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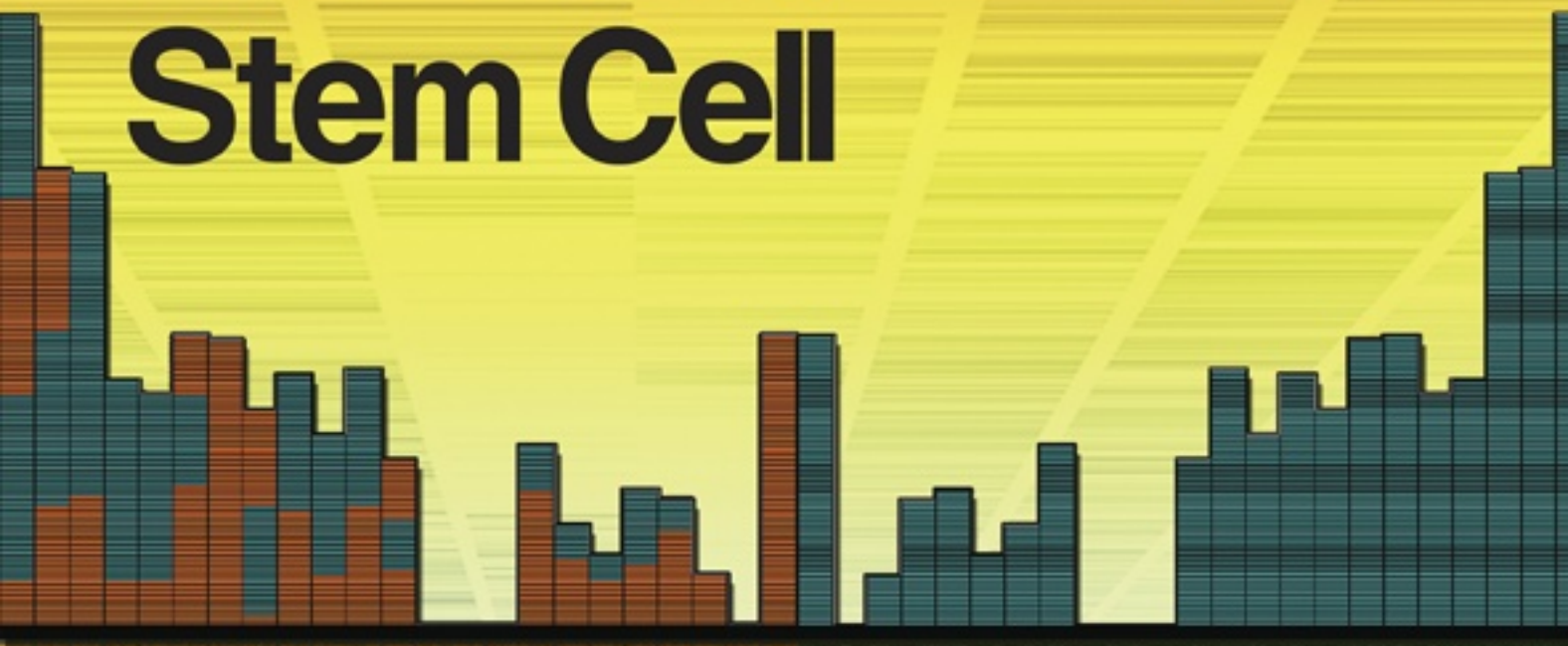

SNP Signature of Parthenogenetic Human ES Cells

Cell Stem Cell
Short Article

Recombination Signatures Distinguish Embryonic Stem Cells Derived by Parthenogenesis and Somatic Cell Nuclear Transfer

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SUMMARY

Parthenogenesis and somatic cell nuclear transfer (SCNT) are two methods for deriving embryonic stem (ES) cells that are genetically matched to the oocyte donor or somatic cell donor, respectively. Using genome-wide single nucleotide polymorphism (SNP) analysis, we demonstrate distinct signatures of genetic recombination that distinguish parthenogenetic ES cells from those generated by SCNT. We applied SNP analysis to the human ES cell line SCNT-hES-1, previously claimed to have been derived by SCNT, and present evidence that it represents a human parthenogenetic ES cell line. Genome-wide SNP analysis represents a means to validate the genetic provenance of an ES cell line.

INTRODUCTION

ES cells can be derived by several methods. Numerous human ES cell lines have been derived from excess embryos generated in the course of in vitro fertilization (IVF) for the treatment of infertility. Parthenogenetic ES (pES) cells have been derived from mouse and primate embryos that result from artificial activation of oocytes without fertilization (Cibelli et al., 2002; Robertson et al., 1983). ES cells derived from embryos generated by somatic cell nuclear transfer (ntES) are of considerable interest as a source of ES cells for research and tissue transplantation (Rideout et al., 2002).

SCNT-hES-1 represents the first human ES cell line purportedly generated by somatic cell nuclear transfer (SCNT). In January of 2006, the editors of *Science* retracted the paper by Hwang et al. (2004) (Kennedy, 2006) following findings by the Seoul National University Investigation Committee (SNUIC) of research misconduct (Investigation Committee Report, 2006). To investigate whether the cell line was generated by SCNT or parthenogenesis, the SNUIC commissioned genetic and epigenetic analysis. DNA fingerprint analysis of nuclear donor cells and SCNT-hES-1 indicated that all 40 of 40 informative markers were shared with the oocyte donor, but 32 were heterozygous while 8 were homozygous, leaving the genetic provenance unexplained. Expression analysis of imprinted genes and bisulphate sequencing of imprinted gene loci suggested the line might represent the accidental isolation of a pES cell, but such studies alone are rarely definitive. Because pES cells had been presumed to be predominantly homozygous due to duplication of the haploid genome (Kaufman et al., 1983; Liu et al., 2003), the largely heterozygous pattern of polymorphic markers seemed inexplicable, and thus the nature of the cell line was left in doubt pending further definitive analysis by the scientific community.

