DNA METHYLATION IN THE EARLY PORCINE EMBRYO

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

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ABSTRACT

Reproductive technologies such as *in vitro* fertilization, intracytoplasmic sperm injection, parthenogenetic activation, and somatic cell nuclear transfer are powerful procedures in the production of animals for agriculture, basic research, and biomedical research. Research using these techniques has produced important insights into the basic mechanisms of gametogenesis, embryogenesis, and fetal development. Unfortunately, the production of live animals by using these *in vitro* technologies is very inefficient. One component contributing to this inefficiency is *in vitro* oocyte maturation and *in vitro* culture of early embryos and donor cells for somatic cell nuclear transfer has been shown to have detrimental effects on the epigenetic factor of cytosine methylation in cytosine-guanine dinucleotides. The purpose of this research is to study the dynamics of DNA methylation in porcine gametes, clonal cell lines, adult somatic cells, and early embryos produced by using *in vivo*, *in vitro*, parthenogenetic, and somatic cell nuclear transfer procedures. Differential Methylation Hybridization microarrays were used to study DNA methylation of the aforementioned groups. Bisulfite sequencing was used to confirm the microarray

results. Additionally, the potential of the donor cells to direct development to the blastocyst stage was analyzed.

The CpG methylation remodeling that occurs in the development of the *in vivo* derived blastocyst does not occur in blastocysts produced by using *in vitro* techniques such as parthenogenesis, NT, and *in vitro* fertilization. Specifically, the methylation events that occur in the development of parthenogenetic and nuclear transfer blastocysts are more similar to the *in vivo*-produced blastocysts than the methylation remodeling events in the *in vitro*-produced blastocysts. These results suggest that the *in vitro*-matured oocytes used to produce embryos derived from *in vitro* fertilization, parthenogenesis, and somatic cell nuclear transfer are not capable of epigenetic remodeling required to direct the development of the early embryo.

The developmental potential and methylation profiles were analyzed in cultured clonal cells derived from primary preparations of porcine fetal fibroblast-like cells and for donor cells selected from kidney and mammary cells that were not cultured prior to somatic cell nuclear transfer. The methylation profiles of these donor cells were determined by using Differential Methylation Hybridization microarrays. A wide range of developmental potential was observed for donor cells regardless of whether the cells were in extended culture. Overall, similarities of the donor cell methylation profiles and the methylation profiles of the *in vivo*-derived embryos were inversely correlated to developmental potential. Specifically, donor cells from kidney tissues were found to have methylation profiles with the highest similarity to *in vivo*-derived embryos and the blastocyst rate following nuclear transfer was found to result

in the lowest blastocyst rate of all the donor cells. Conversely, the methylation profiles of the small mammary cells and the clonal cell lines A7 and A8 were found to be the most dissimilar to the in vivo blastocyst, yet these donor cells resulted in the highest rates of blastocyst development. The epigenetic condition of some donor cells is resistant to the detrimental effects of extended culture on donor cells, and there are subpopulations in somatic cells that show variable resistance to epigenetic remodeling following nuclear transfer.

In conclusion, these studies indicate aberrant epigenetic remodeling is a factor in the low efficiency of *in vitro* techniques of reproductive technologies. A surprising result of these studies is that methylation profiles of blastocysts produced by using somatic cell nuclear transfer and parthenogenesis are similar to that of *in vivo*-produced blastocysts. An additional unexpected result was that donor cells with methylation profiles with the highest similarity to *in vivo*-produced blastocysts were found to have the lowest blastocyst rate. These results suggest that suboptimal *in vitro* maturation conditions of oocytes are important factors in the low development of embryos produced by using techniques such as *in vitro* fertilization, intracytoplasmic sperm injection, parthenogenetic activation, and somatic cell nuclear transfer. An extension of this rationale is that the methylation profiles of *in vivo*-matured oocytes or very early *in vivo*-produced embryos may be the optimal target for the methylation profile of donor cells that are capable of efficiently directing embryonic development after nuclear transfer.

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LIST OF ABBREVIATIONS

AI	Artificial Insemination
ANOVA	Analysis of Variance
BSA	Bovine Serum Albumin
BWS	Beckwith-Weidman Syndrome
COBRA	Combined Bisulfite Restriction Analysis
COC	Cumulus Oocyte Complex
DMEM	Dubelcco's Modified Eagle Medium
DMH	Differential Methylation Hybridization
DNMT	DNA methyltransferase
DTT	Dithiothreitol
EGF	Epidermal Growth Factor

ESC	Embryonic Stem Cells
FCS	Fetal Calf Serum
FSH	Follicle Stimulating Hormone
GV	Germinal Vesicle
HBSS	Hank's Balanced Salt Solution
IAP	Intracisternal A Particles
ICM	Inner Cell Mass
ISCNT	Interspecies Somatic Cell Nuclear Transfer
IVM	*In Vitro*-Matured
LH	Lutenizing Hormone
MS-PCR	Methylation Specific-Polymerase Chain Reaction
mSOF	Modified Synthetic Oviduct Fluid
mTBM	Modified Tris-buffered Medium
LOS	Large Offspring Syndrome
NHP	Non-Human Primates
PBS	Phosphate Buffered Saline
PCGIL	Porcine CpG Island Library
PDMH	Porcine Differential Methylation Hybridization
PFF	Porcine Fetal Fibroblast-like
PCR	Polymerase Chain Reaction
PGC	Primordial Germ Cell
RLGS	Restriction Landmark Genomic Scanning

RT	Room Temperature
SAM	S-Adenosyl-Methionine
SCNT	Somatic Cell Nuclear Transfer
SDS	Sodium Dodecyl Sulfate
STE	Sodium Chloride/Tris/EDTA
TCM199	Tissue Culture Medium 199
TE	Trophectoderm
ZP	Zona Pellucida

CHAPTER I

INTRODUCTION

Chromatin structure and gene expression are affected by the epigenetic modification of cytosine methylation at the dinucleotide pair of cytosine and guanosine. Hypermethylation of CpG dinucleotides often occurs at regions of heterochromatin. Also, hypermethylation of the promoter and first exon of genes can occur in a tissue specific manner resulting in differential gene expression. The tissue specific methylation is thought to be partially responsible for the morphological differences in the various cells and tissues of the adult body. A global demethylation event of the maternal and paternal genomes occurs immediately after fertilization and continues through the blastocyst stage. In the mouse, remethylation or reprogramming of the genome occurs around the time of implantation and is maintained in somatic tissues. These tissue-dependent and differentially methylated regions are also an important epigenetic mechanism of differentiation from the single cell type of the early embryo's inner cell mass to the numerous somatic cell types which comprise the adult organism. The purpose of this research is to study the dynamics of DNA methylation in porcine gametes and early embryos produced by using *in vivo*, *in vitro*, parthenogenetic, and somatic cell nuclear transfer procedures. Our hypothesis is that the methylation profiles of *in vitro*-produced blastocysts will be more similar to the methylation profile of *in vivo*-produced blastocysts than to the methylation profiles of

blastocysts produced by parthenogenesis or somatic cell nuclear transfer. An additional purpose of this research is to assess the methylation status of clonal cell lines and adult somatic cells with respect to developmental potential following nuclear transfer. Our hypothesis is that the donor cells with the highest blastocyst rates after somatic cell nuclear transfer will have methylation profiles that are more similar to the methylation profiles of the *in vivo*-produced blastocysts compared to the methylation profiles of the donor cells with the lowest blastocyst rates after somatic cell nuclear transfer.

CHAPTER II

REVIEW OF THE LITERATURE

Nucleic acid methylation can be found in higher plants, fungi, bacteria, and mammals with the methylation of different sites indicative of differences in the functional role of methylation in each of the groups. Bacterial DNA methylation occurs primarily at adenine and cytosine and acts to prevent endonucleases from cleaving endogenous DNA. Conversely, bacterial endonucleases cleave exogenous bacterial and viral DNA that does not have the methylation patterns at the adenine and cytosine nucleotides. In eukaryote species, DNA methylation is primarily found at the CpG dinucleotide. CpG methylation has generally been thought to be a

mechanism for transcriptional regulation (Bird, 2002). Examples of this include genomic imprinting and X chromosome inactivation (Li, 2002). Recently, DNA methylation has been shown to have a role in tissue specific expression (Futscher et al., 2002) thereby demonstrating a mechanism for programming the epigenome in a manner specific to the cell type. Specifically, hypermethylation of the promoter region of the autosomal gene SERPINB5 was correlated with down-regulated expression in bone marrow, liver, kidney, heart, skin fibroblasts, and lymphocytes. Conversely, hypomethylation correlated with up-regulated expression in airway epithelium, mammary epithelium, skin keratinocytes, oral keratinocytes, and prostate keratinocytes. Methylation of DNA is the prominent type of epigenetic marking. Epigenetic reprogramming is an inheritable modification that has a substantial effect on development but that does not result from a change in the DNA sequence (Russo et al., 1996).

DNA Methylation and Genomic Organization

The CpG dinucleotides are not randomly distributed in the genome. Instead CpG dinucleotides are found at a much lower frequency than expected. In part this is due to the spontaneous deamination of methylated cytosines to thymine while unmethylated cytosines are transformed into uracil (Jones and Baylin, 2002). A substitution reaction occurs when uracil is recognized as an extraneous base. Thymine is recognized as a common base and a substitution does not occur. Both of these reactions decrease the frequency of the cPg dinucleotides in the genome.

The CpG dinucleotides are found in normal numbers in areas called CpG islands (Antequera, 2003).The original criteria for a CpG Island was that a DNA sequence must be longer than 200 base pairs and must have a G+C content ≥ 50% and a CpG observed/expected (o/e) ratio≥0.6 (Gardiner-Garden and Frommer, 1987). Recently, more stringent criteria was developed for regions of DNA greater than 500 bp where the G+C content and CpG o/e ratio were increased to 55% and 0.65, respectively (Takai and Jones, 2002). This alternative criterion was implemented to exclude *Alu* repeats. Please note that the o/e CpG ratio of less than one seems to be counterintuitive since CpG islands are thought to be regions with a high concentration of CpG dinucleotides. The expected occurrence of CpG islands is based on the random occurrence of CpG dinucleotides. The actual number of CpG dinucleotides was shown to be 0.83% in mouse genome sequences (Zhao and Zhang, 2006) instead of the expected frequency of 6.25% (1/16). Conversely, the frequency of CpG dinucleotides was shown to be 6.89% in CpG islands. Accordingly, the Takai and Jones (2002) criteria for o/e CpG ratio ≥ 0.65 in CpG islands would include regions where the frequency of CpG dinucleotides is greater than 4.0625% (i.e. 6.25% x 0.65 = 4.0625%).

The CpG islands are generally located in the promoter region of housekeeping genes and in the first exon (Bird, 2002; Wang and Leung, 2004). Genes associated with CpG islands are generally found to be transcriptionally active when the CpG islands are unmethylated and transcriptionally inactive when the CpG islands are methylated (Roberson et al., 2002).

Methods to Determine Methylation Status

Early techniques to identify CpG methylation involved the use of methylation sensitive restriction enzymes and Southern blotting (Bird and Southern, 1978) or methylation sensitive restriction enzymes and PCR mediated by using ligated adaptors (Singer-Sam et al., 1990a; Singer-Sam et al., 1990b). Restriction landmark genomic scanning (RLGS) is a technique by which global CpG methylation is analyzed by using two-dimensional gel electrophoresis and methylation sensitive restriction enzymes (Plass et al., 1999; Costello et al., 2000; Kremenskoy et al., 2003). Each RLGS can display up to 2,000 end-labeled rare cutting restriction sites. The resulting RLGS spots can be cloned and sequenced by using standard procedures (Costello et al., 1997).

Current technologies for determining DNA methylation have primarily taken advantage of the ability of sodium bisulfite to deaminate unmethylated cytosines to create uracil (Frommer et al., 1992). The nascently created uracils are then represented as thymidines in subsequent PCR amplifications. Bisulfite does not react with 5-methyl cytosine (Wang et al., 1980). Methylation specific PCR (MS-PCR) utilizes primers designed specifically for bisulfite treated and untreated DNA. An extension of this approach, combined bisulfite restriction analysis (COBRA), involves the addition of endonuclease restriction digestion at a recognition site that includes CpG dinucleotides of the PCR amplification product. The aforementioned techniques are capable of identifying changes in methylation status at a limited

number of CpG sites. Specifically, only the methylation status of the CpG sites at the primers or in the restriction endonuclease recognition sites are analyzed.

Bisulfite treatment combined with sequencing identifies the methylation status at any CpG dinucleotides that are between primers that flank the region of interest. The most common application of this approach is to treat the DNA with sodium bisulfite, create a plasmid library, and sequence individual clones from the library. The methylation status of every CpG site present in the cloned amplicon can be determined. While this approach provides a measure of the methylation at each CpG dinucleotide, it is a very labor intensive procedure. Pyrophosphate sequencing is a procedure whereby the methylation status of CpG dinucleotides is identified as the nascent DNA strand is extended (Ronaghi, 2001). Briefly, DNA is treated with sodium bisulfite to convert unmethylated cytosines to thymidines in subsequent sequencing reactions. A biotinylated primer is designed to anneal to a particular strand thereby allowing for the isolation of only that strand by using a streptavidin column. The DNA is extended by using a DNA polymerase and the relative amount of cytosines or thymidines at a specific position are quantified. Quantification of the cytosine or thymidine is mediated by luminescence produced by the release pyrophosphate resulting from the integration of the specific dinucleotide. Pyrophosophate is converted to ATP thereby providing energy for the bioluminescent luciferase reaction. This technique provides a rapid, quantitative analysis of the methylation status at multiple CpG sites of about 200 base reads (Ronaghi, 2001).

Microarrays analysis of methylation has the advantage of quickly analyzing a large number of regions. Unfortunately, the resolution and sensitivity of microarray-based methylation analysis is much lower than bisulfite sequencing analysis and pyrosequencing. Methylation analysis can be grouped into two broad categories including those that require bisulfite treatment of the DNA prior to analysis and those that require restriction enzyme digestion of the DNA prior to analysis (van Steensel and Henikoff, 2003). The PCR amplification for the bisulfite treated samples, and PCR amplification or size fractionation for the restriction enzyme digested samples is required to generate the labeled product that will hybridize to the microarrays. Microarray analysis of bisulfite treated DNA requires microarrays that have paired oligonucleotides (19-23 nucleotides in length) that differ at a methylatable position thereby allowing for the discrimination of single base differences in methylation (Adjoran et al., 2002; Gitan et al., 2002). Methylation analysis of restriction enzyme digested DNA is compatible with DNA microarrays that have any regions of overlap on the array (Novik et al., 2002; Wei et al., 2002). The resolution depends on the size of the PCR product that was spotted on the microarray (0.5 to 3kb). Methylation changes associated with breast cancer were originally studied with restriction enzyme treatment of the DNA samples (Huang et al., 1999). More recent methylation microarray studies have also examined methylation in ovarian cancer (Wei et al., 2002; Shi et al., 2003).

DNA Methyltransferases

The addition of a methyl group is accomplished through the activities of enzymes called DNA methyltransferases (**DNMT**s) (Table 2.1). Cytosine methylation involves the transfer of a methyl group from the cofactor S–adenosyl-methionine (**SAM**) to the 5'-carbon of the cytosine pyrimidine ring (Figure 2.1). The first mammalian methyltransferase to be cloned in mice was DNMT1 (Bestor et al., 1988) and serves as a maintenance methyltransferase acting principally on hemimethylated DNA. An oocyte specific isoform of DNMT1 is DNMT1o which is missing the final 118 amino acids of the N-terminus (Carlson et al., 1992; Mertineit et al., 1998). Establishing normal imprints does not require DNMT1o (Howell et al., 2001).

The DNMT2 contains all the conserved methyltransferase motifs and is widely expressed in different tissues (Okano et al., 1998). Targeted deletion of Dnmt2 has demonstrated it is not essential for global *de novo* methylation or for maintenance methylation in embryonic stem cells (ESC). Recently DNMT2 has been shown to methylate small RNAs instead of DNA (Goll et al., 2006). Specifically, DNMT2 methylates cytosine 38 in the anticodon loop of $tRNA^{Asp}$.

The DNMT3A and DNMT3B are primarily involved with *de novo* methylation. Mutations in DNMT3A and DNMT3B are lethal at the postnatal and embryonic stages, respectively (Okana et al., 1999). The offspring with the DNMT3A -/- genotype appear phenotypically normal but die at about 4 weeks after birth. Also, DNM3B -/- embryos appeared to develop normally before E9.5, but these embryos died before E11.5.

