

CLAIMS

1. A method of reprogramming a non-pluripotent cell to generate a pluripotent cell, a cell line or a population thereof, comprising:

(a) introducing to a first cell, in which the first cell is a non-pluripotent cell:

5 one or more first plasmids, in which the first plasmid comprises a replication origin, and a polynucleotide encoding one or more reprogramming factors but does not encode an EBNA or a derivative thereof; in which at least one of the one or more first plasmids comprises a polynucleotide encoding OCT4; in which the introduction of one or more first plasmids induces reprogramming process; and
10 optionally,

one of: (1) a second plasmid comprising a nucleotide sequence encoding an EBNA, in which the second plasmid does not comprise a replication origin or polynucleotide(s) encoding reprogramming factor(s); (2) an EBNA mRNA; and
(3) an EBNA protein;

15 (b) culturing the cells from step (a) to generate a second cell, in which the second cell is a reprogramming cell, in which the reprogramming cell comprises a morphological change from the first cell and is essentially free of EBNA; in which the reprogramming cell does not comprise:

(1) pluripotent cell morphology; and

20 (2) endogenous OCT4 expression;

and,

(c) further culturing the second cell obtained in step (b) for a sufficient amount of time to generate a pluripotent cell.

2. A method according to Claim 1, in which step (a) comprises introducing to the first cell
25 a second plasmid comprising a nucleotide sequence encoding an EBNA, in which the second plasmid does not comprise a replication origin or polynucleotide(s) encoding reprogramming factor(s).

3. A method according to Claim 1, further comprising one or more of the following:

(i) dissociating the pluripotent cells to obtain single cell dissociated pluripotent cells;

- (ii) suspending the single cell dissociated pluripotent cells;
- (iii) sorting the single cell dissociated pluripotent cells by selecting and isolating the cells expressing one or more pluripotency markers to enrich for pluripotent cells expressing the marker(s); or
- 5 (iv) culturing the pluripotent cell in the presence of a GSK3 inhibitor, a MEK inhibitor and a ROCK inhibitor to maintain pluripotency, in which the pluripotent cell maintains pluripotency for at least 5, 10, 15, or 20 passages.
- 4. A method according to Claim 1, in which:
 - (i) culturing in step (b) comprises culturing in the presence of at least one of a TGF β inhibitor, a GSK3 inhibitor, a MEK inhibitor and a ROCK inhibitor;
 - 10 (ii) the first cell is a somatic cell, a progenitor cell, or a multipotent cell;
 - (iii) the first cell is a fibroblast, and in which the morphological change of the second cell comprises MET (mesenchymal to epithelial transition);
 - (iv) the second cell is essentially free of plasmids of step (a);
 - 15 (v) the second cell comprises reprogramming cells of about 4 to 10, 12, 14, 21, 25 days, or to any number of days in-between post the introduction of one or more first plasmids in step (a);
 - (vi) the pluripotent cell has reduced pluripotency reversion or spontaneous differentiation;
 - 20 (vii) the pluripotent cell is essentially free of the polynucleotides of the plasmids of step (a) without the need for selection or extensive passaging the pluripotent cell;
 - (viii) the pluripotent cell has at least one of the properties:
 - (1) high clonality;
 - (2) genetic stability; and
 - 25 (3) ground state pluripotency; and/or
 - (ix) the pluripotent cell comprises reactivated genes associated with extraembryonic cells.
- 5. A method according to Claim 1, in which:

- (i) the second plasmid has a high rate of loss; and/or in which the expression of EBNA is transient and temporal;
- (ii) the replication origin and/or EBNA is EBV-based;
- (iii) the cells are under a feeder-free condition; and/or
- (iv) the ROCK inhibitor is thiazovivin.

