2x Laminin Plates will be prepared following MPR 170.

[0342] Disinfect BSC. Transfer materials/consumables needed.

[0343] Add the appropriate volume of DPBS 1X +/+ (calculated as box-(5) as described in Section 7.2.2) to an appropriately sized conical tube. Record the volume on BPR. Gently pipette the Laminin stock several times to mix.

[0344] Using either a micropipette (for volumes less than 5 mL) or a 5 mL serological pipette (for volumes 5 mL or larger), gently add the appropriate volume of pre-thawed Laminin CT521 stock solution (100 µg /mL) (calculated as box-(4) as described in Section 7.2.2) to the DPBS in Section [0343] . Record the volume on BPR.

[0345] Using a serological pipette, mix the Laminin CT521-DPBS solution pipetting up and down several times gently. Using an appropriately sized pipette, transfer the required volume of Laminin CT521-DPBS solution to the well or vessel of choice (refer to Table 2 or Table 3 for the total required volume). When adding laminin solution to plates, use only a 5- or 10-mL serological pipette. If adding laminin solution to flask, use only a 10- or 25-mL serological pipette. If coating only a limited number of wells per plate, follow the indications described in Figure 1. Gently swirl and/or rock the plate(s)/flask(s) to coat the entire surface.

[0346] **NOTE:** A well/flask that is not fully coated shouldnot be used.

[0347] Seal the plate(s) or flask(s) with Parafilm to prevent evaporation and label the plates with for example, the following information: Plate Laminin Coated – Vessel Type [x/y] (where x denotes the number of wells coated on the plate and y denotes the number of wells on the plate, where applicable); Batch number, if applicable; Date; Operator Initials; Storage Conditions: 4 °C; Expiration Date: 4 weeks from coating date; Record the number of each vessel format prepared on BPR.

[0348] If the plates/flasks are going to be used the same day, incubate in an incubator at 37 °C for 2 hours. Record incubator RB number, start and end time on identify incubator in the BPR.

[0349] If the plates/flask are not going to be used the same day, transfer to the refrigerator at 4°C. Sealed coated plates/flasks are stable up to four weeks (28 days) at 4°C. Mention expiry date / use by date on the plates. Record refrigerator RB number and location on the BPR.

Example 7

Single cell RNA-sequencing

[0350] Single-cell RNA sequencing (scRNA-seq) is a powerful technology that enables the measurement of transcriptomes at the resolution of individual cells. It is a powerful technique that allows researchers to analyze gene expression profiles at the individual cell level. This method provides unprecedented resolution for understanding cellular heterogeneity, identifying rare cell populations, and tracking cellular differentiation processes.

[0351] We used the 10X Genomics Chromium system, which you used, employs a droplet-based approach for scRNA-seq. Here's a brief overview of the process:

- Single cells are encapsulated in Gel Beads in Emulsion (GEMs) along with barcoded oligonucleotides.
- Within each GEM, cell lysis occurs, and mRNA is captured and barcoded.
- The barcoded cDNA is amplified and used to prepare sequencing libraries.
- Libraries are sequenced using next-generation sequencing platforms.

[0352] Described herein is a brief overview of the process and how it is used to identify gene markers and cluster cells using methods like UMAP, particularly in the context of the 10x Genomics platform:

[0353] **Single-Cell Isolation and Library Preparation (10x Genomics):** Thousands of individual cells are encapsulated into microdroplets along with uniquely barcoded beads. Each bead carries a unique oligonucleotide barcode. Within each droplet, polyadenylated mRNA from a single cell is captured on the barcoded bead, followed by reverse transcription to generate cDNA. This barcode identifies all transcripts that originate from the same cell. After breaking the emulsion, the cDNA is amplified, and sequencing libraries are prepared. Each resulting library contains transcript information linked to an individual cell.

[0354] **Sequencing and Data Processing:** Libraries are sequenced, typically on Illumina platforms. Raw reads are demultiplexed using the 10x Genomics Cell Ranger pipeline, which aligns reads to a reference genome, quantifies gene expression, and outputs a cell-by-gene expression matrix.

