

Supplementary Table S1. Primer sequence information (pluripotency genes)

Gene	Primer sequence		Accession# (mouse)
	1 st round PCR	2 nd round PCR	
<i>Dnmt3l</i>	ATGATGGACACCAGAGTTAC AAAAGCCCAAACCTTTTAGT	TGCTGTTCCGGGGGTACCCT AGGCTCAGTACCCGCACTGG	NM_019448.3
<i>Ctnnb1</i>	CATTAATAGCAGCCTTTCTC CTACACCATTACTCGGTTCT	AACCAAGCTGAGTTTCCTAT AGAACATAACACTACTTAAA	NM_007614.3
<i>Lin28a</i>	AAATTTATGAAGACGAGGTG TGTGTTCTATTGCATTTGTC	GGAATCTGGGGTTCATTTGT TTAGCTATCAACCATCACGCT	NM_145833.1
<i>Fbxo15</i>	AATTTACGCAAGTCTGTAT ATCTCCATAAAAGGTCATTG	TCTGTGTAATCCTGGCCTC CACAAATGTAGTGTCTGGGAG	NM_015798.3
<i>Dppa3</i>	AAGATGAGAAGACTTGTTCCG AAATAGCTTTCACATCTGCT	TTGAGCAGAGACAAAAAAGG TGTAAGCTCCTAATTCTTCC	NM_139218.1
<i>Klf5</i>	GATAGACAAGCTGAGATGCT TATAAACTTTTGTGCAACCA	CCGCTACATTGCTTCCAAC TTCTCCAGATCCGGGTTACT	NM_009769.4
<i>Slc38a5</i>	CACACTGGAGTCATCTTCTT AACAGGTAACCTGGACATAGC	ATTGCTCTCCTGTCTTCTTA TTCCCGGCAGGTCCAAATGC	NM_172479.2
<i>Fzd7</i>	TGGTTGAGATAAACATTCCT AAAACAAAAACAGCAAAAAT	TACCCATTCAGGCACACGTT TGAATTAATCTGTTTAAAAACAATA	NM_008057.3
<i>Nanog</i>	CTAGCAATGGTCTGATTCAGAAG TAGTGGCTTCCAAATTCACCTCC	CACCAGTGGAGTATCCAGCAT TGGCAGAGAAGTTTTGCTGCAAC	NM_028016.2
<i>Oct4</i>	CCCAGGGGGGGCTGTATCCTT CACTGAGCTTCTTTCCCATCC	CCCAGGTCCCCACTTTGGCAC ACCCCTGTTGTGCTTTAATCCC	NM_001252452.1
<i>Dppa5a</i>	CTGAAGAACCTGGAATCTC ATTTAGAGTGGTAACCTTGG	GAAGAAGTATCGAGGTCTT GACTGGAACTGGCTTCACT	NM_025274.3
<i>Dppa4</i>	AGTGCCAGCCGATGCTTCGT GATTTCTATCAGTCTCAGCGT	TCCTGGGAACTCGCGTGGTT TTGGGGCACAGCATGTTGTC	NM_028610.2
<i>Klf4</i>	AGTTTTGTCTTCCGATCTACATTG TACATATTTATTAAGGCAAGGAAC	GGTTTATTTCTGTAACATTTTATG TTAAAGGCATACTTGGGAATAAAC	NM_010637.3
<i>Gapdh</i>	GACCTCAACTACATGGTCTACAT GAGTGAGTTGCATATTTCTCGT	TGGAGAAGGCCGGGGCCAC AAACATGGGGGCATCGGCAG	NM_008084.2

Supplementary Table S2. Primer sequence information (reprogramming-related genes)

