~~BRIEF~~ SUMMARY

[0009] The present ~~invention~~disclosure is broadly summarized as relating to reprogramming of differentiated primate somatic cells to produce primate pluripotent cells.

[0009a] In one aspect, there is provided a method of reprogramming primate somatic cells, the method comprising the steps of:

introducing at least one non-viral episomal vector encoding one or more potency-determining factors into primate somatic cells, wherein the at least one vector encodes at least potency-determining factors OCT4, SOX2, and at least one of LIN28 and NANOG, wherein the cells express the introduced OCT4, SOX2, and at least one of LIN28 and NANOG, and further comprise SV40 T Antigen, under conditions sufficient to reprogram the cells; and

culturing the exposed cells to obtain reprogrammed cells having a higher potency level than the primate somatic cells, the reprogrammed cells being substantially genetically identical to a post-natal individual and substantially free of any vector component associated with exposing the potency-determining factors to the somatic cells.

[0010] ~~In a first aspect, the invention is summarized in that a~~ A method for producing a primate pluripotent cell as disclosed includes the step of delivering into a primate somatic cell a set of transgenes sufficient to reprogram the somatic cell to a pluripotent state, the transgenes being carried on at least one episomal vector that does not encode an infectious virus, and recovering pluripotent cells. References herein to a "non-viral" vector or construct indicate that the vector or construct cannot encode an infectious virus.

[0011] Also disclosed is ~~In a second aspect, the invention relates to~~ an enriched population of replenishable reprogrammed pluripotent cells of a primate, including a human primate, wherein, in contrast to existing iPS cells, the at least one vector, including any element thereof having a viral source or derivation is substantially absent from the pluripotent cells. As used herein, this means that the reprogrammed cells contain fewer than one copy of the episomal vector per cell, and preferably no residual episomal vector in the cells. Because asymmetric partitioning during cell division dilutes the vector, one can readily obtain reprogrammed cells from which the vector has been lost. As noted elsewhere herein, on very rare occasions a reprogramming vector can integrate into the genome of the cell, but cells having an integrated vector can be avoided by screening for absence of the vector. Further, in contrast to existing ES cells, the primate pluripotent cells of the ~~invention~~disclosure are substantially

genetically identical to somatic cells from a fetal or post-natal individual. Fetal cells can be obtained from, e.g., amniotic fluid. The cells of the enriched population are not readily distinguished from existing primate ES and iPS cells morphologically (*i.e.*, round shape, large nucleoli and scant cytoplasm) or by growth properties (*i.e.*, doubling time; ES cells have a doubling time of about seventeen to eighteen hours). Like iPS cells and ES cells, the reprogrammed cells also express pluripotent cell-specific markers (*e.g.*, *OCT-4*, *SSEA-3*, *SSEA-4*, *TRA-1-60*, *TRA-1-81*, but not *SSEA-1*). Unlike ES cells, the reprogrammed cells are not immediately derived from embryos. As used herein, "not immediately derived from embryos" means that the starting cell type for producing the pluripotent cells is a non-pluripotent cell, such as a multipotent cell or terminally differentiated cell, such as somatic cells obtained from a fetal or post-natal individual. Like iPS cells, the pluripotent cells

produced in the method can transiently express one or more copies of selected potency-determining factors during their derivation.

[0012] Unless otherwise defined, all 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~~disclosure belongs. Although suitable materials and methods for the practice or testing of the present ~~invention~~disclosure are described below, other materials and methods similar or equivalent to those described herein, which are well known in the art, can be used.

[0013] Other objectives, advantages and features of the present ~~invention~~disclosure will become apparent from the following specification taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0014] FIG. 1A-B illustrate the effect on reprogramming efficiency of different nucleotide sequences that link transgenes on the vector(s) delivered during the reprogramming methods.

[0015] FIG. 2A-C illustrate the effect on reprogramming efficiency of *c-Myc*, *KLF-4*, and SV40 large T antigen gene expression in human newborn foreskin fibroblasts.