[0355] **Identifying Marker Genes:** Expression data are normalized to account for differences in sequencing depth and other technical variations. Marker genes for clusters or specific cell types are identified by comparing the expression profiles of groups of cells (e.g., cluster 1 vs. all other cells). After sequencing, the data is processed to generate a gene expression matrix for each cell. This matrix is then

used to identify marker genes that characterize different cell populations. High expression (and specificity) of a gene within a cluster suggests it may be a marker for that cell population. Known markers can help assign identities to clusters (e.g., T cells, neurons, epithelial cells, etc.), while novel markers may suggest new cell subtypes or states. Differential expression analysis is performed between clusters or cell types. Genes that are significantly upregulated in a specific cluster compared to others are considered potential markers. The FindAllMarkers() function in Seurat or Loupe Browser, that are popular packages for scRNA-seq analysis

[0356] **Clustering and UMAP Analysis**: Dimensionality reduction is used. Uniform Manifold Approximation and Projection (UMAP) is a dimensionality reduction technique widely used for visualizing and clustering scRNA-seq data. Given that the data can include thousands of genes and thousands of cells, techniques like principal component analysis (PCA) followed by Uniform Manifold Approximation and Projection (UMAP) are used to project the high-dimensional data into a lower-dimensional space. UMAP creates a two-dimensional (or sometimes three-dimensional) representation that preserves local and global similarities between cells. Cells with similar expression profiles cluster together in this reduced space. The UMAP plot is used to visualize how cells group based on their gene expression, and each cluster can be annotated based on known or newly This allows for visualization of cellular relationships and identification of distinct cell populations.

scRNA-Seq Sample Prepration, Library Preparation, Sequencing and Bioinformatics

[0357] Single cell suspensions were stained with ViaStain AOPI Staining Solution (Nexcelom Bioscience, Lawrence, MA) and imaged on an EVOS M7000 Imaging System (Thermo Fisher Scientific, Waltham, CA) to determine cell suspension concentration and cell viability. As per the Fixation of Cells & Nuclei for Chromium Fixed RNA Profiling Demonstrated Protocol (10x Genomics, CG000478), $1x10^6$ cells per sample with a viability >80% were fixed at 4°C using the Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit (10x Genomics, Pleasanton, California). Fixed cells were quenched of enzymatic activity, processed for long-term storage at –80°C, and stored for ~1 month.

[0358] Following the Chromium Fixed RNA Profiling Reagents Kit for Multiplexed Samples User Guide (10x Genomics; CG000527), stored samples were thawed and 300,000 cells were used for probe hybridization for 24 hours, using one probe barcode per sample using the Chromium Fixed RNA Kit, Human Transcriptome kit. Post-hybridization wash was performed on individual samples, which were then pooled using an equal number of cells for each sample for a target capture of 10,000 cells per sample. Gel beads-in-EMulsion (GEMs) were generated on the Chromium X Controller (10x Genomics). Cell barcoding, GEM recovery, pre-amplification PCR, and gene expression library construction were performed following the manufacturer's user guide. Libraries were quantified using a Qubit fluorometer (Thermo Fisher Scientific) and library size was measured via the 4200 TapeStation (Agilent Technologies, Santa Clara, CA). Libraries were sequenced on a 10B 200 cycle kit on the NovaSeq X Plus (Illumina, San

Diego, CA) as per the Chromium Fixed RNA Profiling Reagents Kit for Multiplexed Samples User Guide, with a sequencing depth of 40,000 reads/cell.

[0359] Cell Ranger multi v8.0.0 (10X Genomics) was used for barcode identification, read alignment, and UMI quantification with default parameters and aligning to the human reference genome GRCh38. Count matrix from each sample were filtered to exclude low-quality cells and doublets, based on the following criteria: gene counts between 150 and 10,000, total UMI counts between 0 and 50,000, mitochondrial gene expression between 0% and 10%, and a doublet score of $\leq$0.5. UMAP plots were constructed based on gene expression counts, total UMI counts, and mitochondrial gene expression counts. The filtered cells from all samples were then integrated into a unified dataset and analyzed using python package Scanpy. The unified dataset was normalized and log transformed using normalized_total() and log1p() respectively. Dimension reduction and batch correction were done using ScVI. Neighbors was computed using scVI latent representation using neighbors(). Leiden clustering with latent representation from scVI and resolution of 0.3 was subsequently performed to identify cell populations.

[0360] To validate cell type identities, known marker genes from the literature were visualized using a dot plot. Differential gene expression analysis was conducted to compare each cluster against all other cells, highlighting genes that were significantly upregulated in each cluster. The cluster identities were further confirmed through enrichment analysis using Enrichr (Enrichr).

