

Producing primate embryonic stem cells by somatic cell nuclear transfer

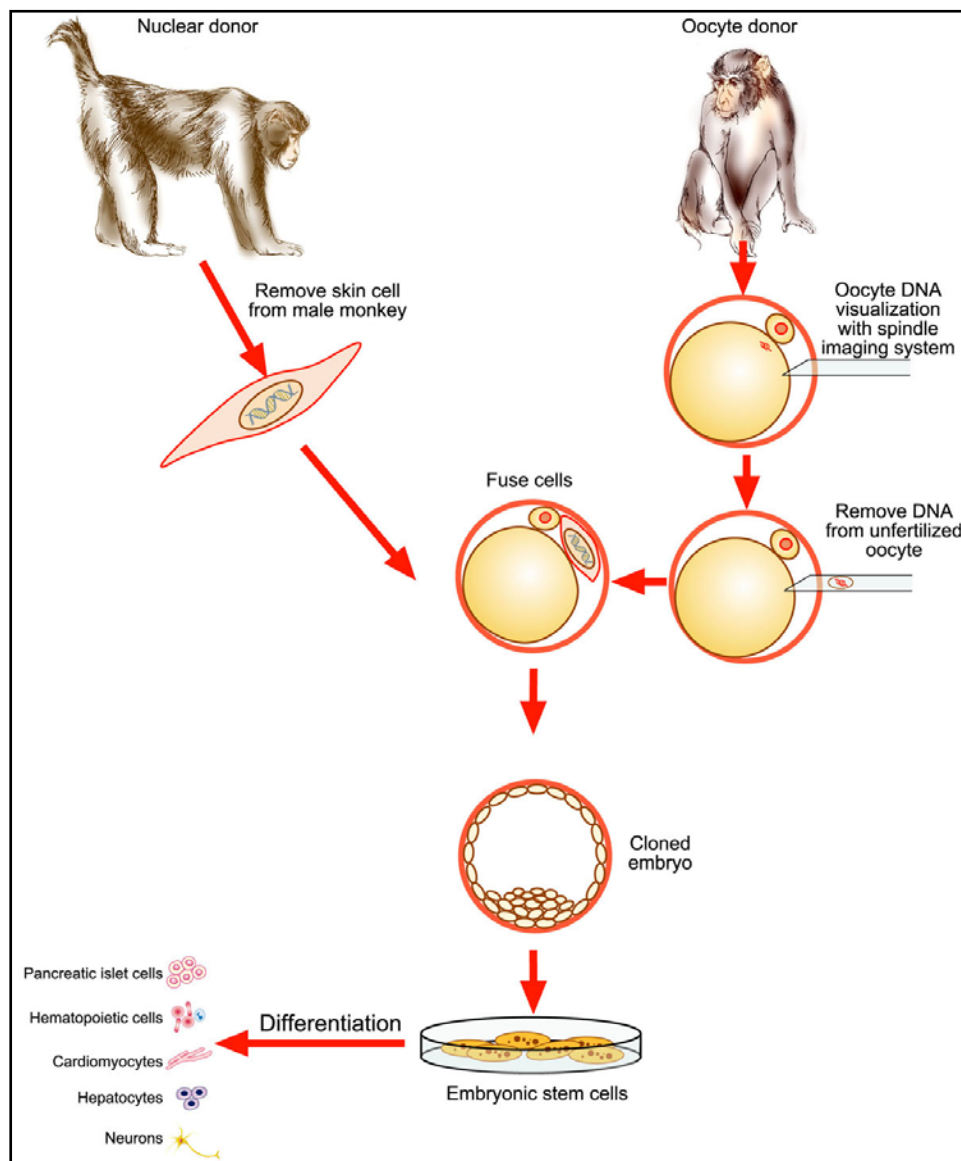
Byrne JA, Pedersen DA, Clepper LL, Nelson M, Sanger WG, Gokhale S, Wolf DP and Mitalipov SM

Microarray data, including CEL and CHP files, can be accessed at the Gene Expression Omnibus (GEO) from <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7748>.

The Supplementary information containing microarray analyses (Supplemental Data 3-7) can be accessed from

<ftp://ftp.ncbi.nih.gov/pub/geo/DATA/supplementary/series/GSE7748>.