Recently, we have analyzed the patterns of genetic recombination in parthenogenetically derived murine ES cell lines (Kim et al., 2007). The most efficient parthenogenetic protocols generate p(MII)ES cells, which show a pattern of recombination that reflects the failure of independent segregation of the sister chromatids during meiosis II. Contrary to expectation, heterozygosity predominates as a result of meiotic recombination, regardless of whether parthenogenesis entails interruption of meiosis II or I (Kim et al., 2007). When derived from hybrid F1 mice, p(MII)ES cells retain pericentromeric homozygosity but show distal regions of heterozygosity. p(MI)ES cells generated by disrupting karyokinesis of meiosis I (Kubiak et al., 1991) show a pattern of recombination that reflects

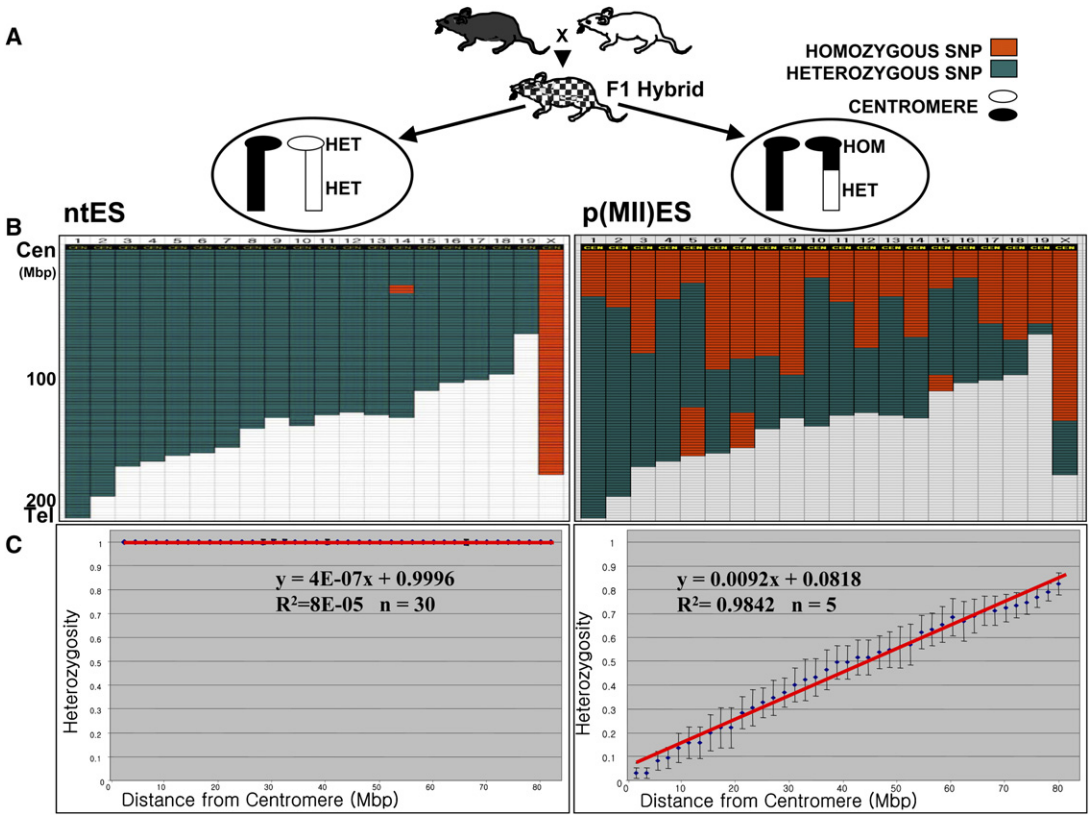

Figure 1. Patterns of Genomic Homozygosity and Heterozygosity in ES Cells Derived by Nuclear Transfer and Parthenogenesis from F1 Hybrid Mice

(A) Schematic of chromosomal genotypes predicted for ES cells of indicated types. Heterozygous region, HET; homozygous region, HOM.

(B) Depiction of SNP genotypes of a representative clone of male ntES cells and female p(MII)ES cells. Chromosome numbers are indicated along the top, and markers are arrayed for the acrocentric murine chromosomes from centromeric (Cen; top) to telomeric (Tel; bottom) in blocks that span a physical distance of 2 Mbp. Distance is marked in megabase pairs (Mbp). Orange blocks, homozygous (HOM) haplotypes; blue blocks, heterozygous (HET) haplotypes.

(C) Graphs show the heterozygosity of SNP markers plotted against SNP marker distance from the centromere. n = 30 for ntES; n = 5 for p(MII). Slope function describing the data is indicated (and rationale is provided in the Experimental Procedures). Error bars represent standard deviation.

the failure of segregation of the parental chromosomes; p(MI)ES cells retain peri-centromeric heterozygosity of genetic markers and have characteristic distal regions of homozygosity. Because ES cells generated by SCNT should be genetic clones of the donor, ntES cells generated from a hybrid F1 mouse should show heterozygosity across all loci, with only occasional deviations due to mitotic recombination or somatic mutation.

Herein, we provide a thorough comparative analysis of five novel pES cells and 30 nuclear transfer-derived murine ES cell lines, as well as SCNT-hES-1 by genome-wide single nucleotide polymorphism (SNP) genotyping. We analyze the murine samples in a manner that facilitates comparison to a single cell line like SCNT-hES-1. Our analysis shows that the recombination pattern of SCNT-hES-1 is distinct from that of an ntES cell line and is consistent with its derivation from a parthenogenetic embryo. Thus, we conclude the derivation of SCNT-hES-1 represented the first reported successful isolation of human pES cells.