[0016] FIG. 3A-C illustrate a suitable construct for carrying transgenes into somatic cells in accord with the method, temporal expression of an episomal vector-mediated transgene, and the effect of vector quantity on cell survival after nucleofection.

[0017] FIG. 4A-D illustrate reprogramming of human newborn foreskin fibroblasts with episomal vector-mediated transgene expression.

[0018] FIG. 5A-B illustrate related constructs harboring an expression cassette useful in the reprogramming methods of the invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0019] The present invention broadly relates to novel methods for reprogramming differentiated primate somatic cells into reprogrammed primate cells that are substantially free of the vectors used in their production by introducing potency-determining factors on a non-viral vector that is present during reprogramming, but is substantially absent from the reprogrammed cells. As used herein, "reprogramming" refers to a genetic process whereby differentiated somatic cells are converted into de-differentiated cells having a higher potency than the cells from which they were derived.

7. The method of Claim 6, wherein the single vector comprises at least one internal ribosomal entry site.

8. The method of Claim 6, wherein the single vector comprises, in order, a first promoter, *OCT4*, IRES2, *SOX2*, a second promoter, *SV40 T* antigen, IRES2, and *KLF4*.

9. The method of Claim 6, wherein the vector comprises, in order, a first promoter, *OCT4*, IRES2, *SOX2*, a second promoter, *c-Myc*, IRES 2, *KLF4*, a third promoter, *NANOG*, IRES2, and *LIN28*.

10. The method of Claim 1, wherein a first non-viral episomal vector comprises, in order, a first promoter, *OCT4*, IRES2, *SOX2*, a second promoter, *SV40 T* antigen, IRES2, and *KLF4*, wherein a second vector comprises, in order, a third promoter, *OCT4*, IRES2, *SOX2*, a fourth promoter, *NANOG*, IRES2, and *KLF4*, and wherein a third vector comprises, in order, a fifth promoter, *c-Myc*, IRES 2, and *LN28*, wherein the promoters need not be identical.

11. The method of Claim 10, wherein each of the first, second, third, and fourth promoters is an elongation factor 1α (EF1α) gene promoter.

12. The method of Claim 1, wherein a first non-viral episomal vector comprises, in order, a first promoter, *OCT4*, IRES2, *SOX2*, a second promoter, *KLF4*, IRES2, *c-Myc*, a third promoter, *NANOG*, IRES2, and *LN28*, wherein a second vector comprises, in order, a fourth promoter, *OCT4*, IRES2, *SOX2*, a fifth promoter, *SV40 T* antigen, IRES2, and *KLF4*, wherein the promoters need not be identical.

13. The method of Claim 12, wherein each of the first, third, fourth, and fifth promoters is an EF1α gene promoter and wherein the second promoter is a cytomegalovirus immediate early gene (CMV) promoter.

14. The method of Claim 1, wherein a first non-viral episomal vector comprises, in order, a first promoter, *OCT4*, IRES2, *SOX2*, a second promoter, *NANOG*, IRES2, and *LIN28*, wherein a second vector comprises, in order, a third promoter, *OCT4*, IRES2, *SOX2*, a fourth promoter, *SV40 T* antigen, IRES2, and *KLF4*, and wherein a third vector comprises, in order, a fifth promoter, *OCT4*, IRES2, *SOX2*, a sixth promoter, *c-Myc*, IRES 2, and *KLF4*, wherein the promoters need not be identical.

~~15. An enriched population of primate induced pluripotent cells substantially genetically identical to a post-natal individual and substantially free of any vector component associated with induction of pluripotency, wherein the primate induced pluripotent cells express pluripotent cell specific markers, have a cell morphology characteristic of undifferentiated cells, and can differentiate into cells of all three germ layers.~~

16. The population of claim 15, wherein the population is produced according to a method comprising the steps of:

introducing at least one non-viral episomal vector encoding one or more potency-determining factors into primate somatic cells, wherein the at least one vector encodes at least potency-determining factors OCT4, SOX2, and at least one of LIN28 and NANOG, wherein the cells express the introduced OCT4, SOX2, and at least one of LIN28 and NANOG, and further comprise SV40 T Antigen, under conditions sufficient to express the potency-determining factors, thereby reprogramming the somatic cells to produce the primate induced pluripotent cells;

the pluripotent cells being substantially free of any vector component associated with induction of pluripotency and substantially genetically identical to a post-natal individual.

