Supplemental Information

Bonk et al. CORRELATION OF DEVELOPMENTAL DIFFERENCES OF NUCLEAR TRANSFER EMBRYOS CELLS TO THE METHYLATION PROFILES OF NUCLEAR TRANSFER DONOR CELLS IN SWINE

Figure S1. Hierarchical support tree including bootstrap analysis with replacement after 1000 iterations by using spots that were significantly different ($P<0.01$) between the donor cells and *in vivo*-produced blastocyst stage embryos.

Figure S2. Methylation status of the Clone S E3 in the liver (A) and clonal cell line A2 (B), Clone X G2 in the liver (A) and clonal cell line A7 (B), B G2 in the liver (A) and clonal cell line A2 (B), Clone HH A7 in the liver (A) and clonal cell line A7 (B), Clone K D3 in the liver (A) and clonal cell line A2 (B), detected by using bisulfite sequencing.

Figure S3. Methylation status of CpG sites of five regions were analyzed by using PDMH microarrays and bisulfite modification PCR sequencing.

Table S1. Bisulfite modification specific primers.

Table S2. Methylation status of CpG sites of five regions were analyzed by using PDMH microarrays and bisulfite modification PCR sequencing.

Table S3. Sequenced clones exhibiting similar methylation profiles in the gametes and blastocysts as determined by Self Organizing Map analysis.

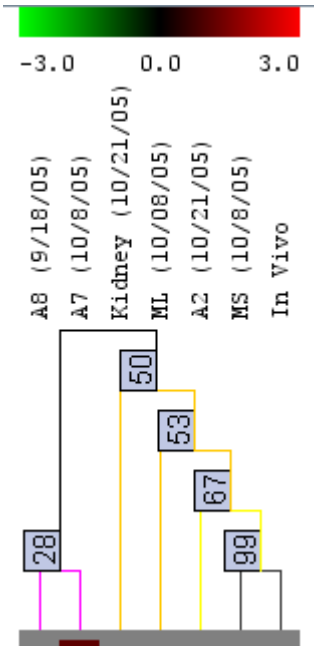

Figure S1. Hierarchical support tree including bootstrap analysis with replacement after 1000 iterations by using spots that were significantly different (P<0.01) between the donor cells and *in vivo*-produced blastocyst stage embryos. Differential methylation in the gametes and blastocysts was previously identified by using PDMH analysis and bisulfite sequencing (Bonk et al., 2007). Larger numbers at the nodes (range=1 to 100) indicates the support of the clustering. The clustering pattern generated by using the Pearson Correlation metric in the TIGR Multiple Array Viewer is partially consistent with the clustering pattern produced by using GeneSpring software. These results demonstrate the consistency of the separation of A7 and A8 cell lines from the rest of the samples.