[0361] Single cell RNA-sequencing was performed on sample data sets from cord blood post wash, cord blood post enrichment, reprogrammed mix of fully reprogrammed, partially reprogrammed and unreprogrammed of cord blood ICF cells, and bona-fide established iPSCs (cord blood-derived).

[0362] iPSCs that have been expanded for 4 to 6 passages were characterized. These iPSCs are at about day 45 to day 65 post reprogramming; and 28 to 42 days pot clone isolation.

[0363] UMAP clustering of single cell data separates distinct sample sets and distinct clusters are found across samples. Dot plot of markers across clusters are show in Figure 6.

[0364] CD genes expressed in the iPSCs include but are not limited to CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, and CD326. (Figures 7-15.)

[0365] Novel genes expressed uniquely in iPSCs include but are not limited to TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, and COBL. (Figures 16-25)

[0366] Known pluripotency genes expressed in the various data sets include but are not limited to GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, and PODXL (CD34 sialomucin). (Figures 26-30.)

[0367] Known blood and hematopoietic stem/ progenitor marker genes expressed include but are not limited to CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, and PTPN22. (Data not shown.)

[0368] Various embodiments of the invention are described above in the Detailed Description. While these descriptions directly describe the above embodiments, it is understood that those skilled in the art may conceive modifications and/or variations to the specific embodiments shown and described herein. Any such modifications or variations that fall within the purview of this description are intended to be included therein as well. Unless specifically noted, it is the intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s).

[0369] The foregoing description of various embodiments of the invention known to the applicant at this time of filing the application has been presented and is intended for the purposes of illustration and description. The present description is not intended to be exhaustive nor limit the invention to the precise form disclosed and many modifications and variations are possible in the light of the above teachings. The embodiments described serve to explain the principles of the invention and its practical application and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed for carrying out the invention.

[0370] While particular embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that, based upon the teachings herein, changes and modifications may be made without departing from this invention and its broader aspects and, therefore, the appended claims are to encompass within their scope all such changes and modifications as are within the true spirit and scope of this invention. As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are useful to an embodiment, yet open to the inclusion of unspecified elements, whether useful or not. It will be understood by those within the art that, in general, terms used herein are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). Although the open-ended term "comprising," as a synonym of terms such as including, containing, or having, is used herein to describe and claim the invention, the present invention, or embodiments thereof, may alternatively be described using alternative terms such as "consisting of" or "consisting essentially of."

[0371] Unless stated otherwise, the terms "a" and "an" and "the" and similar references used in the context of describing a particular embodiment of the application (especially in the context of claims) may be construed to cover both the singular and the plural. The recitation of ranges of values herein is

merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein may be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (for example, "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the application and does not pose a limitation on the scope of the application otherwise claimed. The abbreviation, "e.g." is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example." No language in the specification should be construed as indicating any non-claimed element essential to the practice of the application.

[0372] "Optional" or "optionally" means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not.

[0373] Groupings of alternative elements or embodiments of the present disclosure disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs), comprising:

(a) providing the ICF;

(b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising: one or more plasmids encoding MYCL, LIN28, POU5F1, p53 shRNA, SOX2, LTAg, and KLF4, and optionally, EBNA1;

(c) adding a cell suspension having the ICF to (i) each well of a multi-well plate, wherein the multi-well plate was previously coated with CT521 laminin, or (ii) a plate, wherein the plate was previously coated with CT521 laminin, or (iii) a flask, wherein the flask was previously coated with CT521 laminin;

(d) incubate the multi-well plate, the plate, or the flask at about 32°C-42°C and about 3-7% CO_2; and

(e) feeing the cells beginning on day 2-4.

2. The method of claim 1, wherein reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs), comprises:

(a) providing the ICF;

(b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising:

a first plasmid encoding MYCL-LIN28A, EBNA1,

a second plasmid encoding POU5F1, p53 shRNA, EBNA1,

a third plasmid encoding EBNA1,

a fourth plasmid encoding SOX2-KLF4, EBNA1, and

a fifth plasmid encoding POU5F1-SOX2-LTAg-KLF4, EBNA1;

(c) adding the cell suspension to each well of a multi-well plate, wherein the multi-well plate was previously coated with CT521 laminin;

(d) incubate the multi-well plate at about 32°C-42°C and about 3-7% CO_2;

(e) feeing the cells beginning on day 2-4.