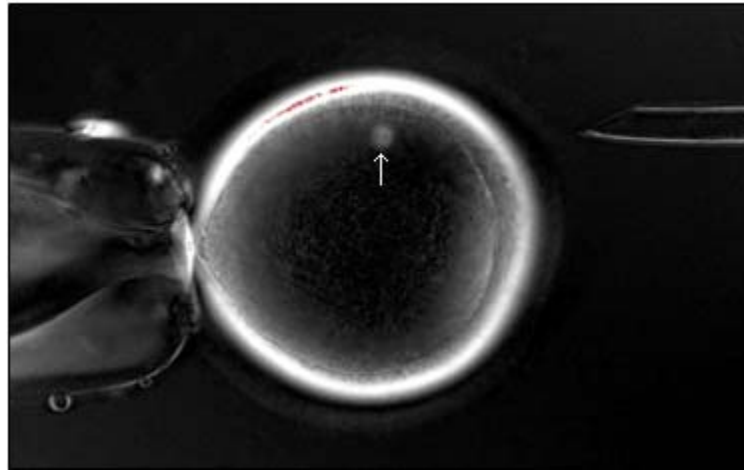
Supplementary Figures and Legends



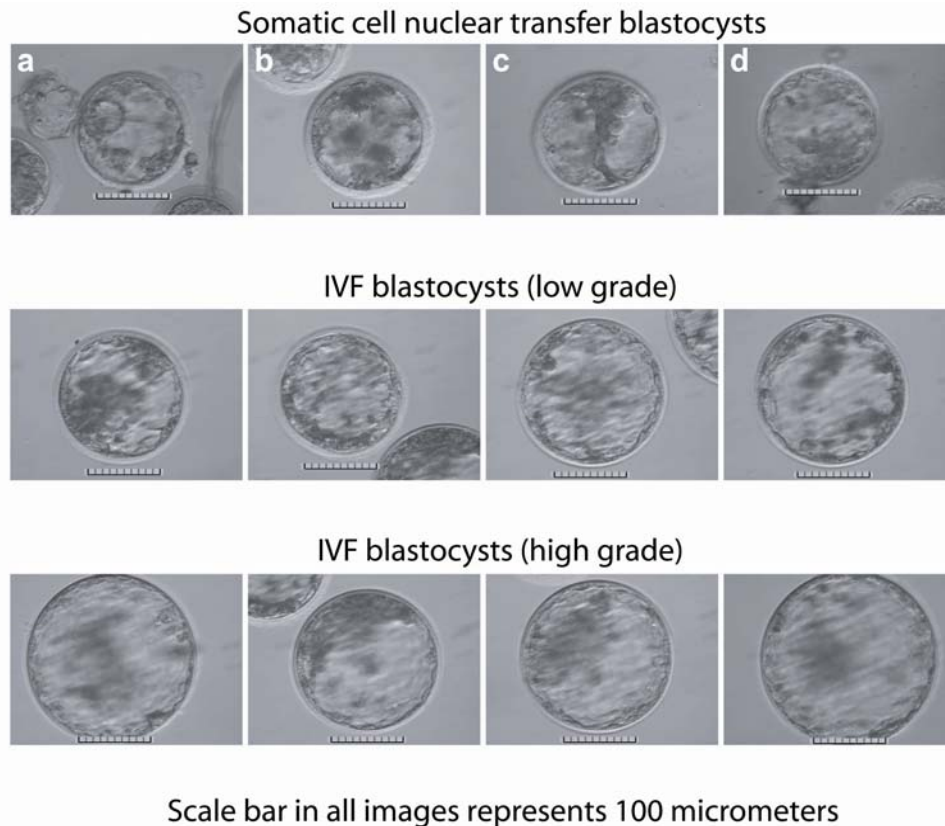
Supplemental Figure 1. A schematic diagram demonstrating the experimental steps involved in the reprogramming of adult primate somatic cells into pluripotent embryonic stem cells. Donor somatic nuclei from skin cells were introduced into enucleated oocytes and the resulting embryos gave rise to embryonic stem cell lines that were genetically identical to the nuclear donor male.



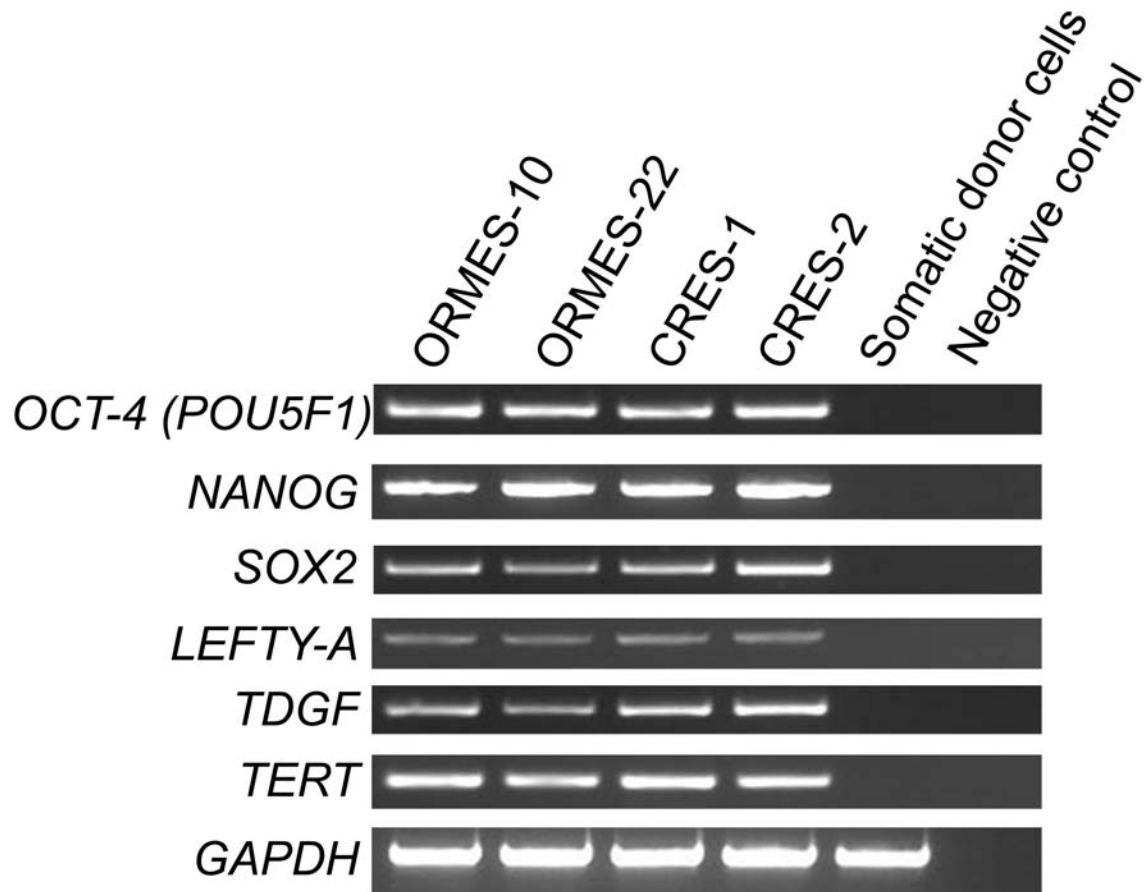
Supplemental Figure 2. Semos, adult rhesus macaque male (Male #1), the somatic nuclear donor for the CRES-1 and CRES-2 cell lines.