The DNMT3L isoform does not appear to be capable of methyltransferase activity (Aapola et al., 1999; Hata et al., 1999; Bourc'his et al., 2001). Despite the lack of methyltransferase activity, DNMT3L may play a role as a regulator or a cofactor for other methyltransferases in *de novo* methylation. Heterozygous progeny of *Dnmt3l* -/- females did not develop past 9.5 postcoitum. Interestingly, these fetuses exhibited biallelic expression of the imprinted genes *Snrpn*, *Necdin*, *Zfo127*, *Kcnq1ot1*, and *Peg3*. The DNMT3L isoform appears to be involved with maternally repressed imprinted genes but not paternally repressed genes (Bourc'his et al., 2001).

DNA Demethylation

After fertilization, the paternal genome undergoes active demethylation, presumably by enzymatic demethylation, and the maternal genome undergoes passive demethylation. Removal of the methyl group from 5-methylcytosine by from methyl binding domain protein 2 (MBD2) has been suggested (Bhattacharya et al., 1999) but paternal demethylation occurs normally in the 1-cell embryos derived (MBD2) null crosses (Hendrich et al., 2001). Although MBD2 has been identified as a demethylase (Bhattacharya et al., 1999) other labs have not been able to replicate these results. The MBD4 isoform is also suggested as a demethylase because of its role in DNA repair (Wu et al., 2003) but the active demethylation of the paternal genome seemed to occur as expected in MDB4 null fertilized oocytes (Santos and Dean, 2004). Therefore, the best evidence for the existence of a demethylase enzyme is the rapid

demethylation of the paternal genome immediately after fertilization (Reik and Walter, 2001).

DNA Methylation and Transcription

The traditional view that methylation represses transcription may be a function of the density of the methylated cytosines (Hsieh, 1997) or that the position of the methylated cytosines is more important (Chen et al., 2001). Methylated cytosines may repress transcription by interfering with the binding of trans-acting factors (Bell and Fensfeld, 2000). An alternative explanation is that methylation sensitive binding proteins have a role in repressing transcription. Transcriptional repressors that act via DNA methylation include the methyl binding proteins (MECP2 and MBD 1-3), a family of 4 proteins that share the common motif of a methyl binding domain (Hendrich and Bird, 1998; Hendrich et al., 2001). The methyl binding is present in MBD4 domain but does not act to repress transcription.

The chromatin structure of methylated DNA is further modified by the recruitment of another transcription corepressor. A corepressor mSin3A interacts with MECP2 that forms a complex with histone deacetylases (Nan et al., 1998). Histone deacetylases remove the acetyl groups from the amino terminal lysine residues of at least histone proteins H3 and H4 (Grunstein, 1997). The generalized configurations of the aforementioned mechanisms of transcriptional inhibition are shown in Figure 2.2 (Klose and Bird, 2006).

DNA Methylation and Imprinting

Genomic imprinting is a system unique to higher plants, marsupials, and eutherian mammals. Imprinting is an epigenetic mechanism whereby certain genes are expressed in a parent-of-origin-dependent manner. In 1984, imprinting was first suggested as a potential mechanism to explain differences between the maternal and paternal genomes (McGrath and Solter, 1984; Surani et al., 1984). Both groups showed that embryos with either two maternal or two paternal pronuclei did not develop to term.

The current number of imprinted genes is 106 for the mouse, 54 for humans, and 3 for pigs. Table 2.2 show the genes that are currently known to be imprinted in the mouse and human (www.otago.ac.nz/IGC) 22/12/05). Three genes have been identified in the pig as being expressed in an imprinted manner. These genes include IGF2 (Jeon et al., 1999; Nezer et al., 1999; Nezer et al., 2003), IGF2AS (Braunschweig et al., 2004), and IGF2R/M6PR (Killian et al., 2001) (Table 2.3). The three imprinted pig sequences have also been found to be imprinted in the mouse and humans. Recently, Luedi at al (2005) used a machine learning approach to identify 600 (2.5%) potentially imprinted genes from 23,788 annotated autosomal mouse genes.

DNA Methylation and Development

Two stages of DNA remodeling have been identified in germ cell development and in the development of preimplantation embryos (Morrison et al., 2005). Figure 2.3 shows the maternal and paternal genome methylation levels in murine gametes and early embryo. In the mouse, demethylation of the primordial germ cells (PGCs) occurs at 10.5 to 12.5 days of gestation, about the time these cells populate the gonads (Hajkova et al., 2002; Lee et al., 2002). Imprinted as well as non-imprinted sequences are demethylated around this time but some repetitive sequences such as intracisternal A particles (**IAP**) sequences resist demethylation (Lane et al., 2002). Remethylation of the male germ line appears to begin at E15 to E16 and later. Female germ cells re-establish methylation of imprinted and non-imprinted regions during early postnatal development until Metaphase II (Lucifero et al., 2004). Female germ cells appear to acquire imprints at the diplotene stage of prophase I (Kono et al., 1996; Bao et al., 2000; Lucifero et al., 2002; Obata and Kono, 2002) including the paternally expressed gene *Snrpn* (Lucifero et al., 2002). Conversely, the maternal methylation for IGF2r, Peg I, and Peg3 is established in metaphase II oocytes (Lucifero et al., 2002).

In the mouse, a second global demethylation event of the maternal and paternal genomes occurs immediately after fertilization and continues through the blastocyst stage. The male pronucleus undergoes active demethylation within 4 hours after fertilization (Mayer et al., 2000; Santos et al., 2002). The ooplasm of porcine *in vivo*-produced 1-cell embryos has been reported to demethylate the paternal genomes

of polyspermic embryos with two and four paternal pronuclei (Fulka et al., 2006). Conversely, the paternal pronucleus does not appear to undergo active, global demethylation immediately after fertilization in sheep (Wilmut et al., 2002) or in the rabbit (Beaujean et al., 2004; Shi et al., 2004). While global and rapid demethylation does not occur, demethylation of the centromeric satellite DNA *Rsat IIE* and the promoter region of the single copy gene surfactant protein A appears to undergo passive demethylation until the blastocyst stage *of in vivo*-produced embryos (Chen et al., 2004). A contrasting pattern was observed in somatic cell nuclear transfer (**SCNT**)-produced embryos where the promoter region of the surfactant protein A gene was rapidly demethylated until the 8-/16-cell stage, followed by remethylation of the sequence to the blastocyst stage. The *Rsat IIE* sequence appeared to be relatively unchanged throughout early development.

The maternal genome undergoes passive demethylation until the blastocyst stage (Rougier and Pequignot et al., 1998). Passive demethylation of the maternal genome occurs progressively as the embryo develops from the zygote to the 8-cell stage in the mouse embryo. Each cellular division results in the production of a nascent strand of DNA that is not remethylated. The failure to remethylate the nascent strand is possibly due to the exclusion of Dnmt1o from the nucleus until the 8-cell stage (Howell et al., 2001). The step-wise reduction in methylation continues until the morula stage (Santos et al., 2002). Methylation of imprinted regions may be mediated by histone modifications (Yang et al., 2003). Specifically, inactive alleles with methylated imprint control regions have been shown to have methylation of lysine 9

on histone H3 while H3 and H4 are hypoacetylated. In contrast, the unmethylated, active allele is hyperacetylated at histone H3 and H4 and methylated at lysine 4 of histone H3.

The demethylation dynamics of the sperm genome has been shown to be directed by the quality and source of the ooplasm of the recipient oocyte. There also may be some species-specificity since Chen et al. (Chen et al., 2006) found that pig–to-rabbit and rabbit-to-pig interspecies somatic cell nuclear transfer (**ISCNT**) resulted in the differential demethylation of repetitive sequences. Specifically, the methylation level of the rabbit repetitive sequence *Rsat IIE* was significantly lower ($P<0.05$) in the 1-cell embryo produced from rabbit to pig ISCNT than in the rabbit donor cells. The methylation levels of *Rsat IIE* was previously shown to be unchanged after rabbit to rabbit SCNT (Chen et al., 2004). Conversely, pig to rabbit ISCNT resulted in an increase in the methylation level of porcine satellite I sequence to 83.9% at 1-cell stage embryo from 66.3% methylation in the porcine cumulus cells that were used as donor cells (Chen et al., 2006).

Remethylation or reprogramming of the genome appears to occur around the time of implantation in the mouse and is maintained in somatic tissues. *De novo* remethylation is differentially applied in the cells of the inner cell mass (ICM) and trophectoderm (TE) (Dean et al., 2001; Santos et al., 2002). The TE, which contributes to the extraembryonic tissues including the placenta, is hypomethylated relative to the cells of the ICM which develop into the fetus and eventually into the adult organism.

Studies of methylation levels in the maternal and paternal genomes and of the early embryo have primarily consisted of 5-methyl cytosine immunofluorescence (Dean et al., 2001; Beaujean et al., 2004; Fulka et al., 2004). Accordingly, studies of this nature yield qualitative results rather than precise, quantitative estimates of methylation levels. Other studies have identified methylation levels at specific sequences and it is not possible to extrapolate the results of these studies as representative of all changes in methylation. Restriction landmark genomic scanning (RLGS), where methylation sensitive restriction enzymes are used to identify differential methylation, was used to identify the methylation status in murine ESCs, embryoid bodies, fetal tissue, teratomas, and adult kidney and brain tissues (Kremenskoy et al., 2003). A panel of 259 differentially methylated regions was identified as differentially methylated in at least one of these cell types or tissues. Based on the methylation status at these 259 sites, the methylation status was shown to be 51.4% in ES cells, 40.2% in fetal tissues, and 53.7% and 48.6% in the kidney and brain tissues, respectively.

Effects of *In Vitro* Culture on DNA Methylation

Khosla et al. (Khosla et al., 2001) showed that the inclusion of fetal calf serum (**FCS**) in the culture medium of preimplantation embryos can influence methylation and expression of imprinted genes, thereby resulting in developmental abnormalities. Fetuses cultured in the chemically defined media M16+FCS as early embryos showed decreased growth and transcriptional abnormalities. Specifically, at Day 14 the

fetuses had significantly lower weights than the control fetuses ($P<0.001$). The fetuses that were initially cultured in M16+FCS also had decreased expression of the imprinted genes *H19*, *IGF2*, *Grb7*.

Culture of *in vivo* produced bovine embryos in modified synthetic medium without serum or cumulus cell coculture (mSOF) resulted in placentas of day 70 fetuses that were abnormal compared to the *in vitro*-produced embryos cultured in medium with serum and cumulus cell co-culture and *in vivo*-produced conceptuses (Miles et al., 2005). Specifically, placentas from the mSOF group were heavier, had the least placental fluid, had fewer placentomes, and had the lowest placental efficiency (fetal weight/placental weight). Fewer placentomes suggests a smaller interface between the placenta and the uterus since the number of placentomes is determined by the number of caruncles in the uterus. In the mouse, aberrant placental imprinting and expression was observed following *in vitro* culture in Whitten's medium (Mann et al., 2004). The normally silent paternal allele of H19 was transcriptionally active in 65% of the cultured blastocysts presumably due to hypomethylation. Additionally, there was aberrant expression of silent alleles for *H19*, *Ascl2*, *Peg3*, and *Xist* in placental tissues but normal expression was retained in the embryo. Whitten's medium does not contain FCS. Therefore, the aberrant methylation observed after *in vitro* culture may result by an alternative mechanism than that induced by FCS.

Assisted reproductive techniques, which inherently use *in vitro* culture procedures, have been implicated in a 9-fold increase in the development of

Beckwith-Wiedmann Syndrome (**BWS**) over the general population (Halliday et al., 2004). Prenatal and postnatal overgrowth due to aberrant imprinting on 11p15.5 in BWS (DeBaun et al., 2003). Specifically, BWS is associated with aberrant methylation of IGF2, H19 and LIT1 genes.

Studies to date on the effect of *in vitro* culture on DNA methylation show that a specific cell culture medium may cause aberrant methylation and transcription. Most studies have examined imprinted genes probably as a result of the familiarity and prior knowledge of these genes. The results of these studies also show that the effect of a specific medium or component of the medium is species-specific with respect to the effect on methylation status and transcription. Further, global effects have not been identified regarding a specific medium, or component of the medium, for all genomes or even a specific gene or region across genomes. Therefore, extrapolation of *in vitro* culture effects on methylation, based on previous research, is highly speculative and should be done with extreme caution.

DNA Methylation and Somatic Cell Nuclear Transfer Derived Embryos

The precise causes of the phenotypic abnormalities observed in cloned animals are not known but are thought to be related to epigenetic defects because the offspring of cloned animals appear to be normal (Tamashiro et al., 2002; Zhang et al., 2004). The first transgenic pig produced in the Prather lab was produced by *in vitro* maturation (**IVM**), *in vitro* fertilization (**IVF**), and *in vitro* culture to the blastocyst stage before surgical embryo transfer to a surrogate recipient (Cabot et al., 2001).

This animal was born with a flexor tendon contracture commonly observed in offspring produced by using *in vitro* techniques such as IVF and somatic cell nuclear transfer (**SCNT**). Of the 4 clones produced by SCNT using donor cells from this gilt, only one had a flexor tendon contracture (Park et al., 2001). Conversely, none of the 24 offspring produced from the original transgenic animal had the flexor tendon contracture (Prather et al., 2003) suggesting that the cause of the abnormalities may have been epigenetic and epigenomic remodeling of the germ line repaired the defects introduced during the initial *in vitro* culture of the reconstructed embryo.

Martin et al. (2007) showed Day 14 SCNT embryos to have more nucleoli and a higher mitotic index than Day 14 *in vivo*-produced embryos and manipulation control embryos. Also, the SCNT embryos developed more slowly than *in vivo*-produced embryos. Additional research is needed to determine if these morphological changes are related to aberrant DNA methylation or if these changes are a means of compensating for the detrimental effects of the SCNT procedure. For an SCNT embryo to produce viable offspring requires DNA remodeling and transcriptional reprogramming that approximates the *in vivo*-produced embryo. Normally, these processes occur under *in vivo* conditions and operate on the genomic and epigenomic substrates of the sperm and oocyte instead of a differentiated somatic cell.

A number of obstacles decrease the efficiency of the SCNT procedure to produce offspring. The obligatory step of culturing the donor cells prior to the SNCT procedure is a likely opportunity to introduce aberrant methylation changes. SCNT and *in vitro* culture of livestock embryos is associated with pre- and post-natal loss

due to large offspring syndrome (**LOS**). Specifically, decreased gene expression due to epigenetic changes in M6P/IGF2R is linked to LOS (Young et al., 2001). *In vitro* culture has been shown to disrupt imprinting in preimplantation embryos resulting in biallelic expression of the H19 gene, which normally is expressed from the maternal allele (Sasaki et al., 1995; Doherty et al., 2000). A subsequent study demonstrated that imprinted expression of *Ascl2*, *Snrpn*, *Peg3* and *Xist* is largely retained in mouse embryos recovered at E9.5, but these genes were transcriptionally active in the normally silent allele (Mann et al., 2004). Conversely, biallelic expression was detected in placental tissues for these genes. The authors suggest that the mechanisms that maintain imprinting may operate with greater fidelity in the embryo than in tissues that originate in the trophectoderm.

The inefficiency of the epigenetic reprogramming is further demonstrated by the analysis of transcription and methylation status of imprinted genes in the preimplantation stage mouse cloned embryo (Mann et al., 2003). Only 4% of the SCNT derived embryos reproduced the expression of the imprinted genes *H19*, *Meg3*, *Igf2r*, *Ascl2*, and *Snrpn* similar to in *vivo* derived blastocysts. The cloned embryos were also found to have substantial loss of allele-specific DNA methylation at the imprinting control regions of the *Snrpn* and *H19* genes.

Abnormal DNA methylation and transcription in most cloned embryos is likely a function of the aberrant regulation of DNA methyltransferases during the early stages of development (Chung et al., 2003). Specifically, cloned preimplantation mouse embryos were found to have DNMT1s, the somatic form of Dnmt1, present in

the cytoplasm of all the blastomeres beginning at the 8-cell stage. A post-transcriptional mechanism normally prevents the appearance of DNMT1s until after implantation (Carlson et al., 1992; Mertineit et al., 1998; Ratnam et al., 2002). Also, Dnmt1o primarily remained in the cytoplasm rather than translocating to the nucleus of the eight-cell stage embryo as occurs with *in vivo*-produced embryos (Chung et al., 2003).