RESULTS

To determine the recombination patterns of ntES and pES cells, we performed genome-wide SNP analysis (Moran et al., 2006) in 30 euploid ntES cell lines generated from hybrid strains of mice using a variety of donor cells and compared the results with five newly derived p(MII)ES cell lines (Figure 1). Cell lines derived from embryos produced by nuclear transfer from a hybrid F1 mouse show complete heterozygosity at all informative SNP markers (Figure 1B, left panels; and see Figure S1 in the Supplemental Data available with this article online), except for rare occurrences of mitotic recombination or gene conversion (e.g., Figure 1B, chromosome 14; Donahue et al., 2006). There is no discernible relationship between rates of marker recombination and marker distance from the centromere (Figure 1C). Analysis of these five newly derived murine p(MII)ES cell lines shows the characteristic pericentromeric homozygosity (Figure 1B, right panel) and increasing heterozygosity as marker distance

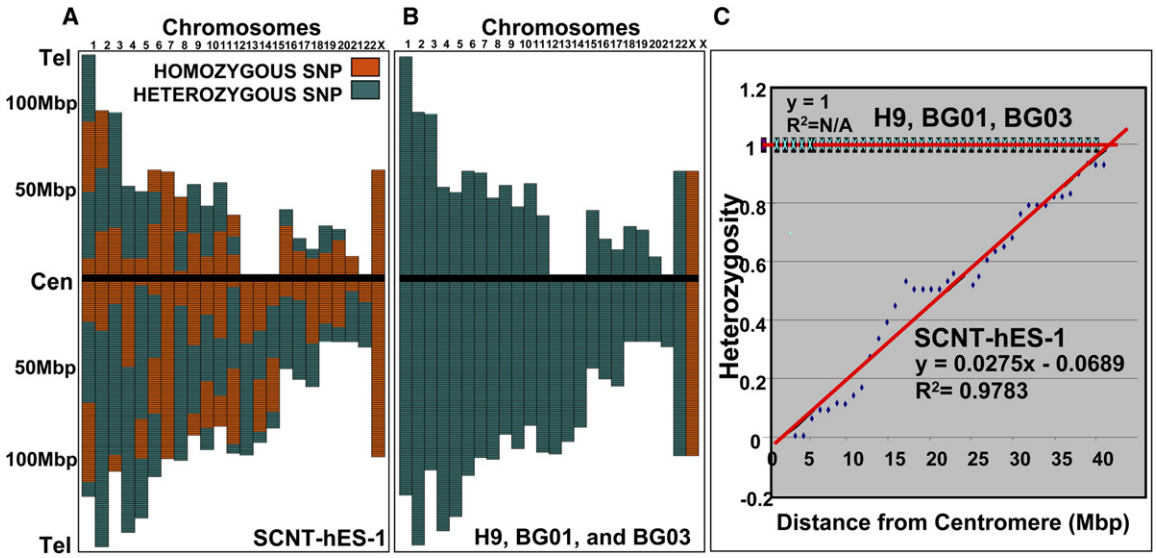

Figure 2. SNP Genotype Data for SCNT-hES-1 and Three Representative Human ES Cell Lines

Genome-wide SNP mapping was performed using the GeneChip Human Mapping 500K SNP Array. (A) SCNT-hES-1. Genotyping data are depicted as in Figure 1, except that short p arm of the human chromosomes project superiorly, while long q arm projects inferiorly. Note pericentromeric regions of homozygosity for each chromosome. Conversion to homozygosity near telomeres is a reflection of the high frequency of double recombination in human chromosomes; (B) genotyping data are shown for three human ES cell lines (H9, BG01, and BG03) generated from fertilization embryos. The patterns of panheterozygosity were identical for all three lines (excepting the X chromosome data, which show homozygosity in the male line BG01); thus the data are presented as a composite. Orange blocks, homozygous (HOM) haplotypes; blue blocks, heterozygous (HET) haplotypes. (C) Heterozygosity of SNP markers plotted against SNP marker distance from the centromere for the four cell lines. Slope function is indicated. Error bars represent standard deviation.

increases from the centromere (Figure 1C, right panels), which show the existence of an identical pattern regardless of genetic background (B6D2F1) and ES cell isolation method.

We used the GeneChip Human Mapping 500K SNP Array set (Affymetrix) to investigate the patterns of marker heterozygosity across all chromosomes of SCNT-hES-1, based on the hypothesis that derivation by SCNT would reveal genome-wide heterozygosity, whereas parthenogenesis would be reflected by large blocks of homozygosity, with the relationship of these blocks to the centromere indicative of an interruption of either meiosis I or II. For comparison, we determined the genome-wide patterns of marker heterozygosity for the human ES cell lines H9, BG01, and BG03, which were derived from embryos created by IVF and confirmed to have normal karyotype. Genotyping data for the hemizygous X chromosome from the male human ES cell line, BG01, served as a control for genotyping error rates. Across this single X chromosome, 2.3% of genotypes were reported as heterozygous (241 out of 10,536 calls). The error rates across this chromosome fit a normal distribution, with >99% of the blocks of 1000 markers showing an error rate < 5%. Thus, we assigned homozygosity to any block of 1000 SNPs (with a median distribution of one SNP per 2.5 kb) where the heterozygous SNP frequency was at or below 5.0% (50 per 1000). (In such an analysis, the random variable is calculated to be 49.429 in a normal distribution with an error rate of 1%, meaning that as many as 49 individual heterozygous SNPs per 1000 could occur by chance alone.) Using this parameter, all of the X chromosome regions from BG01 fit the criteria of a homozygous chromosome, and none of the other regions in chromosomes from H9, BG01, and BG03 were called homozygous regions (Figure 2B). Differences between the heterozygous and homozygous samples were evaluated by χ^2 analysis and revealed a high degree of significance ($p < 0.0001$).

We analyzed the genotyping data for SCNT-hES-1 using the assumptions described above. Chromosome by chromosome, homozygosity predominates at pericentromeric markers, and heterozygosity at more distal markers (Figure 2A). When the SNP heterozygosity data for SCNT-hES-1 are plotted with respect to the marker distance from the centromere (Figure 2C), one observes the pattern characteristic of mouse p(MII)ES cells (Figure 1C). This analysis suggests that SCNT-hES-1 is indeed a human p(MII)ES cell line.