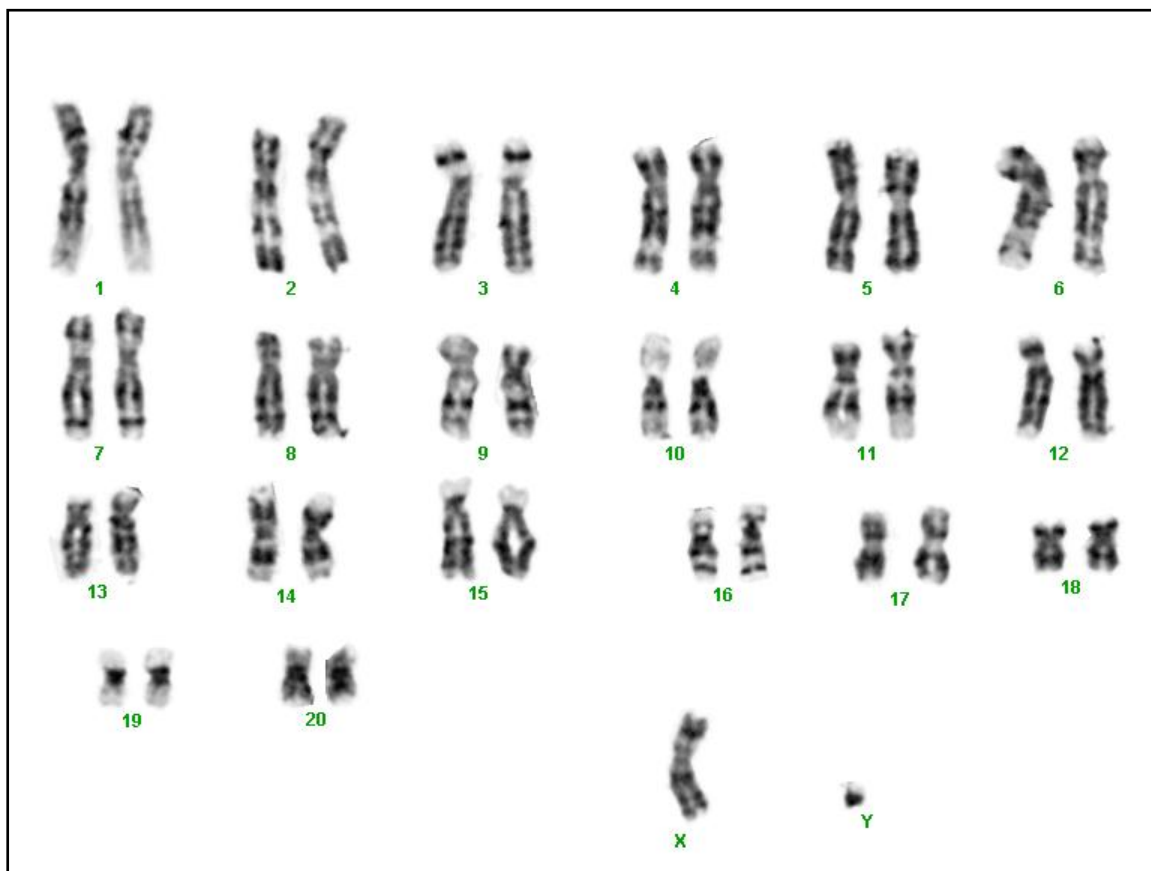
Supplemental Figure 3. Visualization of the meiotic spindle in a rhesus monkey MII oocyte using the Oosight™ spindle imaging system during enucleation. The white arrow highlights the position of the spindle.



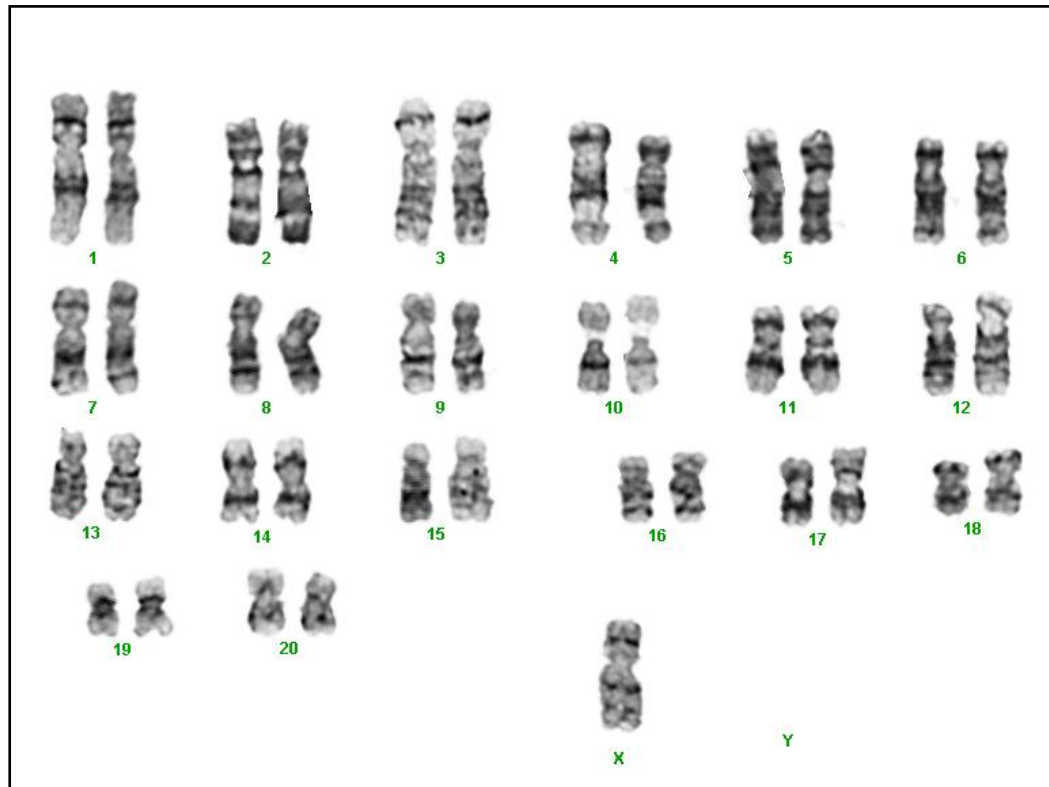
Supplemental Figure 4. Rhesus monkey embryo morphology. The first row demonstrates the morphology of rhesus monkey SCNT expanded and hatching blastocysts; the second row, low grade IVF-produced expanded blastocysts; and the third row, high grade IVF-produced expanded blastocysts. The CRES-1 line was derived from the SCNT blastocyst shown in Figure 4a and the CRES-2 line was derived from the SCNT expanded blastocyst shown in Figure 4b.