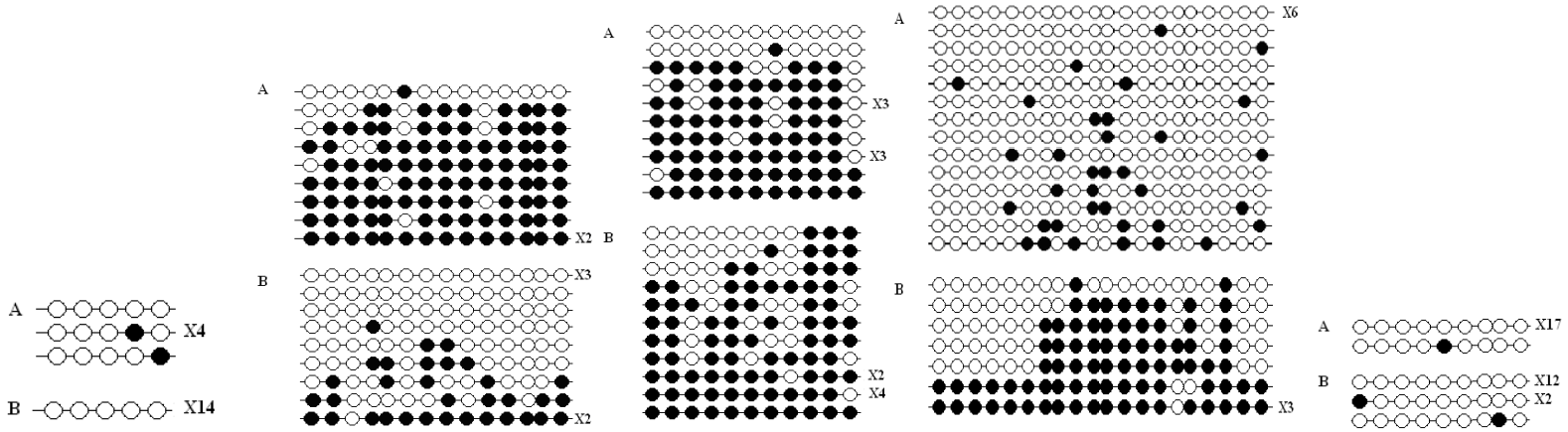

Figure S2. Methylation status of the Clone S E3 in the liver (A) and clonal cell line A2 (B), Clone X G2 in the liver (A) and clonal cell line A7 (B), B G2 in the liver (A) and clonal cell line A2 (B), Clone HH A7 in the liver (A) and clonal cell line A7 (B), Clone K D3 in the liver (A) and clonal cell line A2 (B), detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence.

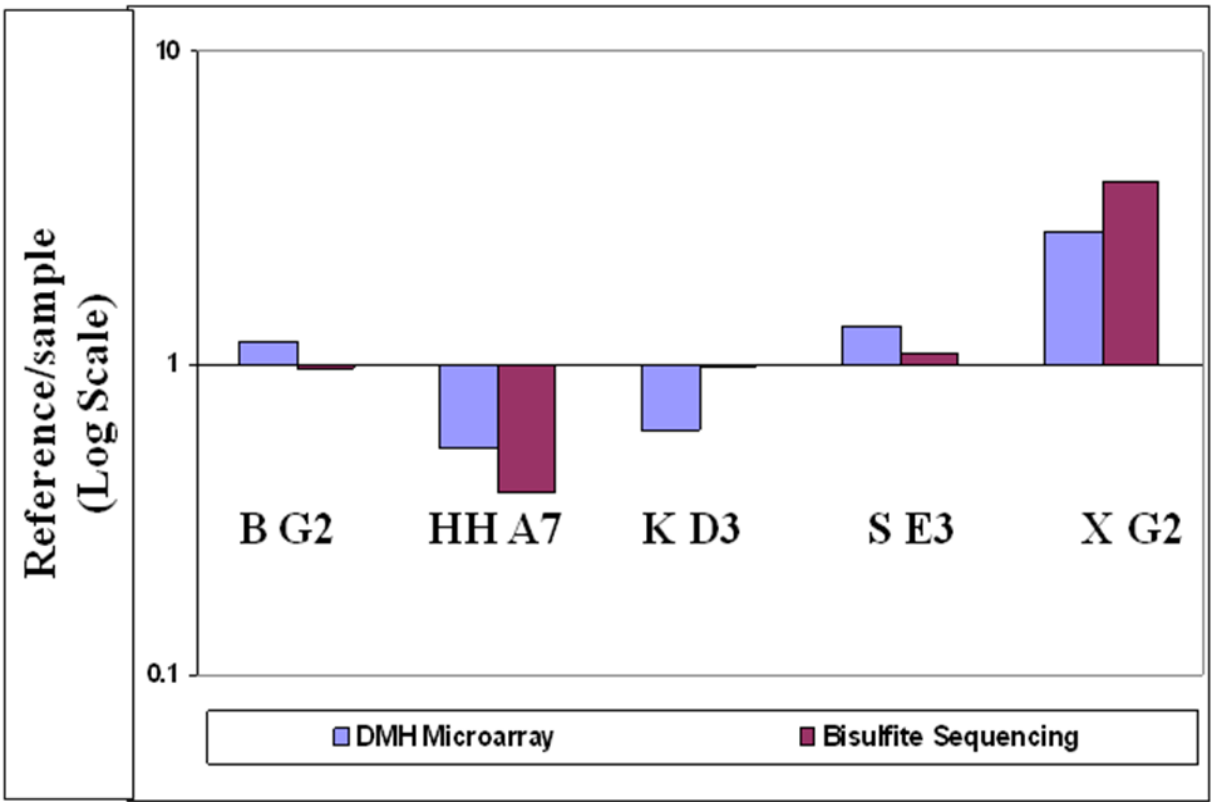

Figure S3. Methylation status of CpG sites of five regions were analyzed by using PDMH microarrays and bisulfite modification PCR sequencing. Similar ratios were obtained when methylation was analyzed by either method thereby validating the microarray data.