Interestingly, chromosomes 7 and X show patterns of complete homozygosity in SCNT-hES-1 (Figure 2A). The hybridization signal for the human SNP genotyping array showed monoallelic intensity for the X chromosome markers and biallelic intensity for the markers on chromosome 7 (Komura et al., 2006). Cytogenetic analysis showed a single copy of the X chromosome, and two copies of chromosome 7 (Figure S3). The original analysis reported for SCNT-hES-1 revealed an XX karyotype, suggesting that the subline of SCNT-hES-1 cells studied here has undergone X chromosome loss. Prior DNA

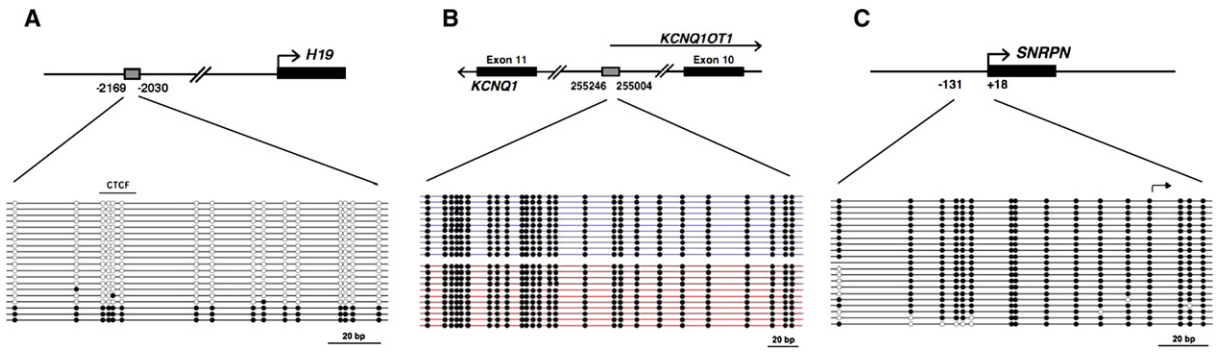

Figure 3. Bisulphite Sequencing of Three Differentially Methylated Regions in SCNT-hES-1 Cells

Circles represent the position and methylation status of individual CpG sites (filled, methylated; open, unmethylated), and each line represents a unique clone of DNA. The numbering of the first and last CpG sites for *H19* and *SNRPN* DMRs is relative to the transcriptional start sites shown, and the numbering for *KCNQ1OT1* DMR is according to the *KCNQ1* sequence (AJ006345). A polymorphism in the *KCNQ1OT1* DMR distinguished the two alleles (blue and red lines). (A) H19. (B) KCNQ1OT1. (C) SNRPN.

fingerprinting analysis of a highly polymorphic marker on chromosome 7 showed heterozygosity (D75820; 08, 11; SNUIC; Seoul National University Investigation Committee, 2006), whereas a repeat fingerprint analysis of the subline studied here shows homozygosity (08-08), suggesting that our line sustained loss of a single copy of chromosome 7 and duplication of the remaining one, a phenomenon that has been reported in cultured cell lines (Donahue et al., 2006). Except for these differences, DNA fingerprint analysis of the subline of SCNT-hES-1 studied here using a set of 16 polymorphic markers distributed across multiple chromosomes matched the fingerprinting data reported for SCNT-hES-1 by the SNUIC (Table S1), thereby confirming the identity of our line of SCNT-hES-1 as the isolate reported by Hwang and colleagues.

Mammalian cells carry parent-of-origin patterns of DNA methylation at imprinted gene loci due to differential modification in male and female gametes, and parental-specific DNA methylation is subsequently maintained throughout development. To provide an additional assay that can distinguish parthenogenetic from biparental cell types, we analyzed the methylation status of three differentially methylated regions (DMRs) in differentiated SCNT-hES-1 cells by bisulphite treatment followed by sequencing. The normally paternally methylated *H19* DMR on chromosome 11 was predominantly unmethylated (3/20 DNA strands methylated; significantly different from the expected 10/20, $p = 0.002$, χ^2 test), whereas the normally maternally methylated *KCNQ1OT1* and *SNRPN* DMRs on chromosomes 11 and 15, respectively, were both fully methylated (22/22, $p = 3 \times 10^{-6}$ and 21/21, $p = 5 \times 10^{-6}$, respectively; Figure 3). Importantly, a polymorphism was identified that distinguished the two *KCNQ1OT1* DMR alleles, thereby revealing that both alleles were fully methylated. This epigenotype contrasts with normal differential methylation patterns observed at the same DMRs in hES cells derived from fertilized embryos (Rugg-Gunn et al., 2005a) and is characteristic of parthenogenetic cells that contain two maternal genomes and no paternal genome. This epigenetic assessment confirms our genome-wide SNP analysis, thereby providing more evidence that SCNT-hES-1 was derived from a parthenogenetically activated embryo.

We have described a strategy for isolating murine pES cells that are genetically matched to the oocyte donor at major histocompatibility complex (MHC) loci (Kim et al., 2007). The mouse MHC cluster is located ~32 Mbp from the centromere on chromosome 17. This region is predicted to be 37.6% heterozygous in p(MII)ES cells (Figure 1C) and 87.2% heterozygous in p(MI)ES cells (Kim et al., 2007). We observed MHC heterozygosity in 33% of p(MII)ES cells (24/72) and 87% of p(MI)ES cells (13/15) (Kim et al., 2007), in close agreement with our prediction.

By applying a similar analysis in human samples, we can determine the probability that any given human pES cell line will be genetically identical at the maternal histocompatibility loci to the oocyte donor. The recombination frequency of the human genome is higher than the mouse genome (Kong et al., 2002), and the human female genetic map is 72% larger than the male due to a higher frequency of recombination in female meiosis (Kong et al., 2002). The female human chromosome 6, which contains the human MHC cluster, has 241.55 cM of genetic distance over 190.87 Mb of physical distance (an average of 1.26 cM/Mb) (Kong et al., 2002). Thus, human chromosome 6 will reach peak heterozygosity, and thus sustain at least one crossover, within 39.7 Mb from the centromere. The genotyping data available for SCNT-hES-1 demonstrate that peak heterozygosity is indeed reached at the predicted physical distance around 38.9 Mb from the centromere (Figure 2C). The human MHC cluster is located 28.3–31.5 Mb from the centromere on chromosome 6. Thus, we predict that 70.9% of human p(MII)ES cells will show heterozygosity at the MHC loci and thereby match the oocyte donor in an autologous manner (Figure 2C).

