

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 comprising an Epstein-Barr nuclear antigen (EBNA) binding site, and a polynucleotide encoding one or more reprogramming factors but does not encode an EBNA; in which at least one of the one or more first plasmids comprises a polynucleotide encoding OCT4; in which the introduction of the one or more first
10 plasmids induces reprogramming process; and

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 cell 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; and 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 comprising an EBNA binding site 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) the first cell is a somatic cell, a progenitor cell, or a multipotent cell;
 - 10 (ii) the first cell is a fibroblast, and in which the morphological change of the second cell comprises MET (mesenchymal to epithelial transition);
 - (iii) the second cell is essentially free of plasmids of step (a);
 - (iv) the second cell comprises reprogramming cells of 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
 - 15 step (a);
 - (v) the pluripotent cell has reduced pluripotency reversion or spontaneous differentiation;
 - (vi) 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;
 - 20 (vii) the pluripotent cell has at least one of the properties:
 - (1) high clonality;
 - (2) genetic stability; and
 - (3) ground state pluripotency; and/or
 - (viii) the pluripotent cell comprises reactivated genes associated with extraembryonic
 - 25 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 non-pluripotent cell or a population thereof comprising (a) one or more first plasmids,
 5 in which the first plasmid comprises a replication origin comprising an EBNA binding site,
 and a polynucleotide encoding one or more reprogramming factors, but does not encode an
 EBNA; in which at least one of the one or more first plasmids comprises a polynucleotide
 encoding OCT4; and (b) one of:

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

(2) an EBNA mRNA; and

(3) an EBNA protein;

in which the non-pluripotent cell does not comprise:

15 (1) pluripotent cell morphology; and

(2) endogenous OCT4 expression; and

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

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

20 (i) the non-pluripotent cell are 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 non-pluripotent 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;

25 (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 non-pluripotent cell is essentially free of first and/or second plasmids
 upon culturing;

(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;

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

(1) high clonality;

(2) genetic stability; and

(3) ground state pluripotency; and/or

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

8. A composition comprising a non-pluripotent cell or a population thereof according to Claim 6 or 7.

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

15 10. A composition according to Claim 9, in which (i) the ROCK inhibitor is thiazovivin; and/or(ii) the medium is feeder-free.

11. A method according to any of Claims 1 to 5, in which the pluripotent cell comprises a genomically engineered pluripotent cell or a cell line produced therefrom.

20 12. A method according to any of Claims 1 to 5, in which 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.

13. A method according to any of Claims 1 to 5, further comprising differentiating the pluripotent cell to obtain a derived non-natural cell.

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

25 (i) is an 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

(ii) 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 counterpart.

15. A composition according to any of Claims 8 to 10 for use in preparing a medicament for therapeutic use, in which the medicament comprises a pluripotent cell obtained from the non-pluripotent cell, or a derived non-natural cell differentiated from the pluripotent cell, and optionally one or more additional therapeutic agents.

16. A composition for use according to Claim 15, in which the pluripotent cell is a genomically engineered pluripotent cell.

17. A composition for use according to Claim 15 or 16, in which the pluripotent cell is allogeneic or autologous.

18. 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 comprising an EBNA binding site, and a polynucleotide encoding one or more reprogramming factors but does not encode an EBNA; in which at least one of the one or more first plasmids comprises a polynucleotide encoding OCT4; and one of:

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

(2) an EBNA mRNA; and

(3) an EBNA protein.

19. A system according to Claim 18, in which the second plasmid has a high rate of loss; and in which the expression of EBNA is transient and temporal.

20. A system according to Claim 18, 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 corresponds to, or is derived from, the wild-type replication origin of EBV;
 - (v) the EBNA is EBV-based;
 - (vi) 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;
 - (vii) the polynucleotides encoding reprogramming factors are comprised in a polycistronic construct or non-polycistronic construct;
 - (viii) the polynucleotides encoding reprogramming factors are comprised in a polycistronic construct, and the polycistronic construct comprises a single open reading frame or multiple open reading frames;
 - (ix) 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;
 - (x) the system comprises two or more first plasmids provides a control of reprogramming factor stoichiometry; and/or
 - (xi) 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.
21. A system according to Claim 20, in which:
- (i) the self-cleaving peptide is a 2A peptide selected from F2A, E2A, P2A and T2A;

- (ii) 2A peptides comprised in a first plasmid construct may be the same or different;
- (iii) 2A peptides in neighbouring positions in a first plasmid construct are different;
- (iv) the first and the second plasmid each comprises one or more promoters for expression of reprogramming factors or EBNA, and in which the one or more promoters
5 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
- (v) the first and the second plasmid each comprises a CAG promoter.

- 22. A system according to any of Claims 18 to 21, further comprising a culture medium
10 comprising at least one of a TGF β inhibitor, a GSK3 inhibitor, a MEK inhibitor and a ROCK inhibitor.
- 23. A kit comprising a system according to any of Claims 18 to 22.