6. A reprogramming cell or a population thereof obtained after introducing into a non-pluripotent cell with one or more first plasmids, in which the first plasmid comprises a replication origin, and a polynucleotide encoding one or more reprogramming factors but does not encode an EBNA or a derivative thereof; in which at least one of the one or more first plasmids comprises a polynucleotide encoding OCT4; and optionally with one of:

- (1) a second plasmid comprising a nucleotide sequence encoding an EBNA, in which the second plasmid does not comprise a replication origin or polynucleotide(s) encoding reprogramming factor(s);
- (2) an EBNA mRNA; and
- (3) an EBNA protein;

in which the reprogramming cell comprises a morphological change from the non-pluripotent cell prior to the introduction of the combination of plasmids, and is essentially free of EBNA or the derivative thereof;

in which the reprogramming cell does not comprise:

- (1) pluripotent cell morphology; and
- (2) endogenous OCT4 expression; and

in which the reprogramming cell is capable of establishing stable or self-sustaining pluripotency given a sufficient amount of time to generate a pluripotent cell.

7. A reprogramming cell or a population thereof according to Claim 6, in which:

- (i) the reprogramming cell are about 4 to 10, 12, 14, 21, 25 days, or to any number of days in-between, post the introduction of one or more first plasmids;
- (ii) the reprogramming cell is cultured in the presence of at least one of a TGF β inhibitor, a GSK3 inhibitor, a MEK inhibitor and a ROCK inhibitor;

(iii) the non-pluripotent cell is a fibroblast, and in which the morphological change of the reprogramming cell comprises MET (mesenchymal to epithelial transition);

(iv) the reprogramming cell is essentially free of first and/or second plasmids;

5 (v) the pluripotent cell is essentially free of the polynucleotides of the plasmids without the need for selection or extensive passaging the pluripotent cell;

(vi) the pluripotent cell has reduced pluripotency reversion or spontaneous differentiation;

(vii) the pluripotent cell has at least one of the properties:

10 (1) high clonality;

(2) genetic stability; and

(3) ground state pluripotency; and/or

(viii) the pluripotent cell comprises reactivated genes associated with extraembryonic cells.

15 8. A composition comprising a reprogramming cell or a population thereof according to Claim 6 or 7.

9. A composition according to Claim 8, further comprising a medium comprising a TGF β inhibitor, a GSK3 inhibitor, a MEK inhibitor and a ROCK inhibitor.

20 10. A composition according to Claim 8, in which (i) the ROCK inhibitor is thiazovivin; and/or(ii) the medium is feeder-free.

11. An isolated pluripotent cell or a pluripotent cell line when produced by a method according to any of Claims 1 to 5.

12. A genomically engineered pluripotent cell or a cell line thereof using an isolated pluripotent cell or cell line produced by a method according to any of Claims 1 to 5.

25 13. A derived non-natural cell re-differentiated from an isolated pluripotent cell or cell line according to Claim 11 or from a genomically engineered pluripotent cell or cell line according to Claim 12.

14. A derived non-natural cell according to Claim 13, in which:

(i) the cell is immune-cell like, in which the cell comprises a CD34 cell, a hemogenic endothelium cell, a hematopoietic stem or progenitor cell, a hematopoietic multipotent progenitor cell, a T cell progenitor, an NK cell progenitor, a T cell, a NKT cell, an NK cell, a B cell, or an immune regulatory cell; and/or

5 (ii) the cell is a rejuvenated cell comprising at least one of the following properties: global increase of heterochromatin; improved mitochondrial function; increased DNA damage responses; telomere elongation and decrease of percentage of short telomere; decrease in the fraction of senescent cells; and higher potential for proliferation, survival, persistence, or memory like functions, in comparison to its natural cell
10 counterpart.

15. A composition for therapeutic use comprising a pluripotent cell obtained by a method according to any of Claims 1 to 5, and optionally one or more additional therapeutic agents.

16. A composition for therapeutic use comprising a genomically engineered pluripotent cell according to Claim 12 or a derived non-natural cell according to Claim 13 or 14, and
15 optionally one or more additional therapeutic agents.

