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:

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 or an origin-binding derivative thereof; 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 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;

(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

(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 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

(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;

(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 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);

(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;

(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 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, 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 or an origin-binding derivative thereof; 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 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:

(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:

(i) the non-pluripotent 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 non-pluripotent cell is cultured in the presence of at least one of a TGFβ 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 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;

(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 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 TGFβ inhibitor, a GSK3 inhibitor, a MEK inhibitor and a ROCK inhibitor.

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.

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 TGFβ 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:

(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 or an origin-binding derivative thereof; 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 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 18, 19 or 20, 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;

(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 comprise at least one of CMV, EF1α, 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.

21. A system according to any of Claims 18 to 21, further comprising a culture medium comprising at least one of a TGFβ inhibitor, a GSK3 inhibitor, a MEK inhibitor and a ROCK inhibitor.

22. A kit comprising a system according to any of Claims 18 to 21.

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:

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 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;

(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

(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 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

(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;

(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 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;

(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 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, 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 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:

(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:

(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 TGFβ 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 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;

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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 TGFβ inhibitor, a GSK3 inhibitor, a MEK inhibitor and a ROCK inhibitor.

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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.

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

(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.

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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 comprise at least one of CMV, EF1α, 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 comprising at least one of a TGFβ 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.