Gene	Primer sequence		Accession#
	1 st round PCR	2 nd round PCR	
<i>Jmjd3</i>	CTCACCATCAGCCACTGTG AGCTCTCACAGGGCCAGAT	TGCCAGCAAGAATGCCAAG CCAGAGTCTTGGTGGAGAAA	NM_001017426.1
<i>Jhdm2a</i>	AGCATTCTTGGCTTTGACA CATCAGATCATCAAACCTG	GGCAGCCAGTGTGGTGT CCTTAAGTTTCAACACCATTG	NM_001038695.2
<i>Jhdm3a</i>	TTCTACCAGTGTGAGGTG TCTCATTGAAGCGCATGTC	CATAGTGTGAGTGTGAGTGTG AGTCTAGACTTGACTCTCTT	NM_001161823.1
<i>Tet2</i>	GGAGGAAGGCAGTGACATC ATAAACTGCATTATAACA	CGATACAAGTGCTTCTGCT AAAAAAACCCTAACAAAGTT	NM_001040400.2
<i>Tet3</i>	GGCATGGCTACACTTCAT GGCTCGGCTGCCACTGTGT	TTCTCTCCTCCTCTCCCT GTTCTCATTTTCAAAGAGC	NM_183138.2
<i>Glp</i>	TGGTCAAGTATGAGCTGATGC TGCTATGGTACCTCTTTG	GCGCCCATCAACAAAGCA CCTTGAAGCTTCTTCCCC	NM_001012518.2
<i>Dnmt3a</i>	TCAAGGAGATCATTGATG TGTTCCACACATGAGCA	GCGCACAAGGGAGCGGCTG AGATGGTGCAATAGGACTG	NM_007872.4 NM_001271753.1
<i>Dnmt3b</i>	CCTACCCGGAATGAACAG ACAGGCAAAGTAGTCCTTC	AGCTCGAGCTGCAGGACT AGATCCTTTGAGCTCAGTG	NM_001003960.3
<i>Lsd1</i>	TCTGGCTTGGCAGCAGCTC GTGGCTTCTAGCAACCGGT	ACTTCTGGAAGCCAGGGATC CTTGATCTTGGCCAGTTCCA	NM_133872.2
<i>Dnmt1</i>	CAGTGGCATGAACCGCTTCA GTCTGGGCCACGCATACT	GCTACTGTGACTACTACCG AAAGGTGCATGGTAGCC	NM_010066.4
<i>Setdb1</i>	CACACGCCAGTTCTATGA TCAGGAAGACCCAGTTCTT	GGCCGCTACCTCAATCAC CCGGATTCTCTTGCTGGC	NM_001163641.1
<i>Ezh1</i>	TGCGGCTATGCCTGGTTGG GTTTTGTGTCAAGTCAGC	GCTTTCTTCAGGGTAGCTG TCCTCTTCTCCTTCAGAA	NM_007970.2
<i>Ezh2</i>	CCGAGAAGAGGAAGACTT GTTTCCTTTCTTCTTTCT	ACCATCAGTGTGCTGGAGT CCAATGAGGACTCTAAACA	NM_007971.2
<i>G9a</i>	GACAGAACGCGGTTTGAG CAGGCCTTATGGAAGCGG	CCGCATGGAGGCTCCCAAG ATGCGGGCTCGATGGGCCT	NM_145830.1
<i>Eed</i>	CTGAGTGCTGATTATGATC TATGAGGATCTTCTACTTC	TTCCTGACTTTTCTACCAG ACTTGGAAAGTATCAAATC	NM_021876.3
<i>Wdr5</i>	TTTTTCTCTGTGGTTGTTG CCAGAGACCTTTCCAACCTC	GAACAGGACCTACAGCCCT TCCATACCCACCTTTTATT	NM_080848.2
<i>Brg1</i>	AGGAGTACCTCAACAGCAT TGCTTCTTCTTTTCTTT	TCACAGATCAGTCACAGGC TGCTGTGCTGAGAAGGTAG	NM_001174078.1
<i>Baf155</i>	GCCCGGCAGCAAATGGAGC GGCTAGGGAGTGGCCGAGG	GCACATCAGATGCCGCA GATGTTTCCGGGCATTGGA	NM_009211.2
<i>Dot1</i>	GTGGACAGTCCGCTACAGG TTGCGTTGCCCTCACCTT	GGGGCCTTGCTGCAGACC TTGGCCTCTGTGGTACTGT	NM_199322.1
<i>p53</i>	TTGCGTTGCCCTCACCTT ACAACCTGGCTGGATAGAAT	AGACCAAGAAGGGCCAGTC GGCTGGTGTGGGGACGGG	NM_011640.3

Supplementary Figure legends

Fig. S1. Expression levels of pluripotency genes in NGFP-mEFs and 4F2A-mEFs. **A**, Multiplex RT-PCR results. Each RT-PCR set-up employed about 3,000 cells as a template, which were repeated four times. Wherever PCR bands are present are denoted by gene symbols with purple dots. **B**, Expression levels of pluripotency genes. Error bars, the standard deviation of the mean (STD). *Gapdh* level = 1.0

Fig. S2. Density profiles of gene expressions in single pre-iPSC colonies in NGFP, 4F2A, and mESC groups. Insets, corresponding gel images.

Fig. S3. A quantitative real-time PCR. Complementary DNAs obtained from pooled colonies (20 per group) were analyzed for *Nanog*, *Dppa5a*, and *Dppa4*. One-way ANOVA and the Bonferroni post hoc test were used for statistical analysis. Asterisks ($p < 0.001$) denote a significant difference between samples. Error bars, standard deviation.

Fig. S4. Gene expression profiles (GEPs) of representative pre-iPSC colonies with the lowest (minimal weighted root mean square deviation, [min wRMSD] in green) and the highest wRMSD (max wRMSD in purple) are shown along with the mean GEP (in red) for each group.

Fig. S5. Single-gene RT-PCR using cDNA from J1 mESCs. The size of PCR amplicon for the indicated reprogramming-related genes is indicated below.

Fig. S6. Multiplex RT-PCR for reprogramming-related genes using single pre-iPSC colonies. Above, a representative gel image (left) and the relative PCR band

positions of the genes analyzed (right). Below, gel images of multiplex RT-PCR products. DNA markers are also shown in each gel.