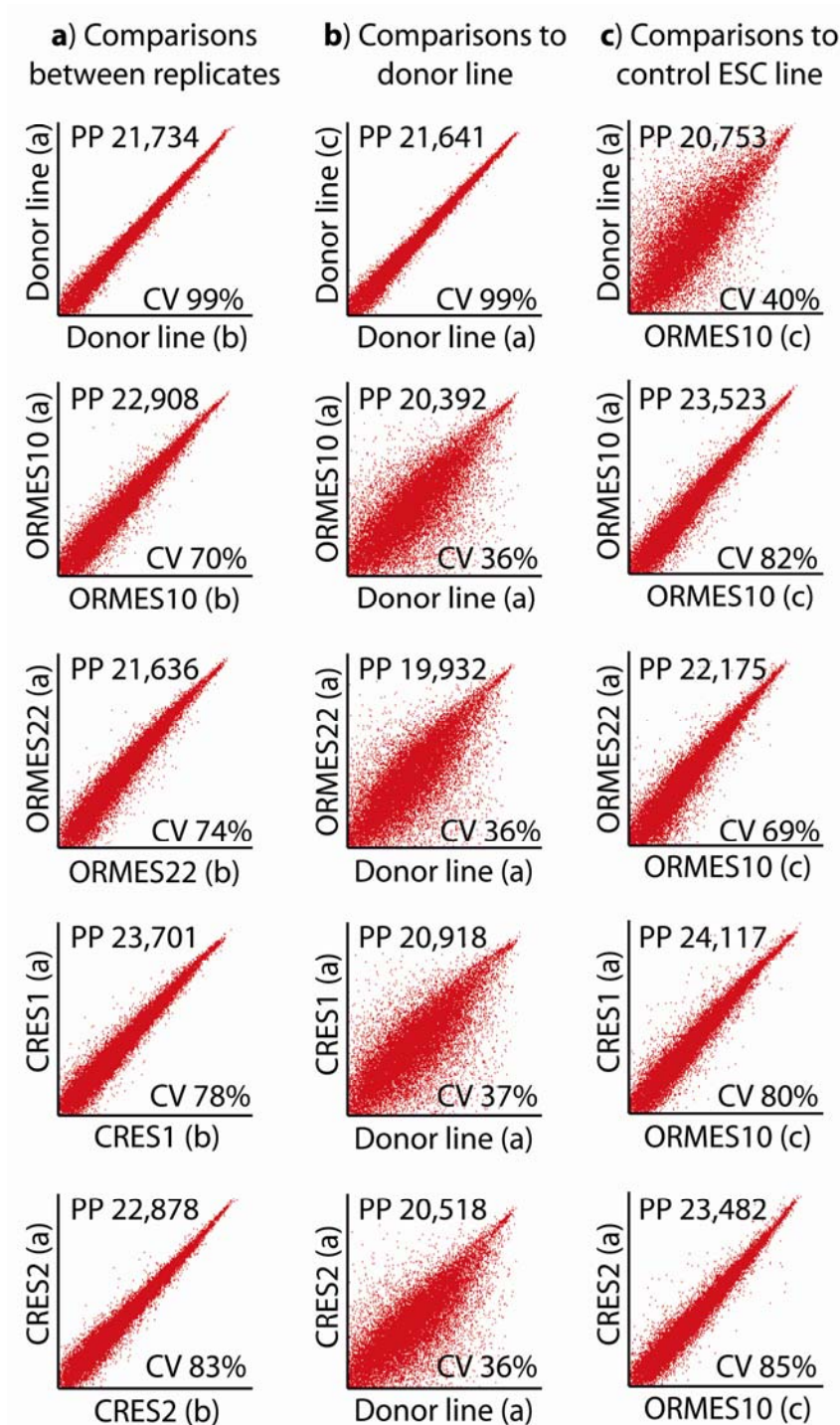
Supplemental Figure 5. RT-PCR analysis of ESC-specific gene expression in CRES cells. *OCT-4*, *NANOG*, *SOX-2*, *LEFTY-A*, *TDGF* and *TERT* represent genes strongly expressed in primate ESCs, but not in somatic cells. *GAPDH* served as a housekeeping control.



Supplemental Figure 6. G-banding analysis of cultured fibroblasts derived from Male #1: Cytogenetic analysis of skin fibroblasts from rhesus monkey nuclear donor Male #1 demonstrated normal euploid chromosome complement (42 XY).