3. The method of claim 1, wherein reprogramming an isolated cell fraction (ICF) from cord blood units (CBUs), comprises:

(a) providing the ICF;

(b) delivering by nucleofection to the ICF a plasmid mixture, the plasmid mixture comprising:

a first plasmid encoding MYCL-LIN28A, EBNA1,

a second plasmid encoding POU5F1, p53 shRNA, EBNA1,

a third plasmid encoding EBNA1,

a fourth plasmid encoding SOX2-KLF4, EBNA1, and

a fifth plasmid encoding POU5F1-SOX2-LTAg-KLF4, EBNA1;

(c) adding the cell suspension to each well of a 12 well plate, wherein the 12 well plate was previously coated with CT521 laminin;

(d) incubate the 12 well plate at about 37°C and about 5% CO_2;

(e) feeing the cells beginning on day 3.

4. The method of any one of claims 1-3, wherein CT521 laminin coated multi-well plate, plate, or flask are made by applying CT521 laminin to the plate, and allowing the plates to coat for at least 8 hours at 2°C-8°C.

5. The method of any one of claims 1-3, wherein CT521 laminin coated multi-well plate, plate, or flask are prepared at least 24 hours before adding the cell suspension and up to 30 days before adding the cell suspension.

6. A method of feeding isolated cell fraction undergoing episomal reprogramming, comprising:

(i) providing isolated cell fraction (ICF) undergoing episomal reprogramming ("cells"), or performing the methods of any one of claims 1-5;

(ii) feeding the cells day 2-4 after nucleofection, by adding mTeSR Plus media or GMP-grade reprogramming media to each well having the ICF undergoing episomal reprogramming;

(iii) feeding the cells on day 3 - day 10 after nucleofection, by removing spent media from each well and adding mTeSR Plus media or GMP-grade reprogramming media to each well;

(iv) feeding the cells on day 9 - day 18 after nucleofection, by removing spent media from each well and adding mTeSR Plus media or GMP-grade reprogramming media to each well;

(v) feeding the cells on day 14 – day 40 after nucleofection, by removing spent media from each well and adding mTeSR Plus media or GMP-grade reprogramming media to each well , wherein in step (v) the cells are cultured for 14-40 days.

7. The method of claim 6, wherein feeding isolated cell fraction undergoing episomal reprogramming, comprises:

(i) providing isolated cell fraction (ICF) undergoing episomal reprogramming ("cells"), or performing the methods of any one of claims 1-5;

(ii) feeding the cells day 2-4 after nucleofection, by adding 0.25-0.75 mL of mTeSR Plus media or GMP-grade reprogramming media to each well having the ICF undergoing episomal reprogramming;

(iii) feeding the cells on day 3 - day 10 after nucleofection, by removing spent media from each well and adding 0.5-1.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well;

(iv) feeding the cells on day 9 - day 18 after nucleofection, by removing spent media from each well and adding 0.5-1.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well or adding 1.5-2.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well;

(v) feeding the cells on day 14 – day 40 after nucleofection, by removing spent media from each well and adding 0.5-1.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well or adding 1.5-2.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well, wherein in step (v) the cells are cultured for 14-40 days.

8. The method of claim 6, wherein feeding isolated cell fraction undergoing episomal reprogramming, comprises:

(i) providing isolated cell fraction (ICF) undergoing episomal reprogramming ("cells"), or performing the methods of any one of claims 1-5;

(ii) feeding the cells day 3 after nucleofection, by adding 0.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well having the ICF undergoing episomal reprogramming;

(iii) feeding the cells on day 4-day 9 after nucleofection, by removing spent media from each well and adding 1 mL of mTeSR Plus media or GMP-grade reprogramming media to each well;

(iv) feeding the cells on day 11- day 16 after nucleofection, by removing spent media from each well and adding 1 mL of mTeSR Plus mediaor GMP-grade reprogramming media to each well or adding 2 mL of mTeSR Plus media or GMP-grade reprogramming media to each well;

(v) feeding the cells on day 17-day 40 after nucleofection, by removing spent media from each well and adding 1 mL of mTeSR Plus media to each well or adding 2 mL of mTeSR Plus media to each well, wherein in step (v) the cells are cultured for 17-40 days.

9. The method of any one of claims 7 or 8, wherein in step (iv) or step (v), when adding 1.5-2.5 mL of mTeSR Plus media or GMP-grade reprogramming media to each well or when adding 2 mL of mTesR Plus media or GMP-grade reprogramming media to each well, feeding the cells can skip one or two days until the next feeding.