The dynamics of methylation remodeling in the early pig embryo and cloned offspring has been studied for several sequences. IVF and SCNT blastocysts show gradual demethylation of centromeric satellite and Pre-1 in the development to the blastocyst (Kang et al., 2001). The demethylation of these sequences was also shown to be similar to the *in vivo*-produced blastocysts. Pre-1 and centromeric satellite sequences were found to have similar methylation levels in healthy cloned pigs and control pigs (Archer et al., 2003). In contrast, the high methylation levels of these sequences is maintained in the development of bovine SCNT-produced embryos through the blastocyst stage (Kang et al., 2001). Additionally, 5-methylcytosine immunofluorescence procedures have shown that porcine paternal and maternal genomes are demethylated during preimplantation development (Dean et al., 2001).

The SCNT procedure involves damaging the zona pellucida (**ZP**) by piercing it with a glass pipette to enucleate the oocyte. Simerly et al. (2003) reported that in non-human primates (**NHP**s), the initial enucleation step which removed the metaphase II spindle-chromosome complex also resulted in a depletion of microtubule motors and centrosome proteins. The resulting SCNT-produced embryos

showed misaligned chromosomes on multipolar spindles and aberrant microtubule patterns. Reconstructed embryos appeared to undergo normal preimplantation development but no pregnancies developed after embryo transfer. The problems observed in NHPs related to enucleation have not been shown to be a critical factor in other species. Although the offspring of many species have been produced by using the same enucleation process, it remains likely that some developmentally important proteins are also removed.

Fusion of donor cell and oocyte membranes is accomplished with application of an electrical pulse. This electrical pulse concurrently induces artificial activation of the oocyte that would have been induced by the fertilizing sperm under *in vivo* conditions. These steps introduce proteins and RNA from the donor cell as well as culture medium that may be detrimental to the development of the reconstructed embryo. For development to proceed, the reconstructed embryo must recapitulate the steps involved in chromatin remodeling of the donor karyoplast that normally occurs with the sperm and oocyte genomes after fertilization. Of course, a critical difference is that the donor karyoplast lacks the chromatin remodeling that is normally performed during gametogenesis. Furthermore, the donor karyoplasts have epigenetic markings that accompany differentiation. Heyman et al. (Heyman et al., 2002) demonstrated the importance of the relative stage of donor cell differentiation by comparing the survival to term of SCNT-produced embryos to the control group of *in vitro*-produced embryos. Embryos reconstructed by using adult fibroblasts or fetal fibroblasts resulted in calves born in 6.8% and 15% of the pregnancies that were

established, respectively. Conversely, the number of calves born from established pregnancies with reconstructed embryos when blastomeres from day 6 *in vivo*- or *in vitro*-produced morula served as donor cells (34.3%) and the control group of *in vitro*-produced embryos (49%) was significantly higher ($P<0.01$). This study supports the idea that the donor cell age and the degree of differentiation is inversely correlated to developmental competency.

The genetic background of the embryonic donor cells has also been shown to be critical in the viability of the reconstructed embryo (Rideout III and Yanagimachi et al., 2000). SCNT-produced embryos using F1 (129SvJaexC57BL/6) ESCs resulted in development of 7 of 34 (21%) embryos to healthy adults. In contrast, 8 of 76 (11 %) SCNT-produced embryos using inbred 129 donor ESCs developed to term but all died within the first day after birth. These results demonstrate the importance of the genetic background of the donor cells in producing viable offspring by using SCNT.

Summary and Research Directions

The study of methylation and DNA started in 1948 when 5-methylcytosine was first identified (Hotchkiss, 1948) thereby introducing the idea of the fifth base. Since then CpG methylation has been shown to be an integral component of gametogenesis, early embryo development, organogenesis, cancer, DNA stability, and cellular senescence. Recent studies in SCNT have demonstrated the relevance of CpG methylation in the development of reconstructed embryos to the ultimate objective of producing healthy, live offspring.

A commonly held view had been that differentiated somatic cells cannot be remodeled and reprogrammed to an undifferentiated state whereby a completely new cell type will result. This theory was disproven by the successful production of fertile offspring from mature murine B and T cells by using a two-step cloning procedure (Hochedlinger and Jaenisch, 2002). It should be noted that the blastocyst rate using these terminally differentiated cells was about 10 times lower when compared to the blastocyst rate when using a heterogeneous populations of donor cells of cumulus cells and fibroblasts (Wakayama et al., 2001). The source of donor cells for most SCNT studies has been a primary culture of somatic cells, often derived from decapitated and eviscerated fetal tissue. From the multitude of cell types present in the initial cell culture results in a population of cells with morphology that is predominantly fibroblast-like. While the morphology of these cells appears to be substantially different than embryonic stem cells, the argument has been made that these cells, while differentiated to some extent, are not fully differentiated and therefore may be a form of stem cells which are more readily reprogrammed to guide the development of the early embryo.

Accordingly, isolating these putative stem cells would enable the production of cloned offspring at a much more efficient level than can be currently achieved. Donor cell selection by using the criteria of morphology and expression is limited in that these characteristics may not be valid indicators of cellular differentiation. Instead, analyses of the mechanisms that direct development and differentiation need to be included in the donor cell selection process. Specifically, the methylation status

prior to the SCNT is likely a critical factor in identifying donor cells that are amenable to epigenetic remodeling and have high potential in directing embryonic development. Analyzing the global epigenetic status of donor cells by using methylation microarrays will be instrumental in further increasing the efficiency of producing live offspring via SCNT.

Table 2.1. DNA Methyltransferases

Gene	Function	Mouse Mutant Phenotype	Reference
Dnmt1	Maintenance DNMT	Aberrant X-linked expression, Lethal to Embryo E8.5, Loss of imprints	Lei et al., 1996 Li, 2002
Dnmt1o	Oocyte specific, imprint maintenance at the 8–cell stage	Loss of parental imprints, Dnmt1o +/- embryo lethal	Howell et al., 2001
Dnmt2	$tRNA^{Asp}$ methylation	N/A Normal	Goll et al., 2006 Okano et al., 1998
Dnmt3a	De novo DNMT	Aberrant spermatogenesis, Offspring die at 4 weeks	Okano et al., 1999
Dnmt3b	De novo DNMT	Embryo lethal at E14.5-18.5, Hypomethylated centromeric minor satellite DNA	Okano et al., 1999
Dnmt3l	Regulation of methylation	Infertility, Imprint loss, Dnmt3l +/- embryo lethal	Bourc'his et al., 2001 Hata et al., 2002

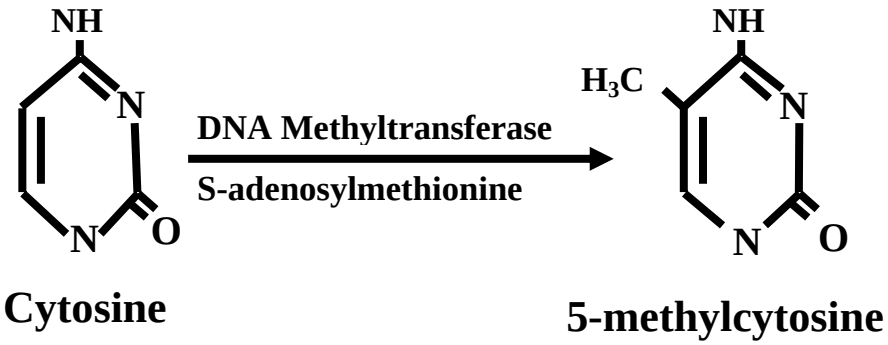

Figure 2.1 Addition of methyl groups to the carbon 5 of cytosine of the CpG dinucleotide

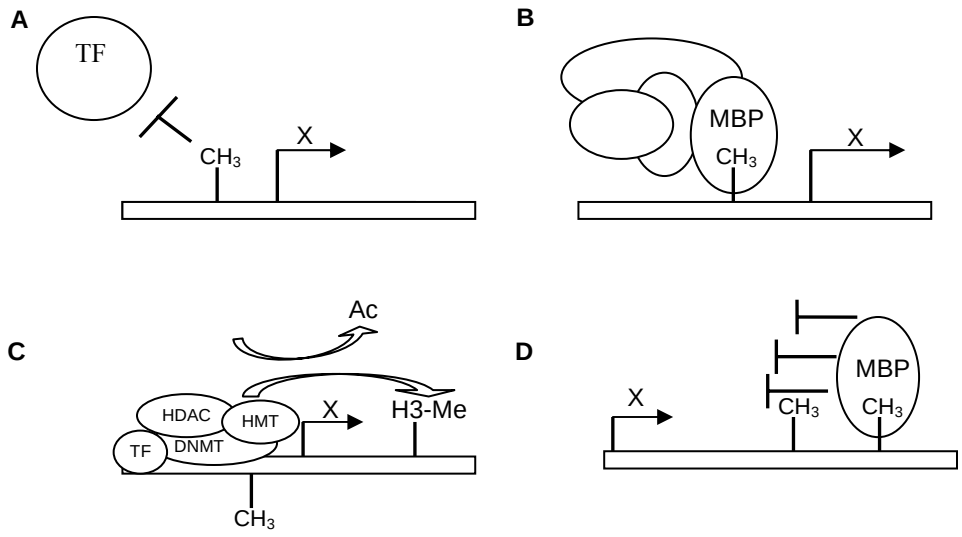

Figure 2.2. Repression of transcription mediated by CpG methylation. A) DNA methylation inhibits transcription factors (TF) from binding to DNA. B) Methyl-CpG-binding proteins (MBPs) binds to methylated DNA and recruits co-repressor molecules to inhibit transcription. C) DNA methyltransferases (DNMTs) associate with histone methyltransferase (HMT) and histone deacetylase (HDAC) thereby mediating transcriptional inhibition and chromatin modification. D) DNA methylation in the gene can inhibit transcriptional elongation (Klose and Bird, 2006).

Table 2.2 Table 2.2 Imprinted human and mouse genes. Abbreviations- AS, antisense transcript; miRNA, microRNAs; misc RNA, RNA of unknown function. CD, conflicting data; I, reported to be imprinted; NI, reported to be not imprinted; NO, no orthologue known; NR, no reports of imprinting status; M, maternal; P, paternal; PD, provisional data; (b) Noncoding RNAs only; (c) Imprinting is isoform dependent; (d) ZIM2 and COPG2 are reported to be oppositely imprinted in human and mouse. (Adapted from www.otago.ac.nz/IGC) 22/12/05)

Chromosome Human (Mouse)	Transcriptional Unit Human(Mouse)	Functional Component	Imprint		Expressed Allele	Protein Name or Description	RNA(b) Description
			Human	Mouse			
1p36 (4 E2)	TP73 (Trp73)		I	NR	M	Tumour related protein	
1p31	DIRAS3		I	NO	P	Ras homolog	
2p15 (11 A3)	COMMD1 (Commd1)		NI	I	M	Copper metabolism gene Murr1	
	(U2af1-rs1)		NO	I	P	U2 small nuclear RNP auxiliary factor	
4q13 (5 E1)	(Mkrn1-ps1)		NO	I	P		Pseudogene
4q22 (6 C1)	NAP1L5 (Nap1l5)		NR	I	P	Nucleosome assembly protein	
6q24 (10 A1)	HYMAI (Hymai)		I	NR	P		misc RNA
6q24 (10 A1)	PLAGL1 (Plagl1)		I	I	P	Zinc finger protein	
6q25 (17 A1)	IGF2R (Igf2r)		NI	I	M	Insulin-like growth factor receptor 2	
	(Air)		NO	I	P		Igf2r AS
	SLC22A2 (Slc22a2)		NR	I	M	Organic cation transporter	
	SLC22A3 (Slc22a3)		NR	I	M	Organic cation transporter	
7p12 (11 A1)	GRB10 (Grb10)		I	I	P/M (c)	Growth factor receptor-bound protein	
7q21 (6 A1)	CALCR (Calcr)		PD	I	M	Calcitonin receptor	
	SGCE (Sgce)		I	I	P	Sarcoglycan, epsilon	
	PEG10 (Peg10)		I	I	P	Retroviral gag pol homologue	
	PPP1R9A (Ppp1r9a)		I	I	M	Protein phosphatase inhibitor	
	PON1 (Pon1)		PD	NI	P	Paraoxonase 1	
	PON3 (Pon3)		NR	PD	M	Paraoxonase 3	

Table 2.2 (continued)

	PON2 (Pon2)		NR	PD	M	Paraoxonase 2	
	ASB4 (Asb4)		NR	I	M	Ankyrin repeat and SOCS box	
	DLX5 (Dlx5)		I	I	M	Homeo box-containing	
7q32 (6 A3)	CPA4 (Cpa4)		I	NR	M	Carboxypeptidase	
	MEST (Mest)		I	I	P	Alpha/beta hydrolase fold family	
	MESTIT1		I	NO	P		MEST AS
	COPG2IT1 (Copgas2)		I	I	P		COPG2 AS
	COPG2 (Copg2)		CD	I	P(M) (d)	Coatomer protein complex subunit	
8q24 (15 D3)	(Peg13)		NO	I	P		misc RNA
10q22 (10 B4)	STOX1		PD	NR	M	Storkhead box 1	
	CTNNA3 (Catna3)		PD	NR	M	Catenin, alpha 3	
10q25 (19 D2)	(Ins1)		NO	CD	P	Insulin I	
10q26 (7 F3)	INPP5F (Inpp5f)	V2 isoform only	NR	I	P	Inositol phosphatase	
11p15 (7 F5)	H19 (H19)		I	I	M		misc RNA
	IGF2 (Igf2)		I	I	P	Insulin-like growth factor 2	
	IGF2AS (Igf2as)		I	I	P		IGF2 AS
	INS (Ins2)		I	I	P	Insulin	
	ASCL2 (Ascl2)		CD	I	M	HLH transcription factor	
	PHEMX (Phemx)		NI	I	M	Tetraspanin superfamily	
	CD81 (Cd81)		NI	I	M	Transmembrane 4 superfamily	
	TSSC4 (Tssc4)		NI	I	M	Tumor suppressing candidate	
	TRPM5 (Trpm5)		PD	NI	P	Ca2+-activated cation channel	
	KCNQ1 (Kcnq1)		I	I	M	Voltage-gated potassium channel	

Table 2.2 (continued)

	KCNQ1OT1 (Kcnq1ot1)		I	I	P		KCNQ1 AS
	KCNQ1DN		I	NO	M	BWRT protein	
	CDKN1C (Cdkn1c)		I	I	M	Cyclin-dependent kinase inhibitor	
	SLC22A1LS		PD	NO	M	SLC22A1LS putative protein	
	(Msuit1, AF313042)		NO	I	M		misc RNA
	SLC22A18 (Slc22a18)		I	I	M	Organic cation transporter	
	PHLDA2 (Phlda2)		I	I	M	Pleckstrin homology-like domain	
	NAP1L4 (Nap1l4)		NR	I	M	Nucleosome assembly protein	
	(Tnfrsf23)		NO	I	M	TNF receptor superfamily	
	OSBPL5 (Osbpl5)		I	I	M	Oxysterol binding protein-like 5	
	ZNF215		PD	NO	M	Zinc finger protein	
11p13 (2 E)	WT1-Alt transcript (Wt1)		I	NR	O	Zinc finger protein	
11p13 (2 E)	WT1AS (Wt1as)		I	NR	P		WT1 AS
11q23 (9 A5)	SDHD (Sdhd)		CD	NR	P	Succinate dehydrogenase, subunit	
12q13 (15 F1)	SLC38A4 (Slc38a4)		NR	I	P	Amino acid transporter	
12q21 (10 C3)	DCN (Dcn)		NR	I	M	Proteoglycan	
13q14 (14 D2)	HTR2A (Htr2a)		CD	I	M	Serotonin receptor	
14q32 (12 F1)	DLK1 (Dlk1)		I	I	P	Delta-like 1 homolog	
	DLK1 downstream transcripts		NR	I	P		misc RNA
	MEG3 (Gtl2)		I	I	M		misc RNA
	miR-337		NR	I	M		miRNA
	LOC388015 (Rtl1)		NR	I	P	Retrotransposon-like 1	
	Anti-PEG11 (anti-Rtl1)	anti-Rtl1	NR	I	M		Rtl1-AS
		miR-431	NR	I	M		miRNA
		miR-433	NR	PD	M		miRNA
		miR-127	NR	I	M		miRNA