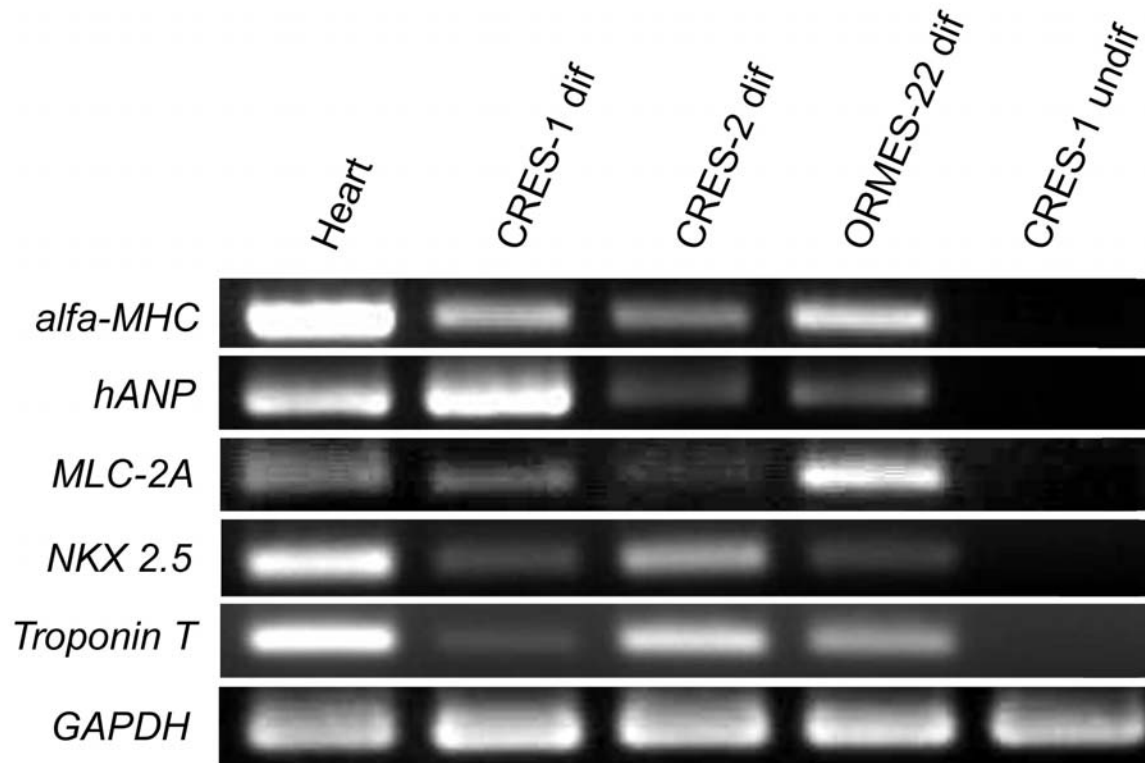


Supplemental Figure 7. G-banding analysis of CRES-1 indicating loss of the Y chromosome. Approximately 12% of cells in CRES-1 population demonstrated this aneuploidy.



Supplemental Figure 8. Microarray analysis of rhesus monkey ESCs. Column a demonstrates selected comparisons between biological replicates of the same cell line, column b demonstrates selected comparisons between the cell lines and the somatic

donor cell line and column c demonstrates selected comparisons between the cell lines and a control IVF-derived ESC line (ORMES-10).



Supplemental Figure 9. Analysis of cardiomyocyte-specific gene expression in differentiated rhesus monkey ESCs. Both the control IVF-derived ESC line (ORMES-22) and the SCNT-derived ESC lines (CRES-1 and CRES-2) were differentiated into contracting cardiomyocytes as described in Methods. Transcripts of cardiac specific genes were detected in the control heart tissue and differentiated ESCs but not in undifferentiated CRES-1 cells.

Supplementary Tables

Supplemental Table 1. Rhesus monkey embryo development

Procedure	Spindle visualization	N	Cleaved # (Mean % $\pm$ SEM)	8-cell # (Mean %* $\pm$ SEM)	Morula # (Mean %* $\pm$ SEM)	CM # (Mean %* $\pm$ SEM)	Blastocyst # (Mean %* $\pm$ SEM)
ICSI** (control)	N/A	40	38 (95% $\pm$ 5.4%)	32 (84% $\pm$ 16%)	29 (76% $\pm$ 14.7%)	29 (76% $\pm$ 14.7%)	19 (50% $\pm$ 13.3%)
SCNT [†]	Hoechst-UV	165	120 (73% $\pm$ 8%)	106 (88% $\pm$ 7%)	32 (27% $\pm$ 9%)	2 (2% $\pm$ 2%)	1 (1% $\pm$ 1%)
SCNT	Oosight TM	304	213 (70% $\pm$ 4.8%)	204 (96% $\pm$ 4.6%)	199 (93% $\pm$ 5.3%)	161 (76% $\pm$ 6.9%)	35 ^{††} (16% $\pm$ 3.6%)

N refers to the number of oocytes used for SCNT and CM refers to the compact morula stage.

* The mean % for the 8-cell to blastocyst stages was calculated based on the number of cleaved embryos.

** ICSI refers to intra-cytoplasmic sperm injection

† This data was derived from our previous SCNT study (Mitalipov et al., Biol Reprod 66, 1367-73. 2002). It should be noted that a variety of different donor cells were used in these studies, none of which resulted in a significant blastocyst formation rate.

†† Of these 35 SCNT blastocysts, 10 failed to expand, 5 collapsed and failed to re-cavitate and 20 were used for ESC isolation.

Supplemental Table 2. Histocompatibility analysis of CRES cell lines and Male #1 based on MHC-linked STR analysis

MHC loci	Male #1 nuclear donor for CRES-1 and -2	CRES-1	CRES-2	Female #1 oocyte donor for CRES-1	Female #2 oocyte donor for CRES-2	Female #3 oocyte donor for ORMES -22	Male #2 sperm donor for ORMES -22	ORMES -22
D6S291	206/208	206/208	206/208	206/208	208/214	206/216	210/216	210/216
G51152	195/218	195/218	195/219	195/209	215/219	210/210	219/0	210/0
9P06	175/175	175/175	175/175	175/175	183/185	175/187	189/189	175/189
DRA	112/134	112/134	112/134	132/136	112/128	110/134	112/134	112/134
MICA	200/200	200/200	200/200	200/200	203/203	200/200	194/194	194/200
246K06	275/283	275/283	275/283	279/279	275/285	283/283	277/285	283/285
162B17A	242/246	242/246	242/246	240/240	238/242	238/242	240/244	238/244
162B17B	295/309	295/309	295/309	295/295	281/303	289/309	293/315	293/309
151L13	301/305	301/305	301/305	299/299	305/309	309/309	303/309	309/309
MOGCA	127/127	127/127	127/127	121/125	121/123	123/127	121/123	123/127
268P23	154/154	154/154	154/154	148/152	148/150	150/154	148/150	150/154
222I18	173/173	173/173	173/173	167/173	167/168	167/173	167/175	173/175
D6S276	227/233	227/233	227/233	225/225	215/217	225/233	215/225	225/233
D6S1691	197/199	197/199	197/199	197/205	203/203	197/216	197/203	197/203

Supplemental Table 3. Single nucleotide polymorphism (SNP) analysis of CRES cell lines