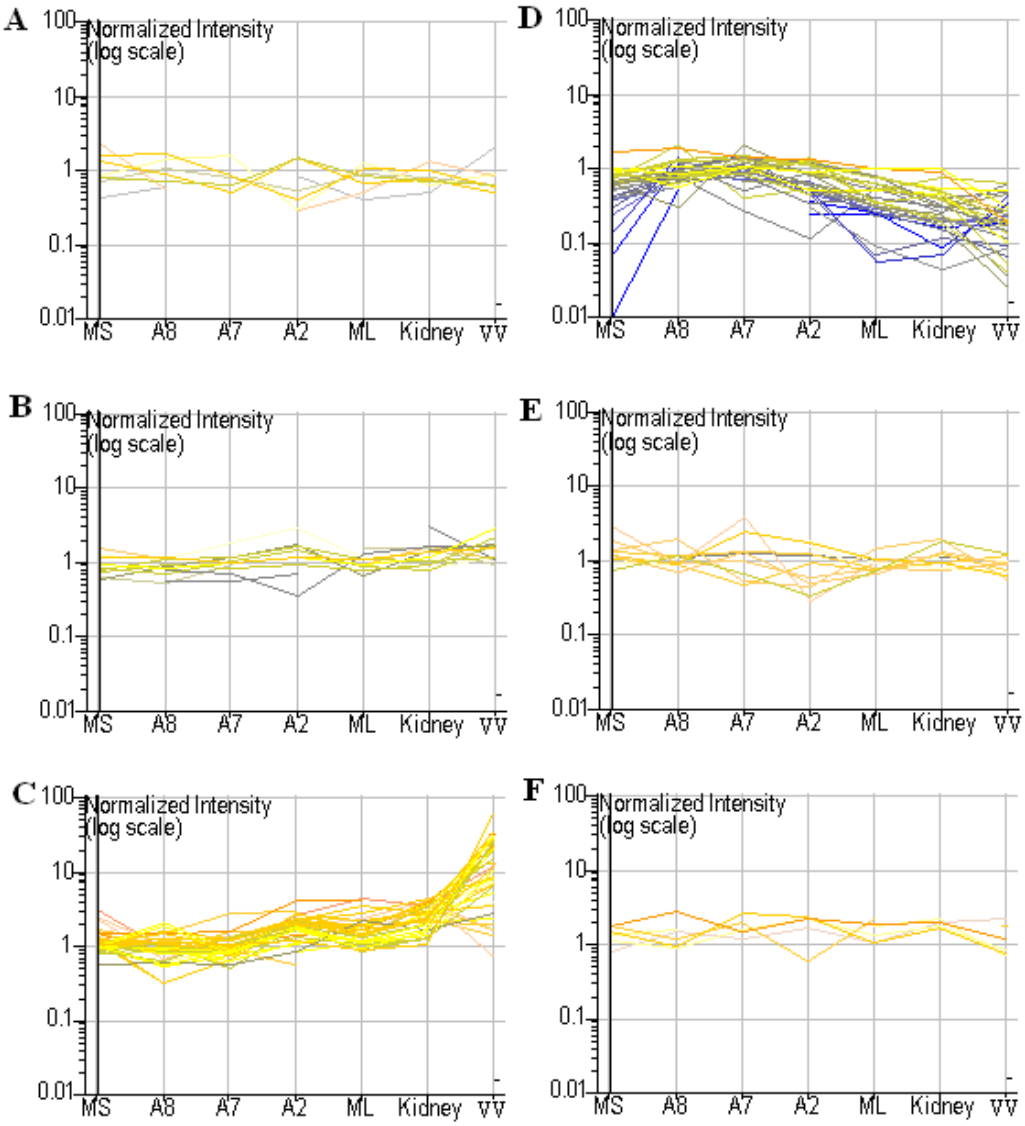

Figure S4. Clones with similar methylation profiles in the donor cells and the *in vivo*-produced blastocysts were clustered by using Self Organizing Map analysis. Representative clones from each of the clusters are listed in Table S3. Hypermethylation levels of the donor cells in (C) are positively correlated decreasing blastocyst rates after SCNT.