17. A composition according to Claim 15 or 16 for use in treating a subject in need thereof.

18. A composition for use in manufacturing a pluripotent cell for application in cell-based therapies, in which the composition comprises a pluripotent cell produced by a method according to any of Claims 1 to 5.

20 19. A composition according to Claim 18, in which the pluripotent cell is allogeneic or autologous.

20. A kit for medicament use comprising a pluripotent cell obtained by a method according to any of Claims 1 to 5.

21. A kit for medicament use comprising a genomically engineered pluripotent cell
25 according to Claim 12 or a derived non-natural cell according to Claim 13 or 14.

22. An *in vitro* system for initiating reprogramming in a non-pluripotent cell, in which the system comprises:

one or more first plasmids, in which the first plasmid comprises a replication origin, and a polynucleotide encoding one or more reprogramming factors but does not encode an

EBNA or a derivative thereof; in which at least one of the one or more first plasmids comprises a polynucleotide encoding OCT4; and optionally one of:

(1) a second plasmid comprising a nucleotide sequence encoding an EBNA, in which the second plasmid does not comprise a replication origin or polynucleotide(s) encoding reprogramming factor(s);

(2) an EBNA mRNA; and

(3) an EBNA protein.

23. A system according to Claim 22, in which the second plasmid has a high rate of loss; and in which the expression of EBNA is transient and temporal.

24. A system according to Claim 22, in which:

(i) the system does not provide EBNA replication and/or continuous expression in the nucleus;

(ii) the system enables a transient/cytoplasmic expression of EBNA for a short duration, and prior to the appearance of pluripotency cell morphology and the induced expression of endogenous pluripotency genes;

(iii) the system enables a transient/cytoplasmic expression of one or more reprogramming factors comprised in first plasmid(s) for a short duration, and prior to the appearance of pluripotency cell morphology and the induced expression of endogenous pluripotency genes;

(iv) the replication origin is selected from the group consisting of a Polyomavirinae virus, a Papillomavirinae virus, and a Gammaherpesvirinae virus;

(v) the replication origin is one selected from the group consisting of SV40, BK virus (BKV), bovine papilloma virus (BPV), or Epstein–Barr virus (EBV);

(vi) the replication origin corresponds to, or is derived from, the wild-type replication origin of EBV;

(vii) the EBNA is EBV-based;

(viii) the one or more first plasmids collectively comprise polynucleotides encoding reprogramming factor(s) comprising one or more of OCT4, SOX2, NANOG, KLF,

LIN28, c-MYC, ECAT1, UTF1, ESRRB, HESRG, CDH1, TDGF1, DPPA4, DNMT3B, ZIC3, and L1TD1;

(ix) the polynucleotides encoding reprogramming factors are comprised in a polycistronic construct or non-polycistronic construct;

5 (x) the polycistronic construct comprises a single open reading frame or multiple open reading frames;

(xi) the system comprises two or more first plasmids, with each first plasmid comprising the same or different reprogramming factors encoded by at least one copy of polynucleotide;

10 (xii) the system comprises two or more first plasmids provides a control of reprogramming factor stoichiometry; and/or

(xiii) the first plasmid comprises more than one polynucleotides encoding reprogramming factors, in which the adjacent polynucleotides are operatively connected by a linker sequence encoding a self-cleaving peptide or an IRES.

15 25. A system according to Claim 22, 23 or 24, in which:

(i) the self-cleaving peptide is a 2A peptide selected from F2A, E2A, P2A and T2A;

(ii) the 2A peptides comprised in a first plasmid construct may be the same or different;

(iii) the 2A peptides in neighbouring positions are different;

20 (iv) the first and the second plasmid each comprises one or more promoters for expression of reprogramming factors and EBNA, and in which the one or more promoters comprise at least one of CMV, EF1 α , PGK, CAG, UBC, and other suitable promoters that are constitutive, inducible, endogenously regulated, or temporal-, tissue- or cell type- specific; and/or

25 (v) the first and the second plasmid each comprises a CAG promoter.

26. A kit comprising a system according to any of Claims 22 to 25.