SNP	Male #1 Nuclear donor for CRES-1 and CRES-2	CRES-1	CRES-2	Female #1 oocyte donor female for CRES-1	Female #2 oocyte donor female for CRES-2
ADRBK2_109	A/A	A/A	A/A	A/A	A/G
AGRP_471	C/C	C/C	C/C	C/C	C/T
CD74_213	C/C	C/C	C/C	C/C	C/T
HTATSF1_636	C/C	C/C	C/C	C/C	C/T
IFNG_312	A/A	A/A	A/A	A/A	A/G
MAOA_116	C/C	C/C	C/C	C/C	C/G
MPDZ_323	A/G	A/G	A/G	A/A	A/A
CCL8_516	A/G	A/G	A/G	G/G	A/A
CD4_558	C/T	C/T	C/T	C/T	T/T
CXCL12_173	C/C	C/C	C/C	T/T	C/C
IL2RA_124	C/T	C/T	C/T	T/T	C/C
INHBB_131	C/T	C/T	C/T	C/C	C/C
LRP8_647	C/T	C/T	C/T	T/T	T/T
PYY_151	C/T	C/T	C/T	T/T	C/T
SASH1_527	A/G	A/G	A/G	G/G	A/G
SIRT1_277	G/T	G/T	G/T	G/G	G/G
SLC6A4_132	G/G	G/G	G/G	C/C	G/G
STAR_522	G/G	G/G	G/G	G/G	G/T
TLR4_735	C/T	C/T	C/T	T/T	T/T
XCL1_320	C/C	C/C	C/C	C/T	T/T
CCR7_397	G/T	G/T	G/T	G/G	G/T
CCRL1_54	A/C	A/C	A/C	A/A	A/A
CD69_294	C/C	C/C	C/C	C/T	C/T
CFTR_796	G/G	G/G	G/G	A/G	A/G
CX3CR1_593	A/A	A/A	A/A	G/G	G/G

IL1_755	A/A	A/A	A/A	A/A	A/T
IL6ST_177	G/G	G/G	G/G	A/G	G/G
CCR9_315	C/C	C/C	C/C	C/T	C/T
SASH1_578	A/A	A/A	A/A	A/A	C/C
FSHR_784	C/C	C/C	C/C	C/G	C/G

Shaded boxes highlight heterozygous loci

Supplemental Table 4. Analysis of somatic specific gene expression in rhesus monkey ESCs

Cell line	Biological replicate	# somatic genes* down regulated compared to donor line replicate A**	# somatic genes* down regulated compared to donor line replicate B**	# somatic genes* down regulated compared to donor line replicate C**
Nuclear donor cells	a	N/A	23 (0.5%)	8 (0.2%)
Nuclear donor cells	b	33 (0.7%)	N/A	67 (1.3%)
Nuclear donor cells	c	52 (1.0%)	89 (1.8%)	N/A
ORMES-22	a	4822 (97%)	4748 (95%)	4756 (95%)
ORMES-22	b	4733 (95%)	4728 (95%)	4756 (95%)
ORMES-22	c	4741 (95%)	4676 (94%)	4721 (95%)
CRES-1	a	4822 (97%)	4851 (97%)	4824 (97%)
CRES-1	b	4807 (96%)	4809 (96%)	4827 (97%)
CRES-1	c	4671 (94%)	4723 (95%)	4727 (95%)
CRES-2	a	4856 (97%)	4822 (97%)	4820 (96%)
CRES-2	b	4752 (95%)	4670 (93%)	4668 (93%)
CRES-2	c	4800 (96%)	4837 (97%)	4812 (96%)

* The general approximation when working with large numbers of probe sets is to assume that each probe set represents hybridization to a single gene. However, multiple probe sets can exist for certain genes, so the actual number of genes included in the analysis is significantly lower than the number of probe sets analyzed.

** The % value represents the proportion of probe sets, out of the 4,998 somatic-specific probe sets, significantly down regulated following comparison analysis.

Supplemental Table 5. Primer information for rhesus monkey genes

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Anneal Temp (°C)	Product size (bp)
<i>POU5F1</i>	TGGAGAAGGAGAAGCTGG AGCAAA	GAAGCTAAGCTGCAGAGCC TCAAA	60	212
<i>NANOG</i>	CAGAACTGTGTTCTCTTCC ACCCA	CGCTGATTAGGCTCCAACC ATACT	60	801
<i>SOX2</i>	CCCCCGGCGGCAATAGCA	TCGGCGCCGGGAGATACA T	60	448
<i>LEFTYA</i>	CCTGTGTCCTTCCATTTCC TGTCT	AAGCCCTTCATCCTTCCTCT TAGC	60	366
<i>TDGF</i>	CATATTGGCCAGTCTGGTC TCGAA	ATTCCTTCAGCACTCTGGTT CCTC	60	303
<i>TERT</i>	AGCTATGCCCGGACCTCC AT	GCCTGCAGCAGGAGGATCT T	60	185
<i>GAPDH</i>	AATCCCATCACCATCTTCC AGGAG	CACCTGTGCTGTAGCCA AATTC	60	217
<i>alpha-MHC</i>	GTCATTGCTGAAACCGAG AATG	GCAAAGTACTGGATGACAC GCT	61	413
<i>hANP</i>	GAACCAGAGGGGAGAGAC AGAG	CCCTCAGCTTGCTTTTATAGG AG	61	406
<i>Mlc2a</i>	ACAGAGTTTATTGAGGTG CCCC	AAGGTGAAGTGTCACAGAG G	61	381
<i>NKX2.5</i>	TGGCTACAGCTGCACTGCC G	GGATCCATGCAGCGTGGAC	57	167
<i>Troponin T</i>	GGCAGCGGAAGAGGATGC TGAA	GAGGCACCAAGTTGGGCAT GAACGA	64	150

MIAME Checklist

EXPERIMENTAL DESIGN

Authors:

Byrne JA, Pedersen DA, Clepper LL, Nelson M, Sanger WG, Gokhale S, Wolf DP and Mitalipov SM

Type of experiment: for example, is it a comparison of normal vs. diseased tissue, a time course, or is it designed to study the effects of a gene knock-out?

We compared the transcriptomes of rhesus monkey embryonic stem cell lines derived from IVF-produced embryos (Oregon Rhesus Macaque Embryonic Stem, ORMES-10 and ORMES-22) with rhesus monkey nuclear transfer embryonic stem cells derived from somatic cell nuclear transfer produced embryos (Cloned Rhesus Embryonic Stem, CRES-1 and CRES-2). We also analyzed the donor adult somatic skin fibroblast cell line used to produce the cloned embryos. Three biological replicates of each cell line (A, B and C) were analyzed.

Experimental factors: the parameters or conditions tested, such as time, dose, or genetic variation.

Three biological replicates of: ORMES-10, ORMES-22, CRES-1, CRES-2 and donor adult somatic skin fibroblasts were analyzed.

The number of hybridizations performed in the experiment.

15 Affymetrix hybridizations performed

The type of reference used for the hybridizations, if any.