We determined the HLA type for SCNT-hES-1 and found it to be homozygous: HLA-A (31, 31), HLA-B (35,

35), HLA-Cw (03, 03), HLA-DRB1 (04, 04), and HLA-DQB1 (0302, 0302). Genetic analysis of the MHC region of SCNT-hES-1 indicates that a crossover event occurred telomeric to the MHC-gene cluster (Figure S2C). Thus, SCNT-HES-1 represents a hemizygous HLA match to the oocyte donor.

DISCUSSION

Both parthenogenesis and nuclear transfer represent strategies for generating histocompatible ES cells for potential therapeutic use. Whereas nuclear transfer could potentially provide a nearly exact match to the nuclear donor's immune identity (matching nuclear, but not mitochondrial, genes), parthenogenesis could provide an exact match to the oocyte donor's genome (both nuclear and mitochondrial). Moreover, parthenogenesis could provide a source of cells that are either heterozygous or homozygous for major histocompatibility alleles, thereby allowing either complete MHC matching to the oocyte donor, or in the case of MHC homozygosity, partial MHC matching to a substantial population of unrelated transplant recipients (Taylor et al., 2005). Parthenogenesis is a more efficient means of generating embryos and ES cell lines than nuclear transfer, and to date human nuclear transfer has not been successfully used to generate an ES cell.

During experimental parthenogenesis in the mouse, cytochalasin is added to prevent the extrusion of the second polar body and to preserve the diploid state. In contrast, in human oocytes cytochalasin is not necessary to retain diploidy (De Sutter et al., 1992; Santos et al., 2003; Taylor and Braude, 1994), and a kinase inhibitor such as 6-dimethylaminopurine (DMAP) suffices to initiate diploid parthenogenetic development (Szollosi et al., 1993). The derivation protocol of SCNT-hES-1 employed DMAP after oocyte activation with a calcium ionophore. Thus, the protocols for generating ntES cell lines typically involve the same steps of artificial oocyte activation as parthenogenesis, and in the case of SCNT-hES-1, there was apparently no enucleation. Alternatively, there was refusion of the first polar body after enucleation (Wakayama et al., 2007). Regardless of the mechanism, the result was development of a diploid parthenogenetic embryo. To rule out a parthenogenetic origin of SCNT-hES-1, Hwang and colleagues offered evidence for expression of two imprinted genes that are normally only expressed from the paternally inherited allele. However, such aberrant expression can result from epigenetic instability, which is frequently observed in mouse pES cells (Dean et al., 1998; Feil et al., 1997). We have shown that methylation analysis of germline-acquired DMRs is a more robust indicator of epigenotype, although this too can alter following extensive in vitro culture (Humpherys et al., 2001; Mitalipov et al., 2007; Rugg-Gunn et al., 2005b).

For trials of nuclear transfer, if the somatic cell nucleus and the recipient oocytes come from different donors, the genomic DNA of any resulting ntES cells can be readily distinguished from parthenogenetic derivatives that might mistakenly arise. However, if nuclear transfer is performed using autologous oocytes from the somatic-cell donor, as in the case of SCNT-hES-1, all genetic markers will be shared, and selection of a small number of markers could mistakenly lead to the conclusion of genetic identity. Importantly, pES cells differ from ntES cells and ES cells generated from fertilized embryos in that certain regions of the genome show homozygosity and are thus only haploidentical to the oocyte donor. Genome-wide SNP genotyping is a reliable means of distinguishing parthenogenetic derivatives from those derived by nuclear transfer, because parthenogenetic embryo development incurs a diagnostic recombination signature that reflects the unique chromosomal dynamics of meiosis. Distinguishing ntES cells from those derived from fertilization embryos requires unequivocal demonstration of genetic identity to the somatic cell donor, or in cases where the somatic cell donor and oocyte donor differ, demonstration that the mitochondrial DNA is distinct from the somatic cell and instead derives from the oocyte.

The evidence indicates that SCNT-hES-1 represented the first reported isolation of a human pES cell. Despite the feasibility of generating patient-specific ES cells from females by parthenogenesis for use in research and potential therapy, concerns about their safety and differentiation efficiency remain. Mouse parthenogenetic embryos are unable to complete full development due to the absence of paternally expressed imprinted genes, and tissues derived from pES cells appear to have growth defects (Hernandez et al., 2003). However, recent reports show that genetic manipulation of a small number of imprinted genes (H19/Igf2; Dlk1-Gtl2) can enhance the quality of the parthenogenetic embryo and sustain full organismal development (Kono et al., 2004; Wu et al., 2006). Also, stable and functional hematopoietic engraftment has been reported from parthenogenetic cells in mice (Eckardt et al., 2007) and in a rare human parthenogenetic chimera (Strain et al., 1995). If careful genetic and functional analyses of tissues derived from human pES cells show them to be safe and effective, then pES cells might represent a favorable source for tissue replacement therapies.

EXPERIMENTAL PROCEDURES

Cytogenetic and Molecular Analysis

Cytogenetic analysis was performed by the Molecular Cytogenetics Core Facility of Memorial Sloan-Kettering Cancer Center, USA. DNA fingerprinting was performed by Cell Line Genetics, USA with the Powerplex 16 kit (Promega) (Goncalves et al., 2002). HLA typing was performed by the Blood Center of Wisconsin, USA with LABtype SSO kit (One Lambda) (Colinas et al., 2000). Human SNP analysis was performed by Affymetrix USA and the Molecular Genetics Core Facility of Children's Hospital Boston and Harvard Medical School with GeneChip Human Mapping Nsp Sty Array kit (Affymetrix) (Komura et al., 2006); mouse SNP analysis was performed at the Broad Institute NCRR Center for Genotyping and Analysis using the Illumina multiplexed allele extension and ligation method (Golden Gate) with detection using oligonucleotide probes covalently attached to beads that are assembled into fiber-optic bundles (Bead Array) (Moran et al., 2006).