Table 2.2 (continued)

		miR-434	NR	PD	M		miRNA
		miR-432	NR	PD	M		miRNA
		miR-136	NR	I	M		miRNA
	MEG8 (Rian)	MEG8 (Rian)	NR	I	M		snoRNA host
		miR-370	NR	I	M		miRNA
		(MBII-78)	NO	I	M		snoRNA
		(MBII-19)	NO	I	M		snoRNA
		14q(0)	NR	I	M		snoRNA
		14q(I) (MBII-48)	NR	I	M		snoRNA
		(MBII-49)	NO	I	M		snoRNA
		(MBII-426)	NO	I	M		snoRNA
		14q(II) (MBII-343)	NR	I	M		snoRNA
	(Mirg)	(Mirg)	NR	I	M		miRNA host
		miR-411	NR	I	M		miRNA
		miR-380	NR	I	M		miRNA
		miR-376b	NR	I	M		miRNA
		miR-376	NR	I	M		miRNA
		miR-134	NR	I	M		miRNA
		miR-154	NR	I	M		miRNA
		miR-410	NR	I	M		miRNA
	DIO3 (Dio3)		NR	I	P	Deiodinase, iodothyronine type III	
15q11-q12 (7B5)	(Peg12)		NO	I	P	Gsk-3-binding protein family	
	MKRN3 (Mkrn3)		I	I	P	Makorin, ring finger protein	
	ZNF127AS (Zfp127as)		NR	I	P		MKRN3 AS
	MAGEL2 (Magel2)		I	I	P	MAGE-like protein	
	NDN (Ndn)		I	I	P	Necdin, neuronal growth suppressor	
	(AK014392)		NR	PD	P		Ndn AS
	(Pec2)		NR	I	P		LINE-rich intergenic
	(Pec3)		NR	I	P		LINE-rich intergenic
	(Nccr)		NR	I	P		miRNA host
	SNURF-SNRPN	SNURF (Snurf)	I	I	P	SNRPN upstream reading frame	

Table 2.2 (continued)

		SNRPN (Snrpn)	I	I	P	Small nuclear ribonucleo-protein	
		HBII-436	I	I	P		snoRNA
		HBII-13	I	I	P		snoRNA
		HBII-437	I	NO	P		snoRNA
		HBII-438A	I	NO	P		snoRNA
		PWCR1 (Pwcr1)	I	I	P		snoRNA
		HBII-52	I	I	P		snoRNA
		HBII-438B	I	NO	P		snoRNA
		UBE3A-AS	I	I	P		UBE3A AS
	UBE3A (Ube3a)		I	I	M	Ubiquitin protein ligase	
	ATP10A (Atp10a)		I	CD	M	ATPase, Class V	
	GABRB3 (Gabrb3)		CD	NI	P	Gamma-aminobutyric acid recep to r	
	GABRA5 (Gabra5)		CD	NI	P	Gamma-aminobutyric acid receptor	
	GABRG3 (Gabrg3)		CD	NI	P	Gamma-aminobutyric acid receptor	
15q21 (2 E5)	GATM (Gatm)		NR	I	M	Glycine amidinotrans-ferase	
15q24 (9 E3)	(4930524O08Rik , A19)		NO	I	P		misc RNA
	RASGRF1 (Rasgrf1)		NR	I	P	Guanine nucleotide exchange factor	
18q11 (18A2-B2)	IMPACT (Impact)		NI	I	P	Imprinted and ancient	
18q21	TCEB3C		I	NO	M	Transcription elongation factor	
19q13 (7 A2-B1)	IMP01/ITUP1		I	NR	P		imprinted transcript variant1
	PEG3 (Peg3)		I	I	P	Zinc-finger protein	
	(Zim1)		NO	I	M	Zinc-finger protein	
	ZIM2 (Zim2) I I P(M)d		I	I	P(M)d	Zinc-finger protein	
	USP29 (Usp29)		NR	I	P	Ubiquitin-specific protease	

Table 2.2 (continued)

	ZIM3 (Zim3)		NR	I	M	Zinc-finger protein (human)	No ORF (mouse)
	ZNF264 (Zfp264)		NR	I	P	Zinc-finger protein (human)	No ORF (mouse)
20q11 (2 H1)	NNAT (Nnat)		I	I	P	Neuronatin	
20q13 (2 H3)	L3MBTL (L3mbtl)		I	NI	P	Polycomb group	
20q13 (2 E1-H3)	GNAS (Gnas)	NESP55	I	I	M	Neuroendocrine secretory protein 55	
		GNASXL	I	I	P	Large isoform of GS-a	
		(F7)	NO	PD	M	Hypothetical protein (Mm.358828)	
		Exon-1A	I	I	P		misc RNA
		GS-alpha	I	I	M	Stimulatory G-protein, alpha subunit	
	SANG (Nespas)		I	I	P		GNAS AS
(A7)	(Xlr3b)		NO	I	M	X linked lymphocyte regulated	
	(Xlr4b)		NO	I	M	X linked lymphocyte regulated	
	(Xlr4c)		NO	I	M	X linked lymphocyte regulated	
Xq13 (X D)	XIST (Xist)		NI	I	P		XIST
	TSIX (Tsix)		NI	I	M		XIST AS

Table 2.3. Imprinted genes in the pig. These genes are also imprinted in the human and mouse genomes

Gene	**Expressed Allele**	**Chromosome**	**References**
IGF2-AS	Paternal	2	(Braunschweig et al., 2004)
IGF2	Paternal	2	(Jeon et al., 1999; Nezer et al., 1999; N
IGF2R/M6PR	Maternal	Unknown	(Killian et al., 2001)

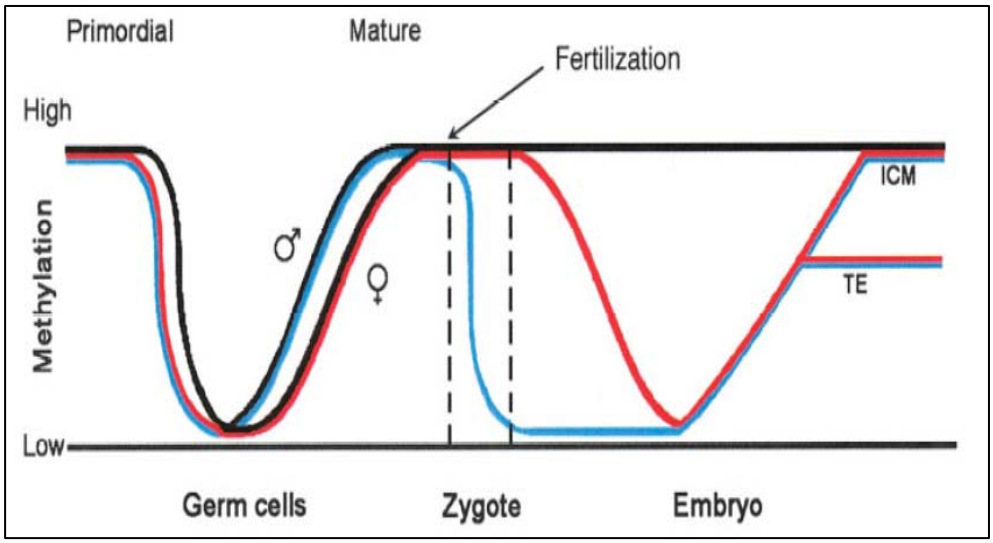

Figure 2.3. Maternal and paternal methylation in murine germ cells, zygote, and early embryo (Dean et al., 2005). Methylation remodeling of the maternal and paternal genomes occur in the development of the germ cells. Immediately after fertilization the paternal genome is demethylated, presumably by an active demethylation mechanism while the maternal genome undergoes passive demethylation to the morula stage when both genomes are remethylated. The methylation status of imprinted genes are shown as a solid black line.

CHAPTER III

ABERRANT DNA METHYLATION IN PORCINE *IN VITRO*-, PARTHENOGENETIC-, AND SOMATIC CELL NUCLEAR TRANSFER-PRODUCED BLASTOCYSTS

ABSTRACT

Early embryonic development in the pig requires DNA methylation remodeling of the maternal and paternal genomes. Aberrant remodeling is detrimental to development and can result in physiological and anatomic abnormalities in the developing fetus and offspring. *In vitro* techniques such as early embryo culture and SCNT have been shown to result in epigenetic and transcriptional profiles that deviate from *in vivo*-produced embryos. Here, we attempted to develop and validate a microarray-based approach to characterize on a global scale the CpG methylation -profiles of porcine sperm, germinal vesicle-stage oocytes, and parthenogenetic-, SCNT-, *in vitro*- , and *in vivo*-produced blastocysts. Our results demonstrate that the parthenogenetic-, nuclear transfer-, and *in vitro*-produced blastocysts fail to undergo the methylation remodeling similar to *in vivo*-produced blastocysts. The relative methylation in the gamete and blastocyst samples were analyzed by using GeneSpring 7.2 showed that 18.5% (921/4992) of the DNA clones were found to be significantly different ($P<0.01$) in at least one of the samples. Furthermore, for the

different blastocyst groups, the methylation profile of the *in vitro*-produced blastocysts was the least similar to the methylation profile of the *in vivo*-produced blastocysts compared to the parthenogenetic- and SCNT-produced blastocysts. The microarray results were validated by using bisulfite sequencing for 12 of the genomic regions in liver, sperm, and *in vivo*-produced blastocysts. Four of the regions analyzed by using bisulfite sequencing showed high methylation in at least one of the samples and the bisulfite sequencing analysis was consistent with 87.5% of these regions with high methylation The remaining 8 regions had low methylation (>10%) and the bisulfite sequencing analysis was consistent with 75.0% of the samples (8/12) of these regions with low methylation. These results suggest that a generalized change in global methylation is not responsible for the low developmental potential of blastocysts produced by using *in vitro* techniques. Instead, the appropriate methylation of a relatively small number of genomic regions in the early embryo may enable early development to occur.

INTRODUCTION

Epigenetic remodeling of the paternal and maternal genomes is necessary for the development of the early embryo. Remodeling of the maternal and paternal genomes occurs immediately after fertilization and continues through the blastocyst stage. In most species, the male pronucleus undergoes active demethylation within 4 hours after fertilization (Mayer et al., 2000; Santos et al., 2002). Conversely, the

paternal pronucleus does not appear to undergo active, global demethylation immediately after fertilization in sheep (Wilmut et al., 2002) or in the rabbit (Beaujean et al., 2004; Shi et al., 2004). The maternal genome undergoes passive demethylation until the blastocyst stage (Rougier and Pequignot et al., 1998). Remethylation or remodeling of the genome appears to occur around the time of implantation in the mouse and is maintained in somatic tissues. *De novo* remethylation is differentially applied in the cells of the inner cell mass (ICM) and trophectoderm (TE) (Dean et al., 2001; Santos et al., 2002). The TE, which contributes to the extraembryonic tissues and the placenta, is hypomethylated relative to the cells of the ICM which develop in to the fetus and, eventually, the adult organism.

Alternative techniques of embryo production, such as *in vitro* fertilization and nuclear transfer, are very inefficient and sometimes result in offspring with severe abnormalities (Carter et al., 2002). The precise causes of the phenotypic abnormalities observed in cloned animals are not known but are thought to be related to epigenetic defects because the offspring of cloned animals appear to be normal (Conway, 1996; Tamashiro et al., 2002; Zhang et al., 2004). For somatic cell nuclear transfer to produce viable offspring requires that DNA remodeling and transcriptional reprogramming approximate that of the *in vivo*-produced embryo. The inefficiency of the epigenetic reprogramming is demonstrated by the analysis of transcription and methylation status of imprinted genes in the preimplantation stage mouse cloned embryo (Mann et al., 2003). Only 4% of the SCNT derived embryos reproduced the

expression of the imprinted genes *H19*, *Meg3*, *Igf2r*, *Ascl2*, and *Snrpn* relative to *in vivo*-derived blastocysts. The cloned embryos were also found to have substantial loss of allele-specific DNA methylation at the imprinting control regions of the *Snrpn* and *H19* genes.

The dynamics of methylation remodeling in the early pig embryo and cloned offspring have been studied for several sequences. The IVF and SCNT blastocysts show gradual demethylation of centromeric satellite and Pre-1 in the development to the blastocyst (Kang et al., 2001). The demethylation of these sequences was also shown to be similar to the *in vivo*-produced blastocysts. Pre-1 and centromeric satellite sequences were found to have similar methylation levels in healthy cloned pigs and control pigs (Archer et al., 2003).

In this study we used a global microarray-based approach, Porcine Differential Methylation Hybridization (**PDMH**), based on a similar tool that has been developed for humans (Huang et al., 1999), to analyze the CpG methylation in porcine gametes, and parthenogenetic-, somatic cell nuclear transfer-, *in vitro*- , and *in vivo*- produced blastocysts. The specific question we were interested in was is there a difference in the global methylation profiles in the parthenogenetic-, SCNT- , *in vitro*-, and *in vivo*- produced blastocysts that explain the developmental differences of these samples? Our hypothesis was that the blastocysts with the highest development rates (*in vitro*-produced blastocysts) would be most similar to the *in vivo*-produced blastocysts and that the blastocysts with the lowest development rates

(parthenogenetic- and SCNT-produced blastocysts) would be least similar to *in vivo*-produced blastocysts.

Our results indicate the remodeling of *in vitro*-produced blastocysts shared the least similarity to the *in vivo*-produced blastocysts despite having higher developmental potential after embryo transfer to a recipient. The parthenogenetic- and SCNT-produced blastocysts exhibited methylation profiles that were more similar to *in vivo*-produced blastocysts.

MATERIALS AND METHODS

Oocyte procurement and *in vitro* maturation

Cumulus-oocyte-complexes (**COCs**) were aspirated from ovaries from prepubertal gilts that were collected from a local abattoir. Germinal vesicle (**GV**) - stage oocytes were collected for PDMH analysis or matured in vitro prior to in vitro fertilization. For PDMH analysis, COCs with numerous layers of intact cumulus cells were vortexed in 0.1% (w/v) hyaluronidase in Hepes-buffered saline for at least 5 minutes to remove the cumulus cells. Denuded GV-stage oocytes were rinsed three times in phosphate buffered saline containing 3 mg/ml Bovine Serum Albumin (**BSA**) (Fraction V) before the removal of the zona pellucida by incubation in 5 mg/ml pronase. The zona free GV-stage oocytes were rinsed three times in DEPC-treated phosphate buffered saline (PBS) and flash frozen in liquid nitrogen prior to storage at -80ºC. For in vitro fertilization the COCs were incubated in Tissue Culture Medium

199 (**TCM199**) (Gibco BRL, Grand Islands, NY) containing 0.14% (w/v) PVA, 10 ng/ml (w/v) epidermal growth Factor (**EGF**), 0.57 mM (w/v) cysteine, 0.5 µg/ml (w/v) porcine follicle stimulating hormone (**FSH**) and 0.5 µg/ml (w/v) porcine lutenizing hormone (**LH**) (Abeydeera et al., 1998). The maturation media was pre-equilibrated in 5% CO_2 in air at 39ºC in a humidified atmosphere overnight. COCs were matured for 40 to 44 hours in 5% CO_2 in air at 39ºC prior to the removal of the cumulus cells by vortexing for three minutes in Hepes-buffered medium with 0.1% (w/v) hyaluronidase. Denuded oocytes were washed and held in modified Tris-buffered medium (**mTBM**) prior to fertilization (Abeydeera and Day, 1997).

In vitro fertilization and embryo culture

Thirty-five oocytes were delivered to 50µl of mTBM under oil and held in 5% CO_2 in air at 39ºC. Freshly collected semen for IVF was diluted 3:1 in Androhep Enduraguard semen extender (Minitub, Verona, WI). The extended semen was washed in Dubelcco's PBS (GibcoBRL) with PVA/TL-Hepes/0.1% (w/v) BSA and centrifuged for 4 minutes at 1900 xg. This wash was repeated two more times and the sperm pellet was resuspended in 100 µl of mTBM. Fresh, extended sperm were diluted to 4 x10^4 per ml. The diluted sperm were preincubated for 2 hours in 5% CO_2 in air at 39ºC. Fifty µl of the diluted sperm was added to the oocytes and incubated for 5 hours in 5% CO_2 in air at 39ºC. The presumptive zygotes were then washed three times and incubated in PZM3 embryo culture medium (Yoshioka et al., 2002) in 5% CO_2 at 39ºC. The blastocysts were removed after 6 days of culture and the zonae

pellucidae of the blastocysts were removed as described. The zona free blastocysts were rinsed 3 times in DEPC-treated PBS and flash frozen in liquid nitrogen prior to storage at -80ºC**.**

Parthenogenetic embryo production

Cumulus cells were removed from in vitro matured oocytes by vortexing for three minutes in Hepes-buffered medium with 0.1% (w/v) hyaluronidase. IVM oocytes were equilibrated in activation medium composed of 0.3 M mannitol, 0.5 mM Hepes, 0.01% (w/v) BSA, 0.1 mM $CaCl_2$, and 0.1 mM $MgCl_2$ for 5 minutes and delivered to an activation chamber with electrodes 1 mm apart containing activation medium. Two 30-µsec electrical pulses of 1.2 kV/cm were conveyed by using a BTX Electro-cell manipulator (BTX San Diego, CA). The activated oocytes were cultured in PZM3 in 5% CO_2 in air at 39ºC (Lai and Prather, 2002; Lai and Prather, 2003).