17. The population of Claim 16, wherein the primate somatic cells are obtained from a fetal or post-natal individual.

18. The population of Claim 16, wherein the primate is a human.

19. The population of Claim 16, wherein the one or more potency-determining factors comprise human OCT-4, human SOX2, human LIN28, human NANOG, human c-Myc, and human KLF4, wherein when the vector comprises c-Myc, the method further comprises the step of exposing the primate somatic cells to SV40 T Antigen.

20. Primate induced pluripotent cells substantially free of any component associated with induction of pluripotency.

TABLE 1 -- Reprogramming genes and translation elements

Gene Symbol	Abbr.	Source	SEQ ID NO	Accession # or sequence
OCT4	O	hESC	1	NM_002701
SOX2	S	hESC	2	NM_003106
NANOG	N	hESC	3	NM_024865
LN28	L	hESC	4	NM_024674
c-Myc	M	hESC	5	NM_002467
KLF4	K	hESC	6	NM_004235
SV40 T	T	pBABE-puro SV40 LT p	7	EF579667
TERT	TERT	pBABE-hygro-hTERT	8	NM_198253
IRES1	1	pIRESpuro3	--	
IRES2	2	pIRES2EGFP	--	
F2A	F2A	(synthesized)	9	
CMV	C		10	
EF1α	E		11	
EF2α	-		12	

TABLE 2: Episomal constructs

#	Name	Size (bp)
1	pCEP4-EGFP	10984
2[b]	pEP4-E-O2S(2)	13523
3[b]	pEP4-E-M2K	14293
4[a]	pCEP4-M2K	13643
5[b]	pEP4-E-K2M	14268
6[a]	pCEP4-K2M	13636
7[b]	pEP4-E-N2K	13819
8[b]	pEP4-E-T2K	15071
9[a]	pCEP4-M2L	12852
10[b]	pEP4-E-N2L	13020
11[b]	pEP4-E-T2L	14284
12[c]	pEP4-E-O2S-C-M2K	16038
13[c]	pEP4-E-O2S-E-M2K	16680
14[c]	pEP4-E-O2S-C-K2M	16010
15[c]	pEP4-E-O2S-E-K2M	16652
16[c]	pEP4-E-O2S-E-N2K	16206
17[c]	pEP4-E-O2S-E-T2K	17458
18[c]	pEP4-E-O2S-E-N2L	15415
19[c]	pEP4-E-O2S-E-T2L	16679
20[c]	pEP4-O2S-C-M2L	15247
21[c]	pEP4-E-O2S-E-K2T	17474
22[c]	pEP4-E-O2S-C-M2L-E-N2K	19956
23[c]	pEP4-E-O2S-C-M2K-E-N2L	19956
24[c]	pEP4-E-O2S-C-K2M-E-N2L	19949
25[c]	pEP4-E-O2S-C-M2L-E-T2K	21220
26[c]	pEP4-E-O2S-C-M2K-E-T2L	21220
27[c]	pEP4-E-O2S-C-K2M-E-T2L	21213
28[c]	pEP4-E-O2S-C-M2L-E-K2T	21224

[a] All linked gene cassettes were cloned into the pCEP4-EGFP between BamHI and NheI restriction sites.

[b] All linked gene cassettes plus the EF1α promoter were cloned into the pCEP4-EGFP between BamHI and SpeI (19) restriction sites.

[c] All expression cassettes were cloned into the pCEP4-EGFP between BamHI and NruI restriction sites.