SNP Data Analysis

In a prior analysis of SNP data from pES cells, we pooled data for each chromosome among multiple pES cells to calculate the relationship between marker heterozygosity and distance of the marker from the centromere (Kim et al., 2007). In order to generate a meaningful comparison of the pattern of genetic recombination in a single cell line (SCNT-hES-1) with murine ntES and pES cell lines, we analyzed the SNP data for five newly derived euploid p(MII)ES cell lines by pooling data for all markers at a given distance from the centromere across all chromosomes in individual cell lines (as illustrated in Figure 1C), thereby reducing the clonal variation we observed in the prior SNP analysis (Kim et al., 2007).

Procurement of SCNT-hES-1 and Handling of Research Materials and Data

DNA and mRNA extracts of SCNT-hES-1 and SCNT-hES-1 cell line were obtained from the Department of Theriogenology and Biotechnology, College of Veterinary Medicine, Seoul National University by Drs. Moore and Pederson under a material transfer agreement between their respective institutions and the Seoul National University. Research data, but not materials, were exchanged among the authors in the preparation of this manuscript.

Supplemental Data

Supplemental Data include three figures, one table, and Supplemental References and can be found with this article online at http://www.cellstemcell.com/cgi/content/full/1/3/346/DC1/.

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Note Added in Proof

During the preparation of this manuscript for publication, another paper appeared describing the derivation of six human parthenogenetic ES cells: Revazova, E.S., Turovets, N.A., Kochetkova, O.D., Kindarova, L.B., Kuzmichev, L.N., Janus, J.D., and Pryzhkova, M.V. (2007). Patient-specific stem cell lines derived from human parthenogenetic blastocysts. Cloning Stem Cells, in press. Published online June 26, 2007. 10.1089/clo.2007.0033.

Cell Stem Cell, Volume *1*

Supplemental Data
Recombination Signatures Distinguish Embryonic Stem Cells Derived by Parthenogenesis and Somatic Cell Nuclear Transfer