Nuclear transfer embryo production

Reconstructed embryos were produced by using somatic cell nuclear transfer techniques as previously described (Lai et al., 2001). Porcine fetal fibroblast-like (**PFF**) cultures were established from a day 30 porcine conceptus. PFFs were cultured in Dubelcco's Modified Eagle Medium (**DMEM**) containing 15% (v/v) fetal calf serum (**FCS**), 100 IU/ml penicillin and 100 µg/ml streptomycin in 5% CO_2 in air at 39ºC. PFFs were harvested with the addition of Hank's Balanced Salt Solution (**HBSS**) with 0.1% (w/v) trypsin and 0.02% (w/v) EDTA.

In vivo embryo collection

The use of animals was conducted in accordance with protocols approved by the University of Missouri Animal Care and Use Committee. Crossbred Landrace gilts were bred on Day 0 of estrus by using artificial insemination (**AI**). Blastocysts were flushed on Day 6 according to previously published procedures (Machaty et al., 1998). Zonae pellucidae were removed from the blastocysts as described and frozen in pools of 5 to 14 blastocysts.

Porcine Differential Methylation Hybridization

Differential methylation hybridization analysis was conducted based on a technique developed for global scanning of methylation changes in the human genome (Huang et al., 1999). Porcine CpG island clones (approximately 8,000) from a Porcine CpG Island Library (**PCGIL**) (United Kingdom Human Genome Mapping Project, Hinxton, Cambridge, United Kingdom) were cultured in 96-well plates. The cloned insert was amplified by polymerase chain reaction (**PCR**) using the library specific primers 3558 (5'- CGG CCG CCT GCA GGT CGA CCT TAA) and 3559 (5'- AAC GCG TTG GGA GCT CTC CCT TAA). The PCR amplification was performed in a 10 µl reaction containing 1X Deep Vent DNA Polymerase Buffer, 10% DMSO, 400 pM of each primer, 100 pm each dATP, dTTP, dCTP, and dGTP , and 0.018 units Deep Vent Polymerase (New England Biolabs, Beverly, MA). The PCR program consisted of a denaturation step at 98ºC for 4 minutes followed by 30

cycles of denaturation at 95ºC for 30 seconds, annealing at 55ºC for 30 seconds and extension for 72ºC for 1 minute. A final extension at 72ºC completed the program. PCR products were stored at -20ºC until needed. Restriction digestion with *Bstu I* was performed using 1.5µl of the PCR reaction in 1X NEB 2 and 0.4 units *Bstu I* at 60ºC for at least 1 hour. The digested and undigested PCR products were run on a 1.5% 0.5X TBE agarose gel. *Bstu I* positive clones where the PCR product was cut, indicating the presence of a Bstu I site (CGCG) in the insert, were reracked and recultured in 96-well plates. Plates with all *Bstu I* positive clones were PCR amplified in a 50µl reaction and purified in Millipore 96-well PCR Purification plates in preparation for printing. The purified PCR products were dried and resuspended in 10µl 50% DMSO (v/v) 1% CHAPS (w/v) (Rickman et al., 2003). The resuspended PCR products were printed on Gold Seal glass microscope slides (Fisher Scientific, Hampton, NH) that were coated with 0.02% (v/v) poly-L-lysine (Sigma, St. Louis, MO) in 0.5X PBS (Eisen and Brown, 1999). The slides were stored for 3 weeks at room temperature under desiccation before printing with a pick and place robot. The printed slides were crosslinked at 120 mJ/cm^2 for 20s (Spectrolinker; Spectronics Corp., Westbury, NY) prior to blocking in 0.018% succinic anhydride (Sigma, St. Louis, MO) and 0.043 M sodium borate (Sigma, St. Louis, MO) in 1-methyl-2-pyrrolidinone (Sigma, St. Louis, MO) (Eisen and Brown, 1999). The slides were stored under desiccation and at room temperature until hybridization. Spotting buffer only (50% DMSO/1% CHAPS) and a whole CpG island library amplification (192 spots for each) served as the negative and positive controls, respectively. The whole

CpG island library amplification was generated by PCR as a means of providing a general positive control on the microarray slides. A scraping of the frozen CpG island library was collected, amplified by using the PCR program shown above and purified by the methods described herein. A total of 384 control spots and 4,992 test spots were printed on the microarray.

DNA Isolation

DNA was three replicates of each of the sample types. DNA was isolated from the pooled blastocysts and GV oocytes by adding H_2O to a final volume of 25 µl and incubating at 98ºC for 15 minutes. DNA from motile sperm was isolated by gently layering the extended semen on a 60%/80% Percoll gradient and centrifuging for 600xg for 10 minutes. The sperm pellet was removed and resuspended in PBS with 0.1% BSA. Contaminating somatic cells were eliminated by incubating the sperm pellet in PBS/0.8% (w/v) Triton X-100/0.8% (w/v) SDS for 10 minutes at room temperature (**RT**). The sperm pellet was rinsed three times in 10mM Tris (pH 8.0)/1mM EDTA/100 mM NaCl **(STE**) and resuspended in 700µl STE followed with the addition of 70µl 20% (w/v) sodium dodecyl sulfate (**SDS**), 25 µl 1M dithiothreitol (**DTT**), and 5µl 20mg/ml proteinase K and incubated at 56ºC overnight. The DNA was purified with phenol chloroform, precipitated with EtoH, and resuspended in 10mM Tris/1mM EDTA (**TE**).

Amplicon Generation, Labeling and Hybridization

Amplicons were produced by digesting DNA from liver (reference), GV-stage oocytes, sperm, or blastocysts with the restriction enzyme Mse I (50 units) in 1X NEB 2, 1X BSA at 37ºC overnight as recommended by the supplier (New England Biolabs, Beverly, MA). The restricted DNA was ligated to PCR linkers produced by mixing oligomers (H-24, 5'-AGG CAA CTG TGC TAT CCG AGG GAT and H-12, 5'-TAA TCC CTC GGA), heating to 65ºC, and cooling to room temperature. DNA was then digested with the methylation sensitive restriction enzyme *BstuI* (NEB) as recommended. The intact DNA fragments were amplified by PCR using Biolase Taq in a 50 ul reaction with H-24 as the linker specific primer. The PCR products were labeled with amino allyl-dUTP using the BioPrime labeling system with modifications. The PCR products were purified with a Qiagen PCR Purification Kit and resuspended in 29 µl H_2O, mixed with 1X BioPrime buffer (with random octamers), dNTPs (2:3 amino allyl-dUTP:dTTP, dATP, dGTP, dCTP), 40 units Klenow, and incubated for 60 minutes at 37ºC. Amino allyl-dUTP incorporated PCR products were purified with the Qiaquick columns using PB buffer, (phosphate washing buffer: 5mM KPO_4, 80% EtoH, pH 8.0) and phosphate elution buffer (4 mM KPO_4, pH 8.5). The samples were dried and resuspended in 0.1 M sodium carbonate buffer (pH 9.0) and mixed with Cy3 for the oocyte, sperm and blastocyst samples or mixed with Cy5 for the liver reference samples. The samples were incubated for 60 minutes at room temperature in the dark. The labeling reactions were purified with Qiaquick columns by using PB buffer, PE buffer, and EB buffer. The labeling

efficiency was then analyzed spectrophotometrically by using the Nanodrop ND-1000 (Nanodrop, Wilmington, DE). Comparable amounts of labeled test sample and liver reference sample were mixed together based on the amount of DNA and the rate of incorporation of the Cy 3 and Cy5 dyes, respectively. The combined samples were dried and resuspended in 26 µl hybridization buffer (50% formamide, 5X SSC, 0.1% SDS). The samples were denatured at 95ºC for 3 minutes and immediately transferred to ice before being applied to a microarray slide with a coverslip. The microarray was incubated at 42ºC for 8 to 12 hours before removing the coverslip in Wash I (1X SSC/0.2% (v/v) SDS), and washing in Wash II (1X SSC/0.2% (w/v) SDS), Wash III (0.1X SSC/0.2% (w/v) SDS), Wash IV (0.1X SSC), and Wash V (H_2O). The slides were immediately centrifuged for five minutes at 1,500xg and scanned with an Axon 4000B scanner.

Microarray Analysis

Microarray images were analyzed with GenePix 4.0. Spots with at least 25% of the pixels possessing intensities greater than one standard deviation higher than the background in either the Cy3 or Cy5 channel were further analyzed with Gene Spring version 7.2. The LOWESS normalized data was analyzed by Analysis of Variance (**ANOVA**) using the Benjamini and Hochberg False Discovery Rate for multiple testing. A P value less than 0.01 was considered significant. Clones (n=106) were initially selected for sequencing based on the presence or absence of a significant difference between the *in vivo*-produced blastocysts and the other samples. The

sequence of the selected clones was determined by amplifying the clone from the appropriate well of the *BstuI* positive clones 96-well plates by PCR using library specific 3558 and 3559 primers. The same cycling protocol used to amplify the insert. The PCR products were purified and sequenced by using the library specific primer 3558. Sequencing was performed at the University of Missouri-Columbia DNA Core facility on an ABI 3730 96-capillary DNA Analyzer with Applied Biosystems Big Dye Terminator cycle sequencing chemistry. Sequence homology was determined by using the National Center for Biotechnology Information nucleotide-nucleotide Basic Local Alignment Search tool. The clonal sequences were identified as homologous when a single gene was exclusively or predominantly identified (n= 41). The scores of these regions ranged from 58 to 910 with an average score (bits) of 178.

Bisulfite Sequencing

Clones were selected for bisulfite analysis because of potential biological significance to embryogenesis (e.g. transcription factors, WNT8B) and repeated detection of the same region in multiple spots in the microarray analysis (e.g. PPOX). In order to ascertain the analytical capacity of the microarray analysis procedures, additional spots were selected based on the presence or absence of a significant difference between the *in vivo*-produced blastocysts and the other biological samples based on the microarray results. This provided an independent method to verify the microarray data. Sperm and liver DNA (1 μg) were treated with sodium bisulfite by using the EZ DNA Methylation Kit (Zymo Research, Orange, CA) according to the

vendors recommendations. In addition, DNA from fifty day 6 *in vivo*-produced blastocysts was treated with bisulfite by using the EZ DNA Methylation-Gold Kit (Zymo Research, Orange, CA) according to the vendor's recommendations. Primers were designed for bisulfite treated DNA by using the MethPrimer software (Li and Dahiya, 2002). Primer sequences are shown in Table 3.1. PCR was performed as shown below:

H_2O	32.5 µl
DNTP	1.3 µl
10X Buffer (TagGold)	5 µl
$MgCl_2$	5 µl
Forward Primer (10 µM)	2 µl
Reverse Primer (10 µM)	2 µl
DNA (Bisulfite treated)	2 µl
AmpliTaq Gold (5u/µl)	0.25 µl
Total	50 µl

The PCR program consisted of a denaturation step at 98ºC for three minutes followed by fifty cycles of denaturation at 95ºC for 15 seconds, annealing at 55ºC for 30 seconds and extension for 72ºC for 30 seconds. A final extension of 72ºC for 5 minutes completed the program.

The PCR reaction was purified by using the Qiaquick columns as described by the vendor. The PCR products were cloned by using the pGEM T-Easy Kit (Promega,

Madison, WI) according to the vendor's recommendations. The vectors were transformed in to DH10B cells (Invitrogen) and grown on LB/IPTG/X-Gal/Ampicillin agar plates. Recombinant colonies were selected for sequencing based on the blue/white screening criteria. The cytosines of the CpG sites were identified as methylated or unmethylated if a C or T was present in the sequence, respectively. The percent methylation was calculated for the respective sequence and the methylation status of the microarrays and bisulfite sequencing were compared. A ratio of liver: donor cell methylation was calculated by using the following formula:

$$Rm = (100-M_S)/(100-M_L)$$

Where: M_S is the average % CpG methylation for a sequence in the sample

M_L is the average % CpG methylation for a sequence in the liver reference

The use of this formula provides a means to calculate a ratio that indicates the relative levels of methylation in a given sequence when one of the samples lacks methylated CpG dinucleotides. 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 were classified as hypomethylated when the ratio was

>1.25. The threshold of hypermethylation and hypomethylation for the microarray results was identified as the smallest deviation from 1 for those regions which were validated by using bisulfite analysis. Specifically, the microarray results for CPG X G2 of the sperm sample was 1.251. Accordingly, microarray results were classified as hypomethylated when the microarray ratio was >1.25 and the sample was classified as hypermethylated when the microarray ratio was <0.75.

RESULTS

Differential Methylation in the GV Oocyte, Sperm, and Blastocysts

The relative methylation in the gamete and blastocyst samples were analyzed by using GeneSpring 7.2 revealed that 18.5% (921/4,992) of the clones were found to be significantly different ($P<0.01$) in at least one of the samples (Fig. 3.1). A complete listing of the clones that were significantly different is shown in Table A.1. Genomic clones were selected to be sequenced based on the relationship between the ratios for the sperm and *in vivo*-produced blastocysts. These samples were selected based on the ample supply of sperm DNA and the reliability of the methylation status of the *in vivo*-produced blastocysts. Of the 104 clones that were successfully sequenced, relevant annotation was available for only 33 of the clones. One sequence was represented twice and another represented three times. Thus, there was little redundancy on the array and most (97.1%: 101/104) of the clones were unique. These sequences were deposited in GenBank (DQ915200-DQ915250).

Validation of the Microarray Analysis by using Bisulfite Sequencing

In order to validate the microarray data, clones (n=12) were selected when the ratios of the sperm and *in vivo*-produced blastocysts were similar and when the ratios were significantly different (Table 3.2). For the selected clones, the methylation status of the *in vivo*-produced blastocysts relative to the liver reference samples was identified as hypomethylated for 6/12 (50.0%), equivalent for 2/12 (16.66%), and hypermethylated for 4/12 (33.33%) of the samples. ANOVA showed there was a significant difference between the *in vivo*-produced blastocysts and the other samples in 32/60 (53.33%) of the comparisons (Table 3.2).

Bisulfite sequencing indicated high levels of methylation for the clones HH A7, WW G4, X G2, and B G2 (Figures 3.2 to 3.5) in liver, sperm or *in vitro*-produced blastocysts. The percent methylation levels of cytosines in the CpG dinucleotides is shown in Table 3.3A for the clones B G2, HH A7, WW G4, and X G2 for the *in vivo*-produced blastocysts. The microarray values are LOWESS normalized Cy5/Cy3 ratios representing the methylation status of the specified clones in the liver (Cy5) and the sperm and *in vivo*-produced blastocyst samples (Cy3) (Table 3.3B). The Bisulfite (Ref/Sample) values represent the relative methylation levels in the liver and *in vivo*-produced blastocyst at selected regions of the specified clones. The Bisulfite (Ref/Sample) values were calculated from the equation shown in the Materials and Methods section. The methylation status, determined by microarray analysis and bisulfite sequencing, of these four regions of the *in vivo*-produced blastocysts is

shown in Figure 3.6. The methylation status, determined by microarray analysis and bisulfite sequencing, of these four regions of the sperm is shown in Figure 3.7. The reference/sample ratios were consistent with the microarray data in 87.5% (7/8) of the regions that were tested (Figures 3.6 and 3.7).

The remaining eight regions showed very little methylation (<10%) in the liver, sperm, and *in vivo*-produced blastocyst samples (Figures 3.8 to 3.15). The low levels of methylated CpGs were consistent with the microarray data in 56.25% of the samples (9/16) (Table 3.4). Table 3.4A shows the percent methylation for the clones CC C1, EEE D4, EE A11, K D3, L E8, O D10, QQ E4, and S E3 in the liver (Reference), sperm, and *in vivo*-produced blastocyst samples. The microarray values are LOWESS normalized Cy5/Cy3 ratios representing the methylation status of the specified clones in the liver (Cy5) and in the sperm and *in vivo*-produced blastocyst samples (Cy3) (Table 3.4B). The Bisulfite (Ref/Sample) values represent the relative methylation levels in the liver and *in vivo*-produced blastocysts at selected regions of the specified clones. The Bisulfite (Ref/Sample) values were calculated from the equation shown in the Materials and Methods section.