TABLE 3: Combinations of episomal constructs tested for reprogramming activity

Equivalent of pCEP4-EGF(µg)	Test #	Plasmids	µg	Morph. Changes	AP+ colony /plate
EXPERIMENT 1					
6.3	1	pEP4-E-O2S-C-M2K	9.2	+/-	0
6.3	2	pEP4-E-O2S-K2Neo	9.3	+/-	0
6.3		pCEP4-M2L	7.4		
6.3	3	pEP4-E-O2S-E-N2K	9.3	+/-	0
6.3		pCEP4-M2L	7.4		
6.3	4	pEP4-E-O2S-E-T2K	10	+++	0
6.3		pCEP4-M2L	7.4		
6.3	5	pEP4-E-O2S-E-TERT2K	10.8	+/-	0
6.3		pCEP4-M2L	7.4		
6.3	6	pEP4-E-O2S-C-M2L	8.7	+/-	0
6.3		pEP4-E-N2K	7.9		
6.3	7	pEP4-E-O2S-C-M2L	8.7	+	0
6.3		pEP4-E-T2K	8.6		
6.3	8	pEP4-E-O2S-C-M2L	8.7	+/-	0
6.3		pEP4-E-TERT2K	9.4		
EXPERIMENT 2					
3.3	1	pEP4-E-O2S-C-M2K	5.0	+/-	0
3.3	2	pEP4-E-O2S-E-M2K	5.0	+/-	0
3.3	3	pEP4-E-O2S-C-K2M	5.0	+/-	0
3.3	4	pEP4-E-O2S-E-K2M	5.0	+/-	0
2.5	5	pEP4-E-O2S(2)	3.0	+/-	0
2.5		pCEP4-M2K	3.0		
2.5	6	pEP4-E-O2S(2)	3.0	+/-	0
2.3		pEP4-E-M2K	3.0		
2.5	7	pEP4-E-O2S(2)	3.0	+/-	0
2.5		pCEP4-K2M	3.0		
2.5	8	pEP4-E-O2S(2)	3.0	+/-	0
2.3		pEP4-E-K2M	3.0		
1.7	9N	pEP4-E-O2S(2)	2.0	+/-	0
1.5		pEP4-E-N2K	2.0		
1.7		pCEP4-M2L	2.0	+/-	0
1.7	10N	pEP4-E-O2S(2)	2.0	+/-	0
1.7		pEP4-E-N2L	2.0		
1.7		pCEP4-M2K	2.0		
1.7	11N	pEP4-E-O2S(2)	2.0	+/-	0
1.7		pEP4-E-N2L	2.0		
1.5		pEP4-E-M2K	2.0		

1.7	12N	pEP4-E-O2S(2)	2.0	+/-	0
1.7		pEP4-E-N2L	2.0		
1.7		pCEP4-K2M	2.0		
1.7	13N	pEP4-E-O2S(2)	2.0	+/-	0
1.7		pEP4-E-N2L	2.0		
1.5		pEP4-E-K2M	2.0		
2.3	14N	pEP4-E-O2S-E-N2K	3.5	+/-	0
2.1		pCEP4-M2L	2.5		
2.5	15N	pEP4-E-O2S-E-N2L	3.5	+/-	0
2.1		pCEP4-M2K	2.5		
2.5	16N	pEP4-E-O2S-E-N2L	3.5	+/-	0
1.9		pEP4-E-M2K	2.5		
2.5	17N	pEP4-E-O2S-E-N2L	3.5	+/-	0
2.1		pCEP4-K2M	2.5		
2.5	18N	pEP4-E-O2S-E-N2L	3.5	+/-	0
1.9		pEP4-E-K2M	2.5		
EXPERIMENT 3					
1.7	9T	pEP4-E-O2S(2)	2.0	++	0
1.4		pEP4-E-T2K	2.0		
1.7		pCEP4-M2L	2.0		
1.7	10T	pEP4-E-O2S(2)	2.0	+	0
1.5		pEP4-E-T2L	2.0		
1.7		pCEP4-M2K	2.0		
1.7	11T	pEP4-E-O2S(2)	2.0	+	0
1.5		pEP4-E-T2L	2.0		
1.5		pEP4-E-M2K	2.0		
1.7	12T	pEP4-E-O2S(2)	2.0	+/-	0
1.5		pEP4-E-T2L	2.0		
1.7		pCEP4-K2M	2.0		
1.7	13T	pEP4-E-O2S(2)	2.0	+/-	0
1.5		pEP4-E-T2L	2.0		
1.5		pEP4-E-K2M	2.0		
2.2	14T	pEP4-E-O2SET2K	3.5	+++	0
2.1		pCEP4-M2L	2.5		
2.3	15T	pEP4-E-O2S-E-T2L	3.5	+	0
2.1		pCEP4-M2K	2.5		
2.3	16T	pEP4-E-O2S-E-T2L	3.5	+	0
1.9		pEP4-E-M2K	2.5		
2.3	17T	pEP4-E-O2S-E-T2L	3.5	+/-	0
2.1		pCEP4-K2M	2.5		
2.3	18T	pEP4-E-O2S-E-T2L	3.5	+/-	0
1.9		pEP4-E-K2M	2.5		
1.9	19	pEP4-E-O2S-E-T2K	3.0	+++	1
2.0		pEP4-E-O2S-E-N2K	3.0	+	