No references used

Hybridization design: if applicable, a description of the comparisons made in each hybridization, whether to a standard reference sample, or between experimental samples. An accompanying diagram or table may be useful.

ESC replicate was compared against each somatic replicate and select comparisons between ESC replicates were performed.

Quality control steps taken.

Isolated total RNA was analyzed on an Agilent Bioanalyzer 2100 to verify that starting material was of good quality. Standard Affymetrix control steps were then taken.

Labeled RNA was hybridized to a Test Array containing probe sets for all Affymetrix Expression Array controls. Background noise, Avg Diff, and ratio of Avg Diff values for probe sets representing the 5' and 3' ends of actin and GAPDH transcripts were used to determine target quality. Quality tested samples were then hybridized to the Rhesus Affymetrix array.

Number of replicates (Biological or Technical)

Three biological replicates per cell line.

URL of any supplemental websites or database accession numbers.

Microarray data, including CEL and CHP files, can be accessed at the Gene Expression Omnibus (GEO) from <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7748>.

The Supplementary information containing microarray analyses (Supplemental Data 3-7) can be accessed from

<ftp://ftp.ncbi.nih.gov/pub/geo/DATA/supplementary/series/GSE7748>.

SAMPLES USED, EXTRACT PREPARATION AND LABELING

The origin of the biological sample (for instance, name of the organism, the provider of the sample) and its characteristics: for example, gender, age, developmental stage, strain, or disease state.

Organism: *Macaca mulatta*

All ESC and somatic cell lines were generated by us at the Oregon National Primate Research Center. Donor somatic fibroblasts were derived from a skin biopsy from a nine year old adult male rhesus monkey.

Samples used in microarray experiment

Sample	Microarray Name	GEO ID	Contents
A_DonorA	A230d1	GSM187389	Donor cell line (Male #1) replicate A
B_DonorB	B232d1	GSM187390	Donor cell line (Male #1) replicate B
C_DonorC	C233d1	GSM187391	Donor cell line (Male #1) replicate C
D_ORMES10a	D177o1	GSM187392	ORMES10 (IVF derived ESCs) replicate A
E_ORMES10b	E184o1	GSM187393	ORMES10 (IVF derived ESCs) replicate B
F_ORMES10c	F187o1	GSM187394	ORMES10 (IVF derived ESCs) replicate C
G_ORMES22a	G169o2	GSM187395	ORMES22 (IVF derived ESCs) replicate A
H_ORMES22b	H171o2	GSM187396	ORMES22 (IVF derived ESCs) replicate B
I_ORMES22c	I185o2	GSM187397	ORMES22 (IVF derived ESCs) replicate C
J_CRES1a	J233c1	GSM187398	CRES1 (SCNT derived ESCs) replicate A
K_CRES1b	K213c1	GSM187399	CRES1 (SCNT derived ESCs) replicate B
L_CRES1c	L215c1	GSM187400	CRES1 (SCNT derived ESCs) replicate C
M_CRES2a	M241c2	GSM187401	CRES2 (SCNT derived ESCs) replicate A
N_CRES2b	N244c2	GSM187402	CRES2 (SCNT derived ESCs) replicate B
O_CRES2c	O245c2	GSM187403	CRES2 (SCNT derived ESCs) replicate C

Manipulation of biological samples and protocols used: for example, growth conditions, treatments, separation techniques.

ESCs were grown at 37°C in 5% CO₂ atmosphere on mitotically inactivated mEF feeder cells in Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12) (Invitrogen) supplemented with 15% foetal bovine serum (FBS) (Hyclone), 0.1 mM β -mercaptoethanol (Sigma), 1% nonessential amino acids (Invitrogen), 4ng/ml FGF2, 2 mM L-glutamine (Invitrogen) and a pH of 7.2 (altered with 4N NaOH). ESC colonies were manually passaged every 4-5 days. ESCs with an undifferentiated morphology were manually removed from culture for total RNA purification.

Protocol for preparing the hybridization extract: for example, the RNA or DNA extraction and purification protocol.

Total RNA was isolated from cell colonies selected for the appropriate ESC morphology (flat monolayer colony with distinctive cobble stem cell morphology and a high nucleocytoplasmic ratio) using the Invitrogen TRIZOL reagent, followed by further purification with the Qiagen RNeasy MinElute Cleanup Kit. The RNA samples were quantified using the NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE) and the quality of the RNA was assessed using Lab-on-a-Chip RNA Pico Chips and the 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

Labeling protocol(s).

Standard Affymetrix Biotin Labeling Protocols (Affymetrix GeneChip Expression Analysis Technical Manual, rev.3. 2001)

External controls (spikes).

Standard Affymetrix external spikes added to hybridization mixture: BioB, BioC, BioD and CreX at 1.5pm, 5.0pm, 25pm and 100pm respectively,

HYBRIDIZATION PROCEDURES AND PARAMETERS

The protocol and conditions used during hybridization, blocking and washing.

Standard Affymetrix Protocols (Affymetrix GeneChip Expression Analysis Technical Manual, rev.3. 2001)

MEASUREMENT DATA AND SPECIFICATIONS

Quantitations based on the images

Original Affymetrix .dat proprietary output files

Type of scanning hardware and software used: this information is appropriate for a materials and methods section.

Software – Affymetrix Microarray Suite 5.0

Scanning Hardware – HP GeneArray Scanner

Type of image analysis software used: specifications should be stated in the materials and methods.

Affymetrix Microarray Suite 5.0 was used for image analysis and feature extraction

A description of the measurements produced by the image-analysis software and a description of which measurements were used in the analysis.

Probe level measurements produced by Affymetrix Microarray Suite 5.0. Analysis performed at level of raw signal. (See Affymetrix Microarray Suite 5.0 reference on web site for description).

The complete output of the image analysis *before* data selection and transformation (spot quantitation matrices).

Original Affymetrix output files

Data selection and transformation procedures.