Figure 3.16 shows a typical arrangement of the location of a CpG island relative to the start of a gene. A differentially methylated CpG island was identified 184 bases upstream of the start of the myeloid leukemia factor 1 gene (WW G4). The methylation profile of WW G4 (myeloid leukemia factor 1 (MLF1) was measured by using PDMH microarrays. This region was hypomethylated in all samples relative to the reference sample except for the SCNT-produced blastocysts where the region was

hypermethylated (Figure 3.17). Regions near the start site of the gene were also identified for QQ E4 and EEE D4.

Analysis Based on Methylation Profiles

Figure 3.18 shows two of the groups that were similar in the methylation status in the sperm, GV oocyte and blastocysts by using Self Organizing Map Analysis. BLAST analyses of clones that are hypomethylated relative to the liver in the *in vivo*-produced sequenced clones are shown in Table 3.5. An ANOVA identified a significant difference between the *in vivo*-produced blastocysts versus the other samples with 134/185 (72.43%) of the samples. BLAST analyses of clones that are hypomethylated relative to the liver in the *in vivo*-produced sequenced clones shown are shown in Table 3.6. An ANOVA identified a significant difference between the *in vivo*-produced blastocysts versus the other samples with 97/165 (58.78%) of the samples. The complete Self Organizing Map Analysis is shown in the Appendix in Figure A.1. The BLAST analysis for the clones shown in Figure A.1 is shown in Table A.2. Figure 3.19 shows a condition tree based on the similarity of the methylation profiles of the sperm, GV oocyte, and blastocysts for the clones where there was a significant difference (P>0.01) in the methylation status in at least one of the samples. The *in vivo*-produced blastocysts clustered with 2 of the 3 SCNT-produced blastocyst replicates while the GV oocyte, sperm, and *in vitro*-produced blastocysts clustered together based on similarity of the methylation profiles. The parthenogenetic blastocysts did not cluster consistently with the other groups.

Additional validation of the hierarchical clustering was performed by using alternate clustering procedure (Figure A.2) The TIGR Multiple Array Viewer software used to do the hierarchical clustering and the bootstrap analysis does not include the same correlation analysis that is used by the GeneSpring software. Specifically, the Standard Correlation used in the GeneSpring software is commonly referred to as Pearson correlation around zero. The TIGR Multiple Array Viewer does not contain this correlation procedure so the Pearson Correlation analysis was substituted. Therefore, caution should be used in attempting to extrapolate the bootstrapping results to the clustering generated by using GeneSpring. The strongest support, based on both clustering procedures, is shown for the clustering of the SCNT-, parthenogenetic-, and *in vivo*-produced blastocysts.

Identification of Putatively Imprinted Genes

Imprinted genes are thought to be resistant to the passive and active demethylation events that occur immediately after fertilization. The methylation status of imprinted genes is subsequently maintained from the gamete to the somatic cells. ANOVA of the sperm and GV oocyte samples identified 28 genes where the methylation status was significantly different ($p<0.05$) as measured by PDMH microarray analysis (Table 3.7). The normalized *in vivo*-produced blastocyst and sperm ratios (reference/sample) and the normalized *in vivo*-produced blastocyst and oocyte ratios (reference/sample) were analyzed by using ANOVA (Table 3.8). The methylation status was maintained in the development from the GV oocyte to the *in*

vivo-produced blastocyst for 11 regions. Specifically, these regions were all hypomethylated in the GV oocyte and in the *in vivo*-produced blastocyst. The methylation status was maintained in the development from the sperm to the *in vivo*-produced blastocyst for 6 regions. In contrast to the GV oocyte where all the putatively imprinted regions were all hypomethylated, 50% (3/6) of the putatively imprinted regions in the sperm were hypomethylated and 50% (3/6) were hypermethylated.

DISCUSSION

Our study shows that the epigenetic remodeling of blastocysts produced by the *in vitro* techniques of parthenogenesis, SCNT, and IVF is incomplete relative to the *in vivo*-produced blastocyst. An unexpected result of this study identified less similarity in hypermethylation of *in vitro*-produced blastocysts compared to the *in vivo*-produced blastocysts than the hypermethylation of parthenogenetic- and *in vitro*-produced blastocysts. Although unexpected, these results are consistent with a recent study that showed that the bovine SCNT-produced blastocysts expression was more similar to *in vivo*-produced blastocysts than the expression patterns of *in vivo*-produced blastocysts and *in vitro*-produced blastocysts (Smith et al., 2005). Also, previous research (Whitworth et al., 2005) has shown that 1696 cDNAs were differentially detected between *in vitro*- and *in vivo*-produced blastocysts. These results are consistent with the differential methylation detected by using microarray

analysis in this study. Further research is needed to demonstrate that the differential expression observed by Whitworth et al., (2005) is caused by differential methylation.

The development rate of *in vitro*-produced blastocysts is comparable to parthenogenetic-produced blastocysts and much higher than SCNT-produced blastocysts. Producing offspring by embryo transfer is more efficient using *in vitro*-produced blastocysts compared to using SCNT-produced blastocysts. This suggests that other factors, other than the lack of hypermethylation detected in *in vitro*-produced blastocysts, relative to the other blastocysts, are responsible for the decreased developmental potential.

Clones were organized according to similar methylation profiles in the gametes and blastocysts by Self Organizing Map analysis. A BLAST analysis identified sequence homology with 10 clones that were hypermethylated relative to the liver in the *in vivo*-produced blastocysts relative to the other samples including glutamate decarboxylase (GAD2), DEAD box (DDX10), Wnt8B (WNT8B), sine oculis homeobox homolog 6 (SIX6), cyclic AMP transcriptional regulator protein (ATF2), protoporphyrin oxidase (PPOX), Zinc finger, CSL domain (ZCSL2), histones H2B.1 and H2A (HIST2H2BE), prostate antigen PARIS-1 (TBC1D2), and splicing factor 3a, subunit 3 (SF3A3) (Table 3.5). The regions analyzed correspond primarily to areas immediately around the start site of the gene. Accordingly, down-regulated expression of these genes is expected based on the premise that hypermethylation interferes with the assembly and binding of transcription factors. A BLAST analysis identified sequence homology with clones that were hypomethylated

in the *in vivo*-produced blastocysts relative to the other samples including aryl hydrocarbon receptor nuclear translocator (ARNT), Coatomer zeta-1 (Zeta-1 COP), myeloid leukemia factor 1 (MLF1), serine/threonine protein kinase Kp78 (MARK3), malignant T cell amplified sequence 1 (MCTS1), methyltransferase-like (LOC533379), FRG1 (FRG1), and forkhead box protein J2 (FOXJ2) (Table 3.6) . Hypomethylation of regulatory regions associated with these genes would be expected to result in up-regulated expression.

The low ratings of the Bootstrapping analysis appear to be a function of the replicate variation. This variation does not affect the reliability of the data when the replicates are averaged as determined by the validation by using bisulfite sequencing. Whereas microarray analysis is generally viewed as a screening tool, bisulfite sequencing is generally viewed as the gold standard in identifying CpG methylation. In this study, when hypermethylation was observed in the bisulfite sequencing reactions, in either the reference or the sample, the microarray analysis was usually (87.5%) consistent with these results. Conversely, differential methylation was identified by the microarray analysis for sequences that were shown to have low levels of methylation by using bisulfite sequencing. These results indicate that PDMH is a valid technique in detecting methylation when the regions are highly methylated (>10%). Furthermore, only 4.17% (1/24) of the samples failed to identify high levels of methylation where the microarray data was not consistent with the bisulfite sequencing. If the PDMH

analysis was a random artifact then about 25% of the bisulfite and microarray results are expected to be contradictory.

It is important to note that the bisulfite sequencing procedure does not test the entire sequence that was used in the microarray. Instead, only about 170 base pairs are assayed with bisulfite analysis whereas the average sequence on the microarray is expected to be about 500 base pairs. When multiple genes are identified from the BLAST analysis of a sequenced clone, there is a region or regions that is/are common to all the identified genes in addition to the unique sequences which define the respective gene. Figure 3.20 shows an example of when multiple genes are identified from a single sequence (QQ A6). BLAST analysis identified: 1) sequences on porcine chromosomes 6, 7, 11, and 17, 2) mRNA expressed in various tissues including liver, thymus, trachea, uterus, and ovary and 3) porcine genes including CKM, ASIP, and KIT. Hypermethylation of any of these similar regions will result in cross-hybridization in the microarray analysis but the methylation may not be detected by using bisulfite sequencing of the original sequence.

Correlation analysis of the methylation profiles produced by the microarrays showed that the sperm and GV oocyte were most similar. Regions with consistent methylation in the liver, oocyte, sperm, and *in vivo*-produced blastocyst are putatively imprinted. Imprinted regions are thought to be resistant to the active and passive demethylation processes in early embryonic development and also resistant to tissue specific epigenetic remodeling. The methylation profile of the *in vitro*-produced

blastocysts clustered with the sperm and GV oocyte instead of with the other blastocysts. This clustering pattern suggests there was incomplete methylation remodeling in the *in vitro*-produced blastocysts relative to the other blastocysts.

Twelve regions were analyzed by using bisulfite analysis to validate the microarray results. The reference/sample ratios were consistent with the microarray data in 87.5% (7/8) of the regions that were tested where high methylation levels were detected. When low levels of methylation were observed, the bisulfite analysis results were consistent with the microarray data in 66.67% of the samples (8/12). Overall, the bisulfite analysis results validated the microarray results for 70.83% (17/24) of the regions that were tested.

Microarray analysis identified differential methylation for 16 regions but these regions were shown to be essentially unmethylated by using bisulfite analysis. In contrast, most (7/8) of the other microarray results were validated by using bisulfite analysis. Furthermore, of the 24 regions that were analyzed, there were no regions where the microarray results and the bisulfite sequencing results were contradictory. Specifically, none of the regions were identified as hypomethylated by using the microarray analysis and also identified as hypermethylated by using bisulfite sequencing, and visa versa. These results suggest there was cross-reactivity in the microarray hybridization whereby differential methylation was identified but bisulfite sequencing showed the sample and the reference to be essentially unmethylated. Amplification of repeated sequences would explain the observed cross-reactivity but sequencing did not generally identify repeated sequences.

There are two possible explanations for the differences between the two techniques. First, the detection procedure used to validate the microarray results in this study (bisulfite sequencing) may not have been sufficient to detect rare, highly methylated strands. The PCR based approach to target production of the microarray analysis could have amplified a low abundance strand to produce a detectable signal after hybridization with the PDMH microarrays. The second explanation is that the low quantity of template DNA available from the blastocysts necessitated the high number of cycles in the amplification step. Additional optimization of the target production or hybridization could minimize this potentially confounding effect.

The hypomethylation detected in the *in vivo*-produced blastocysts distinguishes the methylation profiles from the other samples that were tested. Differential methylation of the embryonic and extraembryonic cells in the early embryo has been detected in the mouse (Santos et al., 2002; Dean et al., 2005). Hypomethylation of the trophectoderm relative to the inner cell mass may indicate a regulatory mechanism for the expression of genes that are critical in implantation. The pig is similar to most other mammals in that the genomes are demethylated during preimplantation development (Dean et al., 2001; Kang et al., 2001). Defects in DNA remodeling and subsequent transcriptional abnormalities in SCNT- and *in vitro*-produced blastocysts, especially of the cells that give rise to the placenta, would account for the high pregnancy loss that occurs around day 25-45 of development in the pig (Lai and Prather, 2003). In this study, the *in vivo*-produced blastocysts were different from the other blastocysts in that there was no time in culture. For example,

previous studies have shown that epigenetic changes caused by *in vitro* preimplantation culture results in biallelic expression of the imprinted gene H19 (Sasaki et al., 1995; Doherty et al., 2000). Aberrant expression of the imprinted genes H19, Ascl2, Snrpn, and Xist was observed while the ICM retained the correct expression (Mann et al., 2004). The authors suggest that the epigenetic remodeling and reprogramming of trophoblast may be more sensitive to the disruptive effect of the culture media than the ICM.

Although the maintenance of imprinted porcine genes was not analyzed in this study, we identified 16 putatively imprinted genes by using PDMH microarray analysis. Ten regions in the GV oocyte and 6 regions in the sperm were found to maintain the hypomethylation or hypermethylation status in the development from the gamete to the *in vivo*-produced blastocyst. Bisulfite analysis or pyrosequencing is needed to confirm the identification of these regions as imprinted. *In vitro* culture has been shown to cause aberrant methylation of imprinted genes (Khosla et al., 2001; Mann et al., 2004). Monitoring the methylation status of imprinted genes in cells or embryos cultured *in vitro* will be important in the identification of *in vitro* culture conditions which support the development of IVF and SCNT embryos.

Our results show that the CpG methylation remodeling is abnormal in blastocysts produced by using *in vitro* techniques. Specifically, there appears to be fewer hypomethylated regions in parthenogenetic-, SCNT-, and *in vitro*-produced blastocysts. Additionally, blastocysts produced by *in vitro* fertilization also appear to lack the methylation events that occur in parthenogenetic-, SCNT- and *in vivo*-

produced blastocysts. Although the methylation profile of the *in vitro*-produced blastocysts is less similar to the *in vivo*-produced blastocyst than those of the parthenogenetic- and SCNT-produced blastocysts, blastocysts produced by *in vitro* fertilization have greater developmental potential to produce offspring after embryo transfer.

Recently, the transcriptional expression patterns of bovine SCNT- and *in vivo*-produced blastocysts were shown to have greater similarity to each other than the similarity of the expression patterns shared by *in vitro*- and *in vivo*-produced blastocysts (Smith et al., 2005). Expression microarray analysis was used to analyze the gene expression profiles of SCNT donor cells, and of individual bovine SCNT-produced blastocysts and blastocysts produced by using AI procedures. Hierarchical clustering analysis showed that the AI- and SCNT-produced blastocysts were more similar to each other than to the *in vitro* fertilization-produced blastocysts. Similar observations have been made in the activation of rRNA synthesis for embryos produced *in vitro*- and SCNT-produced (Bjerregaard et al., 2006). Asynchronous rRNA transcription was observed in the *in vitro*-produced blastocysts from the 4-cell stage through the blastocyst stage. Conversely, the cells in SCNT-produced embryos that developed to the 16-cell stage and the blastocyst stage were shown to be transcriptionally active. The activation of rRNA transcription observed in SCNT-produced blastocysts is consistent with the activation of rRNA transcription for *in vivo*-produced blastocysts. The similarity of transcriptional activity in blastocysts produced by SCNT and *in vivo* procedures is surprising given the low developmental

competence of the SCNT-produced blastocysts after embryo transfer. These results suggest that developmental potential may be controlled by a relatively small number of genes since analogous transcriptional activity in the blastocysts produced by SCNT and *in vivo* procedures do not result in analogous developmental potential.

In conclusion, adaptation of PDMH microarrays enabled the global analysis of differential methylation of the pig genome. While we did not have the resources to sequence each clone on the array we were able to demonstrate the utility of the tool and to justify additional sequencing. The genomic regions that were identified will be useful as markers for understanding the changes in DNA methylation during pig embryogenesis. The use of this tool has permitted us to conclude that the CpG methylation remodeling that occurs in the development of the *in vivo*-derived blastocyst does not occur in blastocysts produced by using *in vitro* techniques such as parthenogenesis, SCNT, and *in vitro* fertilization. Specifically, the methylation events that occur in the development of parthenogenetic and SCNT-produced blastocysts are more similar to the *in vivo*-produced blastocysts than the methylation remodeling events in the *in vitro*-produced blastocysts. Recently, the use of *in vitro* matured oocytes has been shown to result in incomplete demethylation of the paternal genome following *in vitro* fertilization (Gioia et al., 2005). In this study, the parthenogenetic-, SCNT-, and *in vitro*-produced blastocysts were all produced by using *in vitro*-matured oocytes, but only the *in vitro*-produced blastocysts failed to replicate the hypermethylation of specific regions found in the *in vivo*-produced blastocysts. These results suggest that DNA methylation in sperm may be resistant to epigenetic

remodeling directed by *in vitro*-matured oocytes. Conversely, *in vitro*-produced oocytes appear to be able to direct the remethylation in the parthenogenetic- and SCNT-produced blastocyst. This type of analysis will be instrumental in identifying factors that are critical in the efficient production of embryos by using *in vitro* techniques. Examples of these factors includes oocyte maturation media, embryo culture media, and also in the selection of donor cells used in SCNT. Identifying the specific effects of aberrant methylation, as it relates to transcriptional reprogramming, is necessary to fully understand how incorrect genomic remodeling can interfere with the development of the early embryo. Additional research is needed to identify the specific aberrant methylation events that have a direct influence on the developmental deficits observed when producing offspring by using *in vitro* fertilization and SCNT.