10. The method of any one of claims 6-9, wherein

in step (ii), adding the mTesR Plus media or GMP-grade reprogramming media comprises adding the mTesR Plus media or GMP-grade reprogramming media at a rate of 1-4 minutes per 6 wells of a 12 well plate;

in step (iii), removing spent media and adding the mTesR Plus media or GMP-grade reprogramming media is performed at a rate of up to 9 minutes per 6 wells of a 12 well plate;

in step (iv), removing spent media and adding the mTesR Plus media or GMP-grade reprogramming media is performed at a rate of up to 7 minutes per 6 wells of a 12 well plate;

in step (v), adding the mTesR Plus media comprises adding the mTesR Plus media at a rate of 1-4 minutes per 6 wells of a 12 well plate.

11. The method of any one of claims 6-9, wherein

in step (ii), adding the mTesR Plus media or GMP-grade reprogramming media comprises adding the mTesR Plus media or GMP-grade reprogramming media at a rate of 2-3 minutes per 6 wells of a 12 well plate;

in step (iii), removing spent media and adding the mTesR Plus media or GMP-grade reprogramming media is performed at a rate of up to 7 minutes per 6 wells of a 12 well plate;

in step (iv), removing spent media and adding the mTesR Plus media or GMP-grade reprogramming media is performed at a rate of up to 5 minutes per 6 wells of a 12 well plate;

in step (v), removing spent media and adding the mTesR Plus media is performed at a rate of 2-3 minutes per 6 wells of a 12 well plate.

12. A method of isolating iPSC clones from a reprogramming plate, comprising:

(i) feeding the iPSC clones from the reprogramming plate; and

(ii) manually isolating the iPSC clones from the reprogramming plate.

13. A method of isolating iPSC clones from a reprogramming plate, comprising:

(i) feeding the iPSC clones from the reprogramming plate;

(ii) enzymatically isolating the iPSC clones.

14. The method of claim 12 or claim 13, wherein feeding the iPSC clones from the reprogramming plate comprises:

changing media in all wells of a reprogramming plate having one or more colonies, or low-density seeded plate having one or more colonies by removing spent median and replacing with mTeSR Plus per well;

selecting a colony that is not physically in contact with another colony, preferably the selected colony is compact and have morphology similar to iPSC colonies.

15. The method of claim 14, wherein replacing with mTeSR Plus per well comprises replacing with 1 mL of mTeSR Plus per well.

16. The method of claim 12, wherein manually isolating the iPSC clones from the reprogramming plate comprises:

cutting and transferring the selected colony to a new Laminin CT521 coated plate, wherein the laminin has been removed from the coated plate and replaced with mTeSR Plus media;

transferring the plate to 35-39°C CO_2 incubator until the next feeding;

rocking the new plate back and forth and side to side to distribute the clumps.

17. The method of claim 12, wherein manually isolating the iPSC clones from the reprogramming plate comprises:

cutting and transferring the selected colony to a new Laminin CT521 coated plate, wherein the laminin has been removed from the coated plate and replaced with 1 mL of mTeSR Plus media per well;

transferring the plate to 37°C CO_2 incubator until the next feeding;

rocking the new plate back and forth and side to side to distribute the clumps.

18. The method of claim 17, wherein cutting and transferring the selected colony comprises:

cutting the selected colony into small pieces;

nudging clump pieces off the bottom of the well;

transferring the floating pieces to the well containing mTeSR Plus media in the new laminin coated plate.

19. The method of claim 17, wherein cutting and transferring the selected colony comprises:

using an insulin syringe needed to cut the selected colony into small pieces;

using a P1000 tip inside a P20 tip to nudge clump pieces off the bottom of the well;

using a P200 micropipette to transfer the floating pieces to the well containing mTeSR Plus media in the new laminin coated plate.

20. The method of claim 13, wherein enzymatically isolating the iPSC clones comprises:

(a) removing spent media from each well from the reprogramming plate;

(b) adding DPBS-/- to each well;

(c) aspirating the DPBS-/-;

repeating step (c);

(d) adding TrypLE CTS and transferring the reprogramming plate to the incubator and incubate at 37°C for about 5-10 minutes;

(e) gently pipette up and down to break up the clumps;

(f) adding mTeSR plus to the well to neutralize the TrypLE CTS;

(g) transferring cell suspensions to a tube;

repeating steps (d)-(g) for each well and pool into the same tube;

(h) centrifuge the tube at 200xG-400xG for about 5 minutes at room temperature;

(i) removing supernatant from the tube without disturbing the cells pellet;

(j) dislodging the pellet by flicking the tub;

(k) resuspending the pellet in mTeSR Plus with Rock inhibitor;

(l) adding mTeSR Plus with Rock inhibitor;

(m) transferring well mixed cell suspension to a tube.