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Supplementary Figure 1

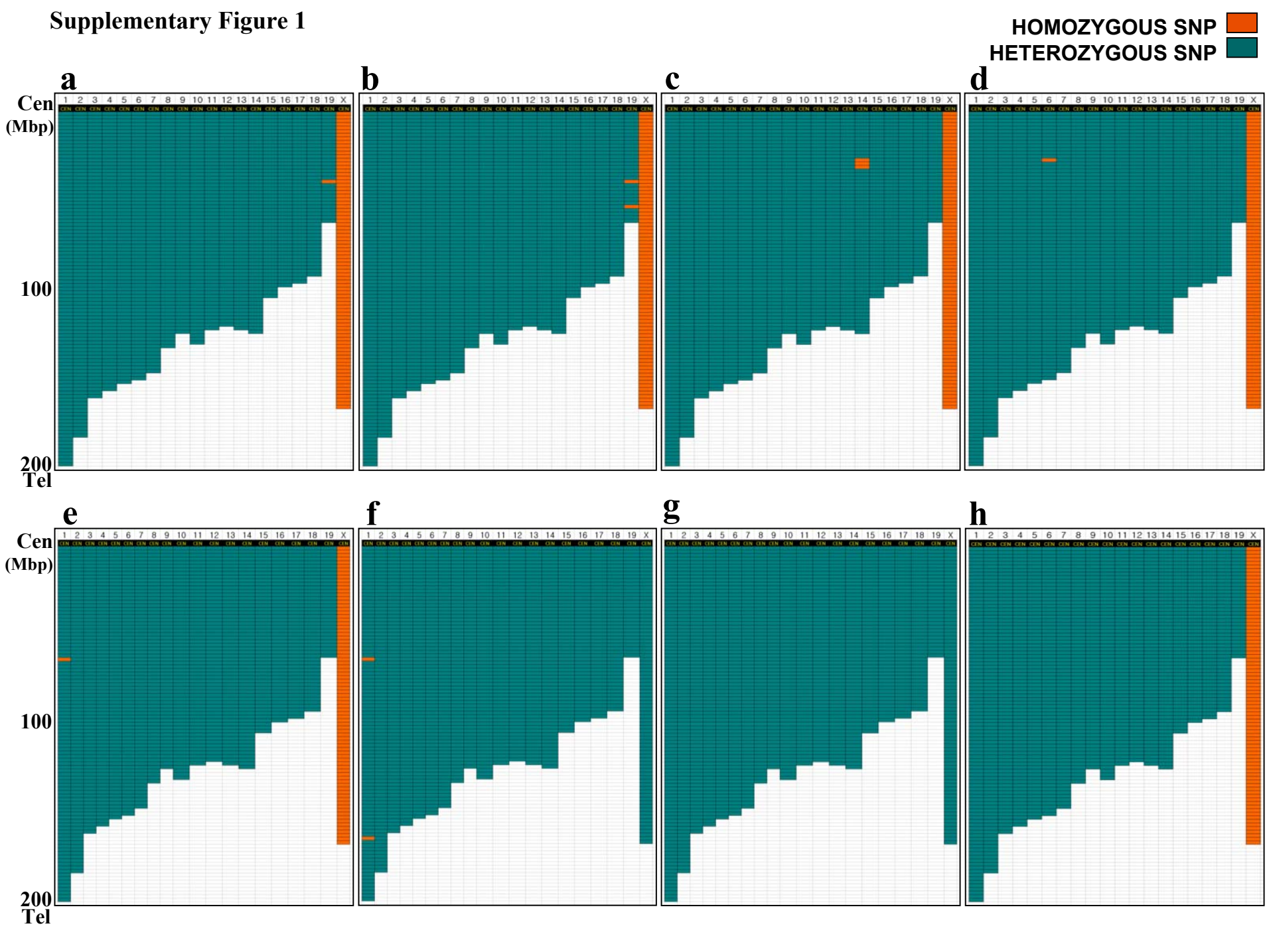


Figure S1. Genome-Wide SNP Genotyping of ntES Cells

Panels show genotypes for each chromosome, from centromere (cen, top) to telomere (tel, bottom), revealing blocks, or haplotypes, of markers. Orange blocks: homozygous (HOM) SNP regions; blue blocks: heterozygous (HET) SNP. **(a)** LN1 (B cell nt-donor cells from C57BL/6N x DBA/2J F1) [1]; **(b)** LN2 (T cell nt-donor cells from C57BL/6N x 129svjae F1) [1]; **(c)** V6.5 NSC B1 (neuronal stem cell nt-donor cells from C57BL/6N x 129svjae F1) [2]; **(d)** ESCC cells (fibroblast nt-donor cells form C57BL/6N x M.cast F1) [1]; **(e)** BCT-1F (fibroblast nt-donor cells from C57BL/6N x C3H/HeJ F1) [3]; **(f)** BCC-5 (cumulus nt-donor cells from C57BL/6N x C3H/HeJ F1) [3]; **(g)** BDC-2, BDC-5, BDC-9, BDC-10, BDC-11, and BDC-13 (cumulus nt-donor cells from C57BL/6N x DBA/2J) BDT-1F (fibroblast nt-donor cells from C57BL/6N x DBA/2J). BCC-1, BCC-3, BCC-4, and BCC-6 (cumulus nt-donor cells from C57BL/6N x C3H/HeJ F1) [3]; **(h)** LN3 (T cell nt-donor cells from C57BL/6N x 129svjae F1) [1]. V6.5 NSC B2 (neuronal stem cell nt-donor cells from C57BL/6N x 129svjae F1) [2]. BDT-2, BDT-3, BDT-5, BDT-6, BDT-7, and BDT-8 (fibroblast nt-donor cells from C57BL/6N x DBA/2J F1). BCT-1, BCT-2, BCT-3, BCT-4, and BCT-5 (fibroblast nt-donor cells from C57BL/6N x C3H/HeJ F1) [3].