The arrays were scanned using the GeneChip laser scanner (Affymetrix) and image processing, standard normalization, and expression analysis were performed with the

Affymetrix GCOS ver. 1.4 software. MAS-5 statistical analysis was performed to calculate the signal log ratio (SLR) for each probe set comparison, and the gene expression fold-changes (FCs) between two samples were calculated from the SLR using the following formula: $FC = (2^{SLR})$. The GCOS 1.4 MAS 5.0 software was used to calculate statistically significant differences in gene expression ($P < 0.002$) between samples. For the primary microarray comparison analysis (Supplemental Data 3) the $\log_{10}$ of the absolute detected signal for each present “P” probe set ($p < 0.05$) was plotted in a scatter graph (Supplemental Figure 8) using Affymetrix GeneChip Operating Software (GCOS) version 1.4. For the Correlation Value (CV) calculations, the microarray data for each individual cell line comparison was filtered to only include probe sets present ($p < 0.05$) in both cell lines. The present probe sets (PP) value details the number of probe sets, post filtering, with a present ($p < 0.05$) signal in both compared cell lines. The Correlation Value (CV) for each cell line comparison was calculated using MAS-5 (Affymetrix microarray suite 5) analysis to calculate the proportion of compared probe sets which demonstrated no significant change “NC” in gene expression (Supplemental Data 3). Each cell line had three biological replicates and the letter after the cell line name details which replicate was utilized in the primary microarray comparison analysis. For the secondary gene specific analysis, comparison analysis was performed between each of the three control ORMES-10 biological replicates and each of the three somatic donor cell replicates, to give a total of nine somatic-ESC comparisons (Supplemental Data 4). The following selection criteria were used to identify rhesus somatic-specific genes: 1) genes that were considered to be present ($P < 0.05$) in all three somatic donor cell replicates and 2) genes that demonstrated statistically significant decrease “D” in gene expression in the ORMES-10 replicates in all nine comparisons with the somatic donor cell replicates following GCOS comparisons with MAS-5 statistical analysis. A total of 4,998 somatic-specific probe sets were identified in this way (Supplemental Data 5). The following selection criteria were used to identify rhesus ESC-specific genes: 1) genes that were considered to be present ($P < 0.05$) in all three ORMES-10 replicates and 2) genes that demonstrated a statistically significant increase “I” in gene expression in the ORMES-10 ESC replicates in all nine comparisons with the somatic donor cell replicates following GCOS comparisons with MAS-5 statistical analysis. A total of 6,178 ESC-

specific probe sets were identified in this way (Supplemental Data 6). It should be noted that the general approximation when working with large numbers of probe sets is to assume that each probe set represents hybridization to a single gene. However, multiple probe sets can exist for certain genes, so the actual number of genes included in the analysis is significantly lower than the number of probe sets analyzed. The somatic-specific and ESC-specific genes identified from this comparison analysis were then used to investigate if the CRES cell lines had successfully down regulated somatic-specific genes (Supplemental Table 4) and successfully upregulated ESC specific genes (Table 2) following comparison analysis with the three somatic donor cell replicates. For the tertiary stemness gene analysis, stemness genes were derived from our previous transcriptional profiling work with rhesus monkey ESC lines (Byrne et al., Biol Reprod 30, 2006). These twelve putative stemness genes had the highest average fold change in gene expression when three undifferentiated biological replicates of ORMES-6 were compared to their *in vitro* differentiated counterparts and all twelve were significantly upregulated in five different rhesus monkey embryonic stem cell lines examined. Comparison analysis was performed between the ORMES and CRES cell line replicates and the donor somatic cell replicates (Supplemental Data 7) and the average fold change (FC) increase in gene expression of the twelve stemness genes in the ORMES and CRES cell lines was calculated (Table 3). All of the normalized microarray data sets generated from these studies can be found on the GEO website, as noted above.

Final gene expression data table(s) used by the authors to make their conclusions after data selection and transformation (gene expression data matrices).

All normalized microarray data sets generated from these studies can be found in on GEO: <ftp://ftp.ncbi.nih.gov/pub/geo/DATA/supplementary/series/GSE7748>.

Supplemental_Data_3_Microarray_Comparisons.xls

Contains the comparison analysis for all 52,865 probe sets for selected ESC and somatic sample comparisons (Supplemental Data 3).

Supplemental_Data_4_Donor_Comparisons.xls

Comparison analysis was performed between each of the three control ORMES-10 biological replicates and each of the three somatic donor cell replicates, to give a total of nine somatic-ESC comparisons (Supplemental Data 4).

Supplemental_Data_5_Somatic_specific_genes.xls

The following selection criteria were used to identify rhesus somatic-specific genes: 1) genes that were considered to be present ($P < 0.05$) in all three somatic donor cell replicates and 2) genes that demonstrated statistically significant decrease “D” in gene expression in the ORMES-10 replicates in all nine comparisons with the somatic donor cell replicates following GCOS comparisons with MAS-5 statistical analysis. A total of 4,998 somatic-specific probe sets were identified in this way (Supplemental Data 5).

Supplemental_Data_6_Embryonic_specific_genes.xls

The following selection criteria were used to identify rhesus ESC-specific genes: 1) genes that were considered to be present ($P < 0.05$) in all three ORMES-10 replicates and 2) genes that demonstrated a statistically significant increase “I” in gene expression in the ORMES-10 ESC replicates in all nine comparisons with the somatic donor cell replicates following GCOS comparisons with MAS-5 statistical analysis. A total of 6,178 ESC-specific probe sets were identified in this way (Supplemental Data 6).

Supplemental_Data_7_Stemness_Genes.xls

Stemness genes were derived from our previous transcriptional profiling work with rhesus monkey embryonic stem cell lines. These twelve putative stemness genes had the highest average fold change in gene expression when three undifferentiated biological replicates of ORMES-6 were compared to their *in vitro* differentiated counterparts and all twelve were significantly upregulated in five different rhesus monkey embryonic stem cell lines examined. Comparison analysis was performed between the ORMES and CRES cell line replicates and the donor somatic cell replicates (Supplemental Data 7).

ARRAY DESIGN

Platform Type

Synthesized Oligonucleotide Array

Surface and Coating Specifications

Glass

Availability of the Array (name/make)

Affymetrix Rhesus Gene array

For each feature (spot) on the array, its location on the array and the ID of its respective reporter (molecule present on each spot) should be given.

See www.affymetrix.com

For each reporter, its type (e.g., cDNA or oligonucleotide) should be given, along with information that characterizes the reporter molecule unambiguously, in the form of appropriate database reference(s) and sequence (if available).

See www.affymetrix.com