21. The method of claim 20, further comprising performing a cell count.

22. The method of claim 20, further comprising transferring a volume of cells comprising about 10,000-30,000 cells to each well of a multi-well plate and rocking the 6 well plate in an incubator at about 37°C and 5% CO_2 to distribute the cells evenly.

23. The method of claim 20, further comprising:

(n) adding MoxiCyte viability reagent to cell suspension, mix and incubate in the dark for about 2-7 minutes;

(o) mixing the cell suspension in the tube;

(p) transferring a volume of cells comprising about 10,000-30,000 cells to each well of a multi-well plate;

(q) rocking the 6 well plate in an incubator at about 37°C and 5% CO_2 to distribute the cells evenly.

24. The method of claim 13, wherein enzymatically isolating the iPSC clones comprises:

(a) removing spent media from each well from the reprogramming plate;

(b) adding about 1 mL of DPBS-/- to each well;

(c) aspirating the DPBS-/-;

repeating step (c);

(d) adding about 1 mL of TrypLE CTS and transferring the reprogramming plate to the incubator and incubate at about 37°C for about 7 minutes;

(e) gently pipette up and down using a P1000 micropipette to break up the clumps;

(f) adding about 1 mL of mTeSR plus to the well to neutralize the TrypLE CTS;

(g) transferring cell suspensions to a 15 mL conical tube;

repeating steps (d)-(g) for each well and pool into the same 15 mL conical tub;

(h) centrifuge the conical tube at about 300x g for about about 5 minutes at room temperature;

(i) removing supernatant from the conical tube without disturbing the cells pellet;

(j) dislodging the pellet by flicking the tub;

(k) resuspending the pellet in about 1 mL of mTeSR Plus with Rock inhibitor;

(l) adding mTeSR Plus with Rock inhibitor to bring the volume to about 4 mL per well dissociated; and

(m) transferring about 20 uL of well mixed cell suspension to a 1.5 mL microcentrifuge tube.

25. The method of claim 24, further comprising performing a cell count.

26. The method of claim 24, further comprising transferring a volume of cells comprising about 10,000-30,000 cells to each well of a multi-well plate and rocking the 6 well plate in an incubator at about 37°C and 5% CO_2 to distribute the cells evenly.

27. The method of claim 24, further comprising

(n) adding 180 uL of MoxiCyte viability reagent to cell suspension, mix and incubate in the dark for about 5 minutes;

(o) mixing the cell suspension in the 15 mL conical tube;

(p) transferring a volume of cells comprising about 20,000 cells to each well of a 6 well plate;

(q) rocking the 6 well plate in an incubator at about 37°C and about 5% CO_2 to distribute the cells evenly.

28. The method of any one of claims 12-27, further comprising passaging the cells.

29. The method of any one of claims 12-27, wherein after 3 passages, iPSC appear.

30. The method of any one of claims 12-27, wherein iPSCs appear in less than 10 passages.

31. The method of any one of claim 12-30, wherein the volume of cells is calculated by using the following formula

$$cell\ suspension\ volume\ (ml) = \frac{20{,}000\ cells}{average\ viable\ cell\ concentration}$$

32. The method of any one of claim 12-31, wherein mTeSR Plus with 10 µM ROCK comprises

$$volume\ of\ 10\ mM\ ROCK\ Inhibitor\ stock\ (uL) = \frac{10\ uM\ x\ volume\ of\ mTeSR\ Plus\ (mL)}{10\ mM}.$$

33. Induced pluripotent stem cells (iPSCs) reprogrammed from isolated cell fraction (ICF) from cord blood units (CBUs),

wherein the iPSCs express one or more genes selected from CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, or CD326, or

wherein the iPSCs express one or more genes selected from TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, or COBL, or

wherein the iPSCs express one or more genes selected from GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, or PODXL (CD34 sialomucin).

34. The iPSCs of claim 33, wherein the iPSCs are generated by any one of the methods of claim 1-32.

35. A composition comprising iPSCs of claim 33; and cell media.