Table S1. Bisulfite modification specific primers.

CPG Clone	Primer position	5'----------3'
B G2	LEFT	TTT TAT TAA TGG GAG GTA GAA TTA G
B G2	RIGHT	TAA AAA CAA AAT TCT CCC AAC CTC
HH A7	LEFT	GTT AAA GTT TGG AGT AAA AGG TG
HH A7	RIGHT	AAT TTA AAA CCC CAT ATT AAA ACC
K D3	LEFT	AAT AAT AAA GTT TTA GGA GGG ATT T
K D3	RIGHT	ATA CTA CCC AAC CCA AAC AAA AAA
S E3	RIGHT	AAA AAA AAT AAC AAT TCC ACC ACC
S E3	LEFT	GTT TAT GGG GAA GTT TAG GGT AGA G
X G2	RIGHT	TAA ACA CTA ACC CAA AAA AAC CTT C
X G2	LEFT	GTT TGG TAG GGG AGT TTG TAG AGT

Table S2. Methylation status of CpG sites of five regions were analyzed by using PDMH microarrays and bisulfite modification PCR sequencing. Similar ratios were derived based on the methylation levels detected in the liver and *in vivo*-produced blastocysts when analyzed by either method. The PDMH values are LOWESS normalized Cy5/Cy3 ratios representing the methylation status of the specified clones in the liver (Cy5) and in the clonal donor cells (Cy3) samples. The Bisulfite Analysis values represent methylation in the liver and in the clonal donor cells at selected regions of the specified clones. The clonal cell line (A2 or A7) is shown in parentheses. The Bisulfite Analysis values were calculated from the equation shown in the Materials and Methods section. Bisulfite Analysis data validates the microarray analysis data for all the samples. The ratios produced from the microarray and bisulfite analysis were classified as consistent when the bisulfite analysis-produced ratio indicated the sample was hypomethylated (>1) or hypermethylated (<1) and matched the hypermethylation status of the microarray-produced data. From the microarray-produced ratios, the samples were classified as hypermethylated when the ratio was < 0.75 and the sample was classified as hypomethylated when the ratio was >1.25.

A B

	Bisulfite Analysis		Reference/Sample	
CpG Clone	Liver	Donor Cell	Bisulfite	Microarray
B G2 (A7)	69.2%	73.9%	0.847	1.180
HH A7 (A7)	9.2%	65.6%	0.379	0.530
K D3 (A2)	0.7%	1.5%	0.992	0.610
S E3 (A7)	6.9%	0.0%	1.074	1.310
X G2 (A2)	80.7%	16.3%	4.337	2.640

Table S3. Sequenced clones exhibiting similar methylation profiles in the gametes and blastocysts as determined by Self Organizing Map analysis. Spots that were significantly different ($P<0.05$) were included in the analysis, BLAST analysis identified 21.1% (22/104) of the clones as having similarity to multiple regions, 38.5% (40/104) of the clones as having no similarity to existing sequenced, and 40.4% (42/104) of the clones as having similarity to sequenced clones as similar to identified or predicted genes.

A				
G A10	396	Human DNA sequence from clone RP11-697G4 on chromosome 6, 5' end of the FOXO3A gene	FOXO3A	AL391646.
PINK A9	157	activating transcription factor 2	ATF2	
NN H8		NS		
B				
QQ E4	149	PREDICTED: Bos taurus similar to peptidyl prolyl isomerase H	PPIH	XM_873469.
II B3	293	BAC		AC016595.
X G10		NS		
U B12		NS		
N G6		NS		
T F3	188	Human GLA gene for alpha-D-galactosidase A (EC 3.2.1.22).	GLA	X14448.
QQ A1		NS		
A C6		Multiple		
E A10		Multiple		
C				
G G10	188	PREDICTED: Canis familiaris similar to DEAD (Asp-Glu-Ala-Asp) box	DDX10	XM_536583
P F6	180	Bos taurus similar to protoporphyrinogen oxidase, Last enzyme of heme synth.	PPOX	XM_593850
AA A1		NS		
UU C10		NS		
D D6		Multiple		
X G2		NS		