Supplementary Figure 2

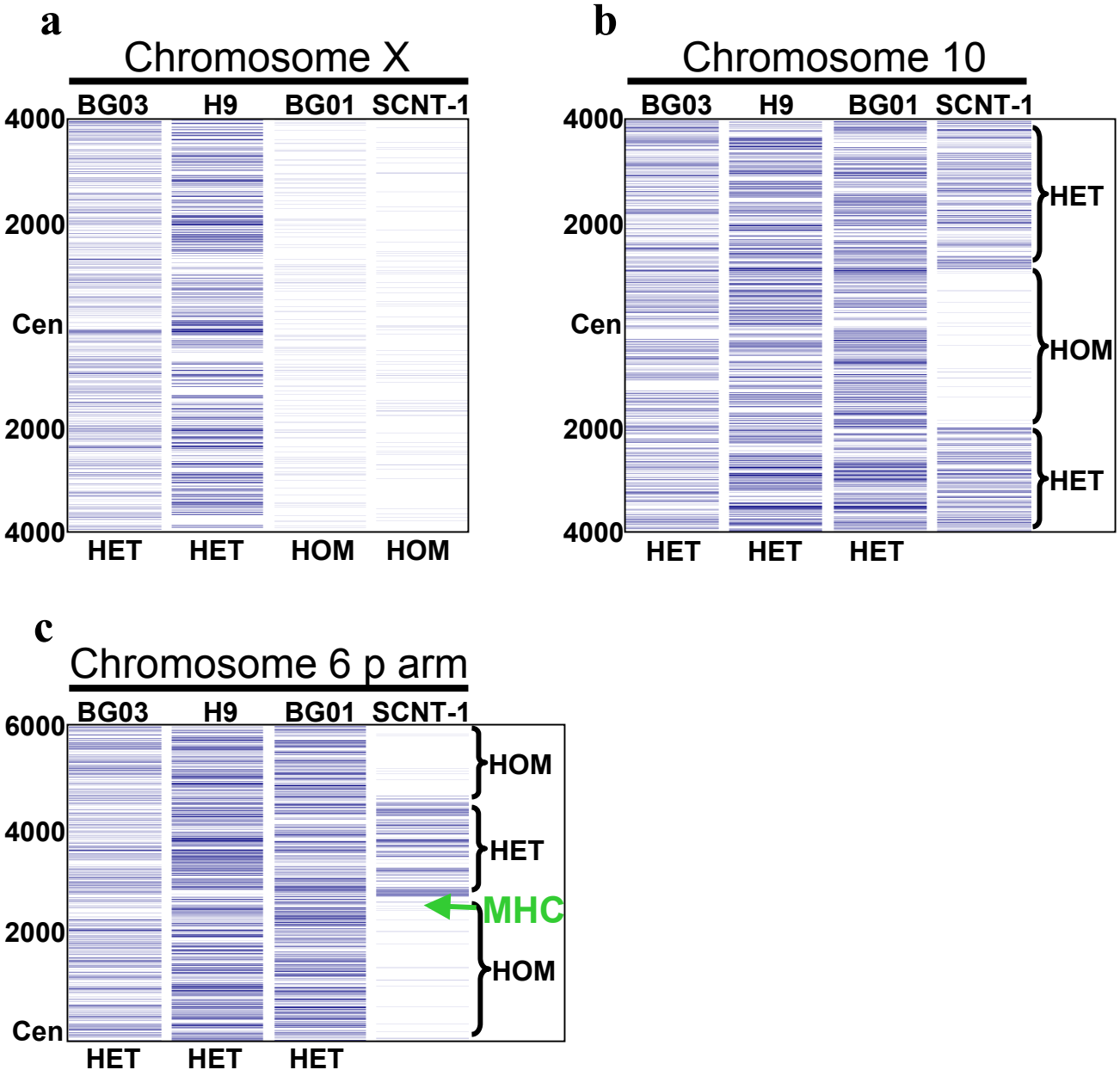
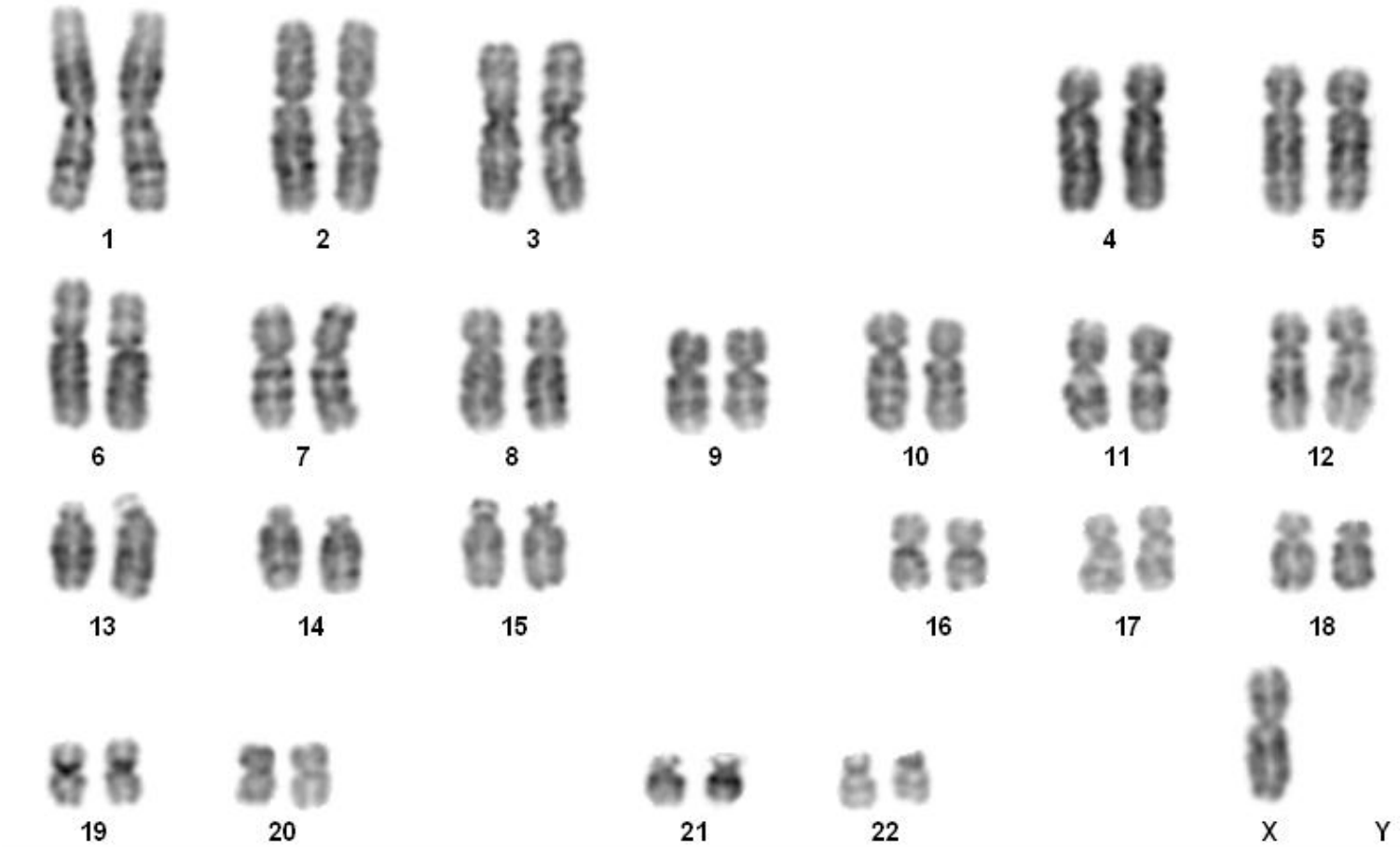


Figure S2. SNP Genotyping of Human ES Cell Lines BG03, H9, BG01, and SCNThES-1

Panels depict results of SNP genotyping data for each chromosome indicated, from centromere (cen) to telomere (p arm, top half; q arm, bottom half). Blue lines indicate indicative heterozygous SNP markers. HOM: homozygous regions (reflected in <5% frequency of heterozygous SNPs); HET: heterozygous SNP regions. 2000, 4000, and 6000 show the number of the SNP markers from the centromere. **(a)** X chromosome control for heterozygosity; BG03 and H9 are predominantly heterozygous female lines with two X chromosomes. BG01 control for assigning homozygosity due to the hemizygous Xchromosome (genotyping error rate of 2.3%); SCNT-hES-1 data is consistent with similar hemizygosity of the X chromosome. **(b)** chromosome 10; A typical pericentromeric homozygosity can be observed only in SCNT-hES-1 **(c)** chromosome 6 p-arm. The green arrow indicates the location of the MHC (human HLA antigen) cluster. The MHC cluster is located on the border of a homozygous region indicating that the cross-over event occurred telomeric to the MHC-gene cluster.

Supplementary Figure 3



45 XO

Figure S3. Karyotype of SCNT-hES-1

Table S1. DNA Fingerprint Analysis of SCNT-hES-1

Marker Name	Chromosome Location	Genotype	Marker Name	Chromosome Location	Genotype
TPOX	2	08-08	vWA	12	17-17
D3S1358	3	16-18	D13S317	13	08-09
FGA	4	21-23	PentaE	15	12-14
D5S818	5	10-11	D16S539	16	09-12
CSF1PO	5	12-13	D18S51	18	15-16
D7S820	7	08-08[1]	D21S11	21	32.2-32.2
D8S1179	8	10-11	PentaD	21	08-12
TH01	11	06-09	Amelogenin[2]	X	X

[1] 08-11 was shown in SNUIC report.
[2] Amelogenin marker was not analyzed in SNUIC report.

Supplemental References

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