17		pCEP4-M2L	2.0		
EXPERIMENT 4					
6	1	pEP4-E-O2S-C-M2K-E-N2L	10.9	+/-	0
4	2	pEP4-E-O2S-C-M2K-E-N2L	7.3	+++	0
2		pEP4-E-O2S-E-T2K	3.2		
6	3	pEP4-E-O2S-C-K2M-E-N2L	10.9	+/-	0
4	4	pEP4-E-O2S-C-K2M-E-N2L	7.3	++	2
2		pEP4-E-O2S-E-T2K	3.2		
3	5	pEP4-E-O2S-E-N2L	4.2	+/-	0
3		pEP4-E-O2S-E-M2K	4.6		
3	6	pEP4-E-O2S-E-N2L	4.2	++	1
2		pEP4-E-O2S-E-T2K	3.2		
3		pEP4-E-O2S-E-M2K	4.6		
3	7	pEP4-E-O2S-E-N2L	4.2	+/-	0
3		pEP4-E-O2S-C-M2K	4.4		
3	8	pEP4-E-O2S-E-N2L	4.2	+	0
2		pEP4-E-O2S-E-T2K	3.2		
3		pEP4-E-O2S-C-M2K	4.4		
3	9	pEP4-E-O2S-E-N2L	4.2	+/-	0
3		pEP4-E-O2S-E-K2M	4.5		
3	10	pEP4-E-O2S-E-N2L	4.2	+/-	0
2		pEP4-E-O2S-E-T2K	3.2		
3		pEP4-E-O2S-E-K2M	4.5		
3	11	pEP4-E-O2S-E-N2L	4.2	+/-	0
3		pEP4-E-O2S-C-K2M	4.4		
3	12	pEP4-E-O2S-E-N2L	4.2	+	0
2		pEP4-E-O2S-E-T2K	3.2		
3		pEP4-E-O2S-C-K2M	4.4		
2	13	pEP4-E-O2S-C-M2L-E-T2K	3.9	+	0
4		pEP4-E-O2S-E-N2K	5.9		
6	14	pEP4-E-O2S-C-M2K-E-T2L	11.6	+	0
3	15	pEP4-E-O2S-C-M2K-E-T2L	5.8	+	0
3		pEP4-E-O2S-E-N2K	4.4		
6	16	pEP4-E-O2S-C-K2M-E-T2L	11.6	+/-	0
3	17	pEP4-E-O2S-C-K2M-E-T2L	5.8	+	0
3		pEP4-E-O2S-E-N2K	4.4		

6	18	pEP4-E-O2S-C-M2L-E-K2T	11.6	+/-	0
3	19	pEP4-E-O2S-C-M2L-E-K2T	5.8	+/-	0
3		pEP4-E-O2S-E-N2K	4.4		
3	20	pEP4-E-O2S-E-K2T	4.8	+/-	0
3		pEP4-E-O2S-E-N2K	4.4		
2		pEP4-E-O2S-C-M2L	2.8		

+/-: No or very few colonies with morphological change were observed (~~fig.~~ FIG. 4B).
+, ++ and +++: Different number (from less to more) of colonies with morphological change were observed.