Table 3.1. Bisulfite Modification Specific Primers

CPG Clone	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
CC C1	LEFT	TTT GAA ATT AGG GTT GTA AGG TAG GT
CC C1	RIGHT	CCA CCC TCT AAC AAA AAA CTC TTA C
EE A11	RIGHT	AAAAATAACTCTAACCAAAATAAAC
EE A11	LEFT	TTTTAGTTAATAGGGAGGTAGTGTA
EEE D4	LEFT	GGT ATT GTA GAA AGT GGG TTT GAG T
EEE D4	RIGHT	AAAAATAATATAAAACCA AAA ATA ACA C
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
L E8	LEFT	GGG TTT TAT TTT GTT TTT TTA AG
L E8	RIGHT	TAT CAC TAA AAA TCA ATC CCC AAA A
O D10	LEFT	GTA GAA GGT AGA TGA TTT TTT TT
O D10	RIGHT	TAA AAC AAA TTT TTC AAA CCC AAA C
QQ E4	RIGHT	ACAAAACTAAAACATCTCTTTACCTAAAAT
QQ E4	LEFT	GTTTGGATTGGGTTTTTTGAT
S E3	RIGHT	AAA AAA AAT AAC AAT TCC ACC ACC
S E3	LEFT	GTT TAT GGG GAA GTT TAG GGT AGA G
WW G4	LEFT	GGT TTT TTA GTT TTT TAT TTG TTT AG
WW G4	RIGHT	AACTAAATCTTACCCTACTTTCTA TAA ATA
X G2	RIGHT	TAA ACA CTA ACC CAA AAA AAC CTT C
X G2	LEFT	GTT TGG TAG GGG AGT TTG TAG AGT

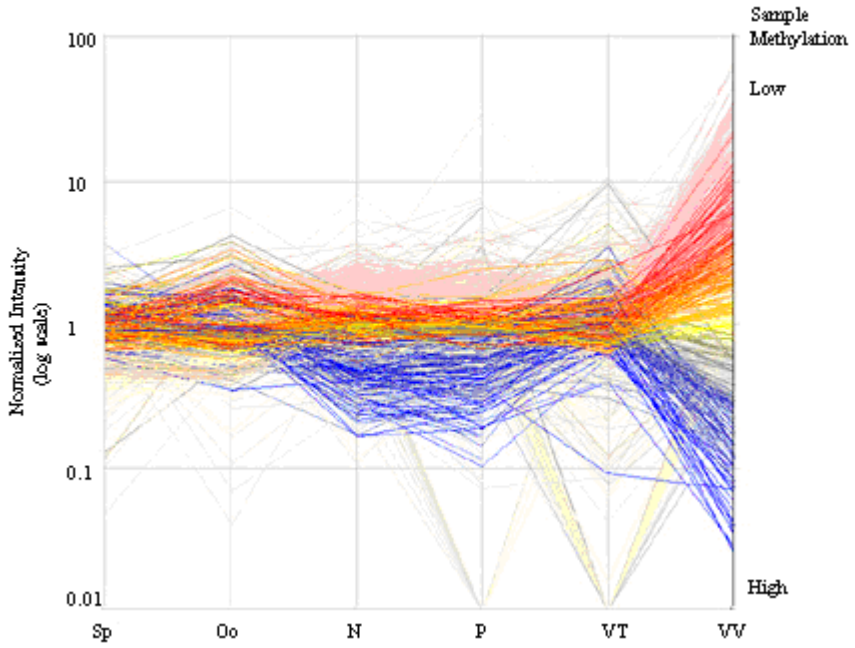

Figure 3.1. Methylation profiles of porcine sperm (Sp), germinal vesicle oocytes (Oo), parthenogenetic- (P), nuclear transfer- (N), *in vitro*- (VT), and *in vivo*- (VV) produced blastocysts generated by using PDMH analysis. This graph shows the Reference/ Sample ratios for the 921 clones that were significantly different ($P<0.01$) in at least one of the sample groups. A Reference/Sample ratio greater than 1 indicates that the reference is hypermethylated relative to the sample and a Reference/Sample ratio less than one indicates that the reference is hypomethylated relative to the sample. Each line represents the methylation status of a single clone at the different stages listed on the X axis. The *in vivo*-produced blastocysts have more genes that are hypomethylated relative to the reference as compared to the other samples. Extensive hypermethylation is measured in the parthenogenetic-, SCNT-, and *in vivo*-produced blastocysts but not in the *in vitro*-produced blastocysts. The clones are colored by the Reference/Sample ratios in the *in vivo*-produced blastocyst sample.

Table 3.2. Microarray reference/sample ratios± standard error of microarray clones identifies the methylation status of the selected clones. A ratio greater than one indicates the region is hypomethylated in the test sample relative to the reference sample. A ratio less than one indicate the region is hypermethylated in the test sample relative to the reference sample. These clones were selected to validate the microarray results by using bisulfite sequencing. A significant difference was detected by using ANOVA between the in vivo-produced blastocysts and the other samples ([a] - $P<0.05$, [b]- $P<0.01$, [c]-$P<0.001$, [d] -$P<0.0001$). There was a significant difference between the *in* vivo-produced blastocysts versus the other samples in 32/60 (53.33%) of the samples. The absence of a standard error indicates data was available for 1 of the 6 replicates. n.d.- no data.

	Sperm	Oocyte	Parth. blast.	SCNT blast.	In vitro blast.	In vivo blast.
B G2	0.960±0.172 [c]	1.043±0.181 [c]	0.992±0.203 [c]	1.748±0.241 [b]	0.878±0.187 [c]	21.520±11.047
CC C1	2.055±0.545 [b]	1.145±0.607	0.354±0.455	0.324±0.293	n.d.	0.375±0.565
EE A11	1.262±0.208 [c]	0.867±0.201 [b]	0.560±0.243 [b]	0.476±0.240 [a]	1.232±0.199 [c]	0.173±0.354
EEE D4	1.074±0.191 [c]	2.825±0.223 [b]	3.381±0.484 [a]	2.544±0.710 [a]	1.463±0.235 [b]	32.965±20.055
HH A7	0.619±0.427 [c]	2.033±0.773 [a]	2.539±2.283 [a]	0.354±0.677	4.885±1.886 [b]	0.558±0.673
K D3	0.593±0.214 [c]	0.411±0.548 [a]	1.674±0.501	1.432±0.291	3.364	2.806±1.259
L E8	1.033±0.229 [a]	0.804±0.253	0.485±0.242	0.552±0.242	0.721±0.446	0.464±0.450
O D10	1.105±0.175 [d]	1.068±0.188 [c]	1.178±0.214 [c]	1.546±0.239 [b]	0.990±0.196 [b]	6.897±0.966
QQ E4	0.462±0.271	0.179±0.320	3.438±1.776	0.965±0.401	n.d.	1.135±0.716
S E3	0.910±0.290	1.691±0.721	1.235±0.574	0.833±0.318	n.d.	0.859±0.578
WW G4	1.506±0.413 [b]	2.378±0.943	2.054±1.158	0.218±1.187	3.959±0.976	5.927±2.425
X G2	1.251±0.203 [b]	1.203±0.185 [b]	1.240±0.328	2.129±0.313	0.938±0.322 [b]	3.596±0.692

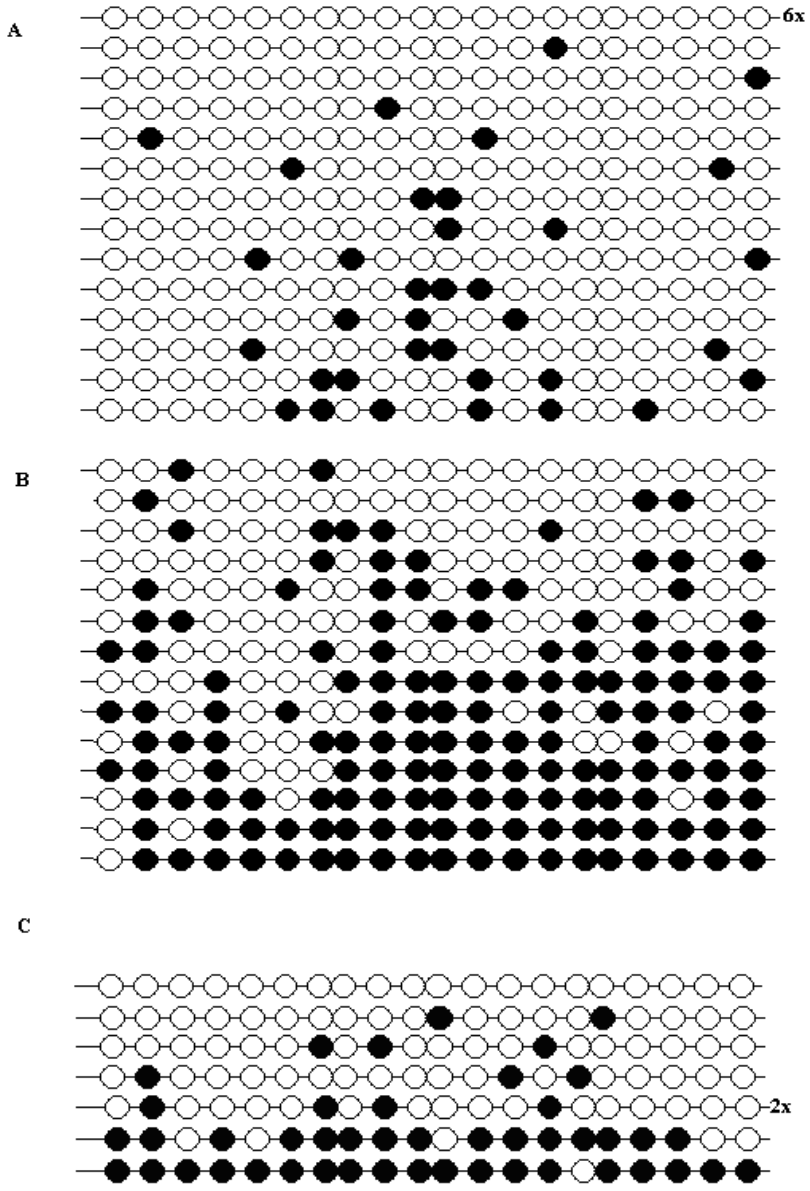

Figure 3.2. Methylation status of the Clone HH A7 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. This region was hypermethylated in the sperm relative to the *in vivo*-produced blastocysts and the liver samples.

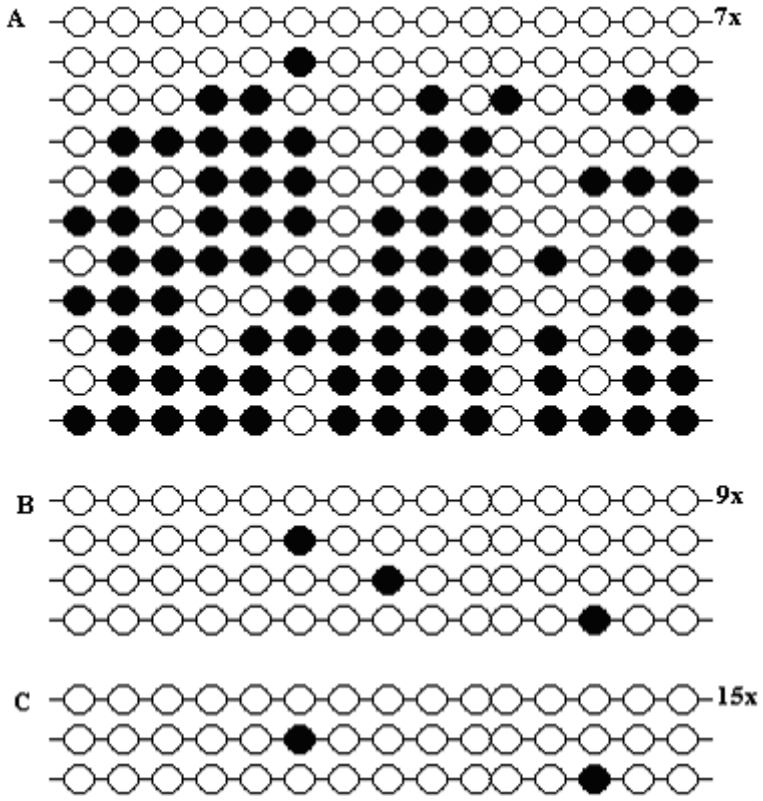

Figure 3.3. Methylation status of the Clone WW G4 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence . This region was hypermethylated in the liver sample relative to the sperm and *in vivo*-produced blastocysts.

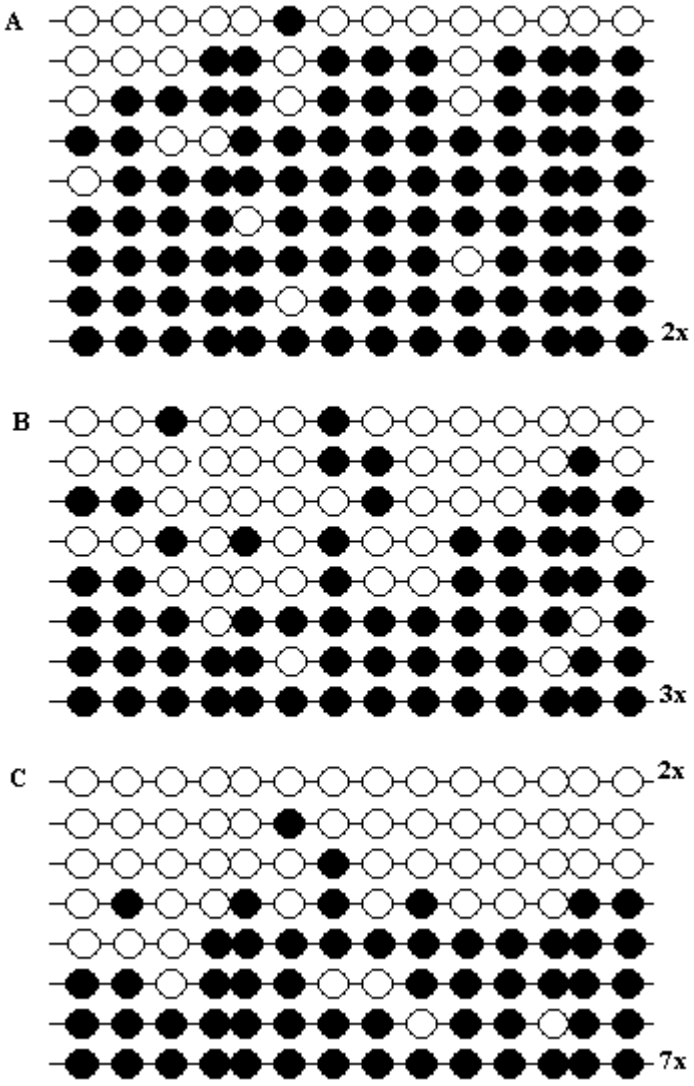

Figure 3.4. Methylation status of the Clone X G2 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. Higher methylation levels were observed for this region in the liver samples relative to the sperm and *in vivo*-produced blastocysts.