Table S3 (continued)

Blue D7		Sus scrofa CC chemokine receptor genes (CCR9)	CCR9	
GGG D4	226	WNT8B gene	WNT8B	Y11108.
EEE E3		Bac matches		
X F12		Homo sapiens prostate antigen PARIS-1 mRNA, complete cds	TBC1D2	
UU H3		NS		
RR G5	597	H. sapiens genes for histones H2B.1 and H2A	HIST2H2 BE	BC069193.
EEE D4	299	Homo sapiens cell division cycle 27 (CDC27) gene, complete cds	CDC27	AY518321.
XX H10		NS		
P H5	200	Zinc finger, CSL-type containing 2	ZCSL2	XM_874300.
XX H12	133		SF3A3	NM_006802.
III D1		Multiple		
LL E4		NS		
D D10		NS		
NN G9		NS		
CCC H12	113	Multiple		
JJ B10	910	Bos taurus similar to Homeobox protein SIX6 (Sine oculis homeobox homolog 6)	SIX6	XM_589185
JJ D12	129	H.sapiens CpG islands		
JJ E10		NS		
B G2		NS		
O D12		Multiple		
BBB H7		Multiple		
EEE B9		Multiple		
F E10		Multiple		
QQ A6		Multiple		
EEE B7		Multiple		
D				
CCC B6		Multiple		
G B8	123	Canis familiaris similar to Coatomer zeta-1 subunit	Zeta-1 COP Not found in Hugo	XM_843171.
EE H2		Multiple		

Table S3 (continued)

L E8		CpG Island plus others		
CC B12		NS		
BLUE E4		NS		
PP G1		NS		
NN F4	151	Mus musculus RIKEN cDNA 2810429O05 gene	Not found in Hugo	NM_134046.
EE H8	159	Homo sapiens aryl hydrocarbon receptor nuclear translocator	ARNT	AY430083.
PINK E8		Multiple		
W E3		NS		
PINK F7		Multiple		
PP C2		NS		
PP E4	293	PREDICTED: Bos taurus similar to malignant T cell amplified sequence 1	MCTS1	XM_593366.
EE A11		Membrane bound O-acyltransferase domain		
PP E2		Multiple		
QQ D3		NS		
Q A2	569	Homo sapiens serine/threonine protein kinase Kp78 (ribosomal)	MARK3	AF159295.
TT G8	121	acidic (leucine-rich) nuclear phosphoprotein 32 family, member E	ANP32E	AL138795.
Q H5	103	PREDICTED: Bos taurus similar to Forkhead box protein J2	FOXJ2	XM_612715
PP E5	145	PREDICTED: Canis familiaris similar to Methyltransferase-like	Not found in Hugo	XM_537604.
N E2	553	Homo sapiens serine/threonine protein kinase Kp78 (ribosomal)	MARK3	AF159295.
II G8		NS		
PP D6		NS		
Pink E2		NS		
FF G1		Bac matches		
Pink E10		Multiple		
W F1		NS		
E				
P H3	73.8	Homo sapiens similar to ankyrin-repeat protein Nrarp	Nrarp Not found in Hugo	BC053618.
III C8	196	Homo sapiens UMPS gene for UMP synthase	UMPS	AY691629.

Table S3 (continued)

M C9	69.1	H. sapiens clone RP11-4181C1 on chromosome 10	MLLT10	AL358780.
T G4	167	Homo sapiens RPL18 gene for ribosomal protein L18, complete cds	RPL18	AB061825.
PP F12		NS		
U G4		Multiple ribosomal proteins		
P D2		NS		
S E3	168	Homo sapiens protoporphyrinogen oxidase (PPOX) gene, exons 2, 3,	PPOX	AY032686.
F				
P G12		NS		
T F1		NS		
PP D2		NS		