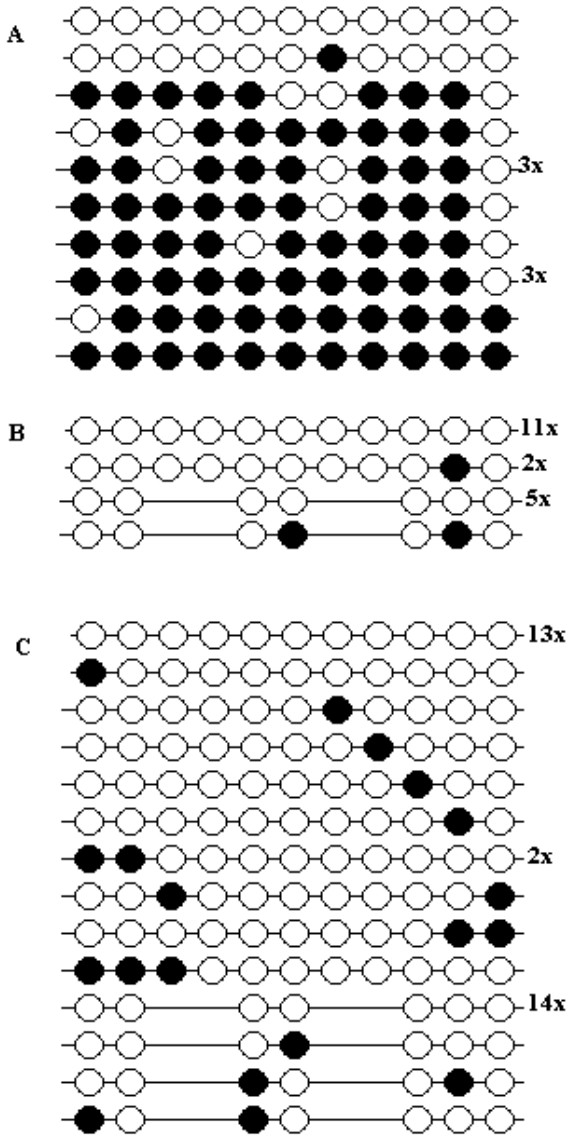

Figure 3.5. Methylation status of the Clone B G2 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. The liver epigenome at this site is hypermethylated relative to the sperm and *in vivo*-produced blastocyst DNA. Also, internal regions of this clone identify an alternate sequence in the sperm and *in vivo*-produced blastocyst. This alternate sequence may be an allele with two modified regions.

Table 3.3. Methylation status of B G2, HH A7, WW G4, and X G2 for *in vivo*-produced blastocysts analyzed by using microarray and bisulfite sequencing analysis. A) Shown here is the percent cytosine methylation at all the CpG dinucleotides that were analyzed by using bisulfite sequencing in the liver DNA, sperm DNA, and *in vivo*-produced blastocyst DNA for the regions analyzed by using bisulfite sequencing. B) Bisulfite analysis data and the microarray analysis data are in agreement for 87.5% (7/8) of 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 methylation 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. The ratios produced from bisulfite analysis data and the microarray analysis data are not in agreement for 12.5% (1/8) of the samples (shown in parentheses). The microarray values are LOWESS normalized Cy5/Cy3 ratios representing the methylation status of the specified clones in the liver (Cy5) and *in vivo*-derived blastocyst (Cy3) samples. The Bisulfite ratios (Ref/Sample) were calculated from the equation shown in the Materials and Methods section.

A

	Bisulfite Analysis		
CPG clone	Liver	Sperm	In vivo blast.
B G2	0.692	0.024	0.053
HH A7	0.092	0.543	0.313
WW G4	0.341	0.018	0.008
X G2	0.807	0.657	0.667

B

	Sperm (Ref/Sample)		In vivo-produced Blast (Ref/Sample)	
CPG clone	Bisulfite	Microarray	Bisulfite	Microarray
B G2	(3.173)	(0.960)	3.078	21.520
HH A7	0.504	0.619	0.757	0.558
WW G4	1.490	1.506	1.505	5.927
X G2	1.778	1.251	1.728	3.596

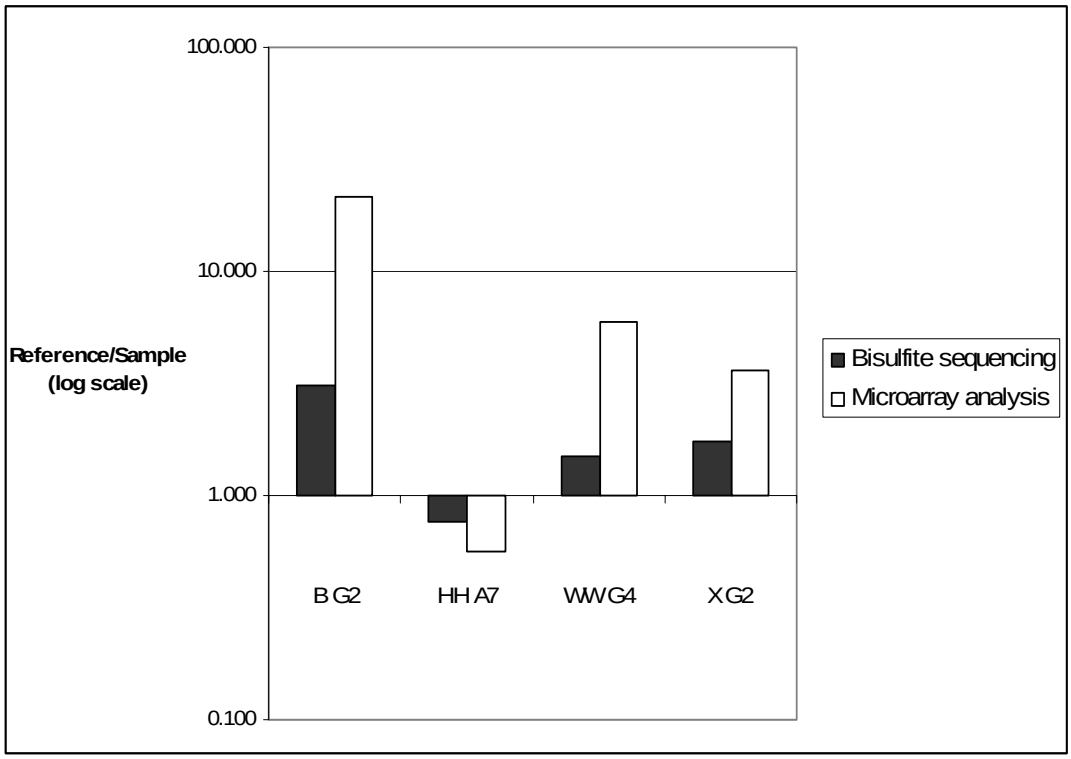

Figure 3.6. Methylation status of *in vivo*-produced blastocysts measured by using microarray and bisulfite analysis. Bisulfite analysis confirmed the microarray data at these four regions in the *in vivo*-produced blastocyst.

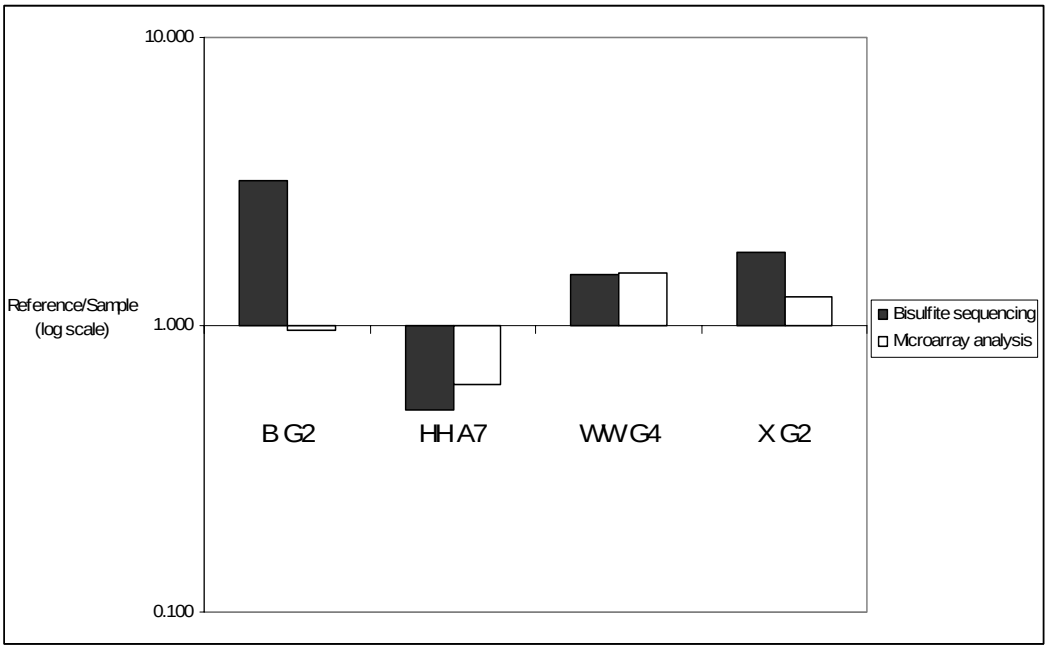

Figure 3.7. Methylation status of sperm measured by microarray and bisulfite sequencing analysis. The methylation status identified by using the PDMH microarrays was confirmed by bisulfite sequencing for all regions except for B G2.

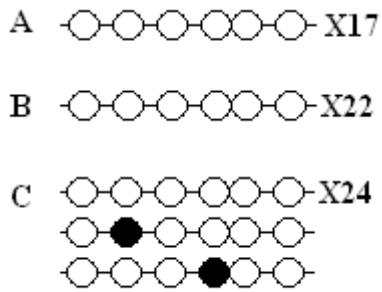

Figure 3.8. Methylation status of the Clone CC C1 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. This region was hypomethylated in the liver, sperm and *in vivo*-produced blastocysts.

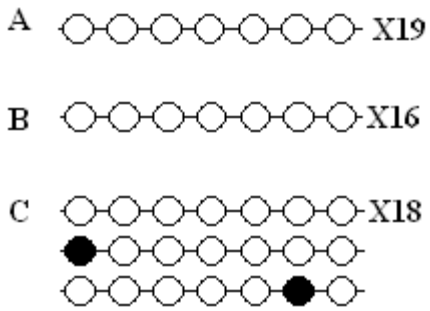

Figure 3.9. Methylation status of the Clone EE A11 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. This region was hypomethylated in the liver, sperm and *in vivo*-produced blastocysts.

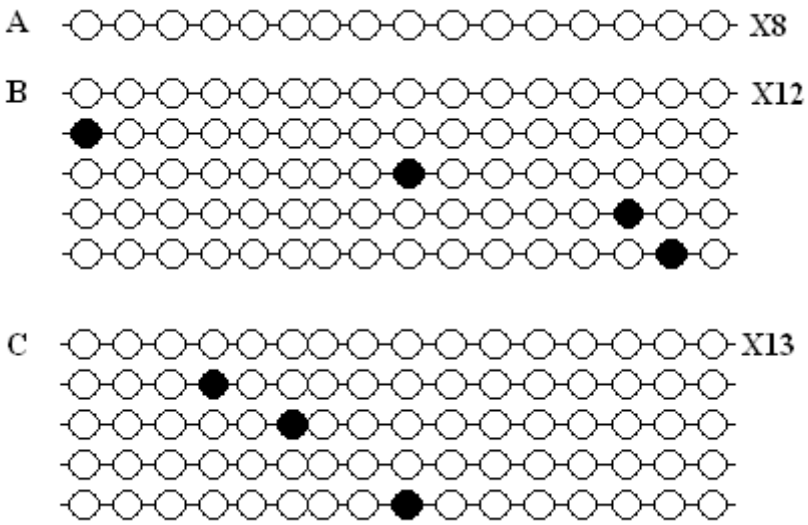

Figure 3.10. Methylation status of the Clone EEE D4 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. This region was hypomethylated in the liver, sperm and *in vivo*-produced blastocysts.

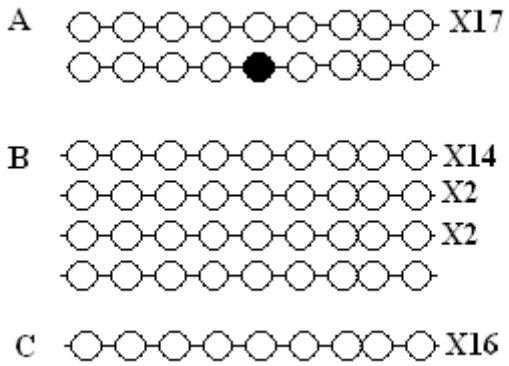

Figure 3.11. Methylation status of the Clone K D3 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. This region was hypomethylated in the liver, sperm and *in vivo*-produced blastocysts.

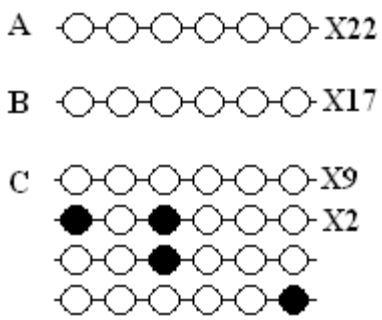

Figure 3.12. Methylation status of the Clone L E8 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. This region was hypomethylated in the liver, sperm and there were low levels of methylation (5.6%) in the *in vivo*-produced blastocysts.

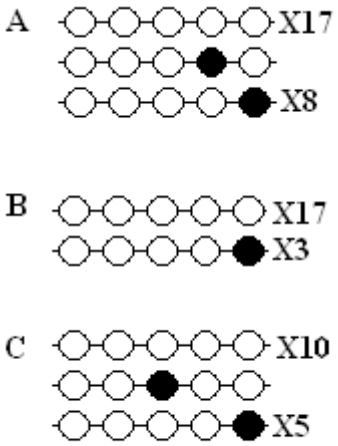

Figure 3.13. Methylation status of the Clone S E3 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. This region was hypomethylated in the liver, sperm and *in vivo*-produced blastocysts.

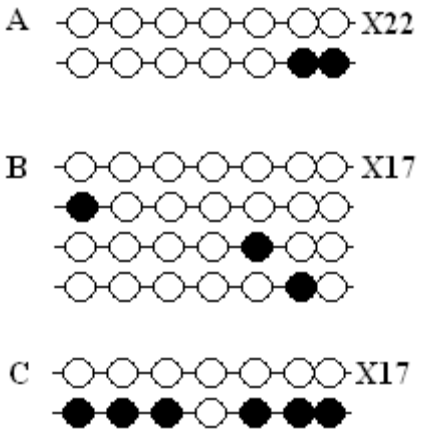

Figure 3.14. Methylation status of the Clone O D10 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. Low levels of methylation (<5%) was observed in this region in the sperm, liver, and *in vivo*-produced blastocysts.

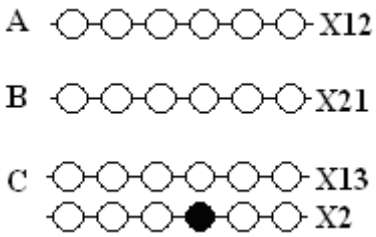

Figure 3.15. Methylation status of the Clone QQ E4 in the liver (A), sperm (B) and *in vivo*-produced blastocyst (C) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. This region was hypomethylated in the liver, sperm and *in vivo*-produced blastocysts.

Table 3.4. Minimal methylation was detected by using bisulfite sequencing in 8 of the 12 clones selected for bisulfite sequencing. A) Shown here is the percent cytosine methylation at the CpG dinucleotides in the liver DNA, sperm DNA, and *in vivo*-produced blastocyst DNA for the regions analyzed by using bisulfite sequencing in clones CC C1, EEE D4, EE A11, K D3, L E8, O D10, QQ E4, and S E3.
B) Bisulfite analysis data and the microarray analysis data are in agreement for 62.5% (10/16) of the samples (shown in bold type). 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 methylation 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. The ratios produced from bisulfite analysis data and the microarray analysis data are not in agreement for 43.75% (7/16) of the samples (shown in parentheses). The microarray values are LOWESS normalized Cy5/Cy3 ratios representing the methylation status of the specified clones in the liver (Cy5) and *in vivo*-derived blastocyst (Cy3) samples. The Bisulfite ratios (Ref/Sample) were calculated from the equation shown in the Materials and Method section.

A

	Percent methylation		
	Liver	Sperm	IVP Blast
CC C1	0.00	0.00	1.54
EE A11	0.00	0.00	1.43
EEE D4	0.00	1.56	1.10
K D3	0.62	0.00	0.00
L E8	0.00	0.00	7.14
O D10	1.24	2.14	4.76
QQ E4	0.00	0.00	2.22
S E3	6.92	3.00	7.50

B

Sperm (Ref/Sample)		IVP Blast (Ref/Sample)	
Bisulfite	PDMH microarray	Bisulfite	PDMH microarray
(1.000)	(2.055)	**0.985**	**0.375**
(1.000)	(1.262)	**0.986**	**0.173**
0.984	**1.074**	(0.989)	(32.960)
(1.006)	(0.593)	**1.006**	**2.806**
1.000	**1.033**	(0.929)	(0.464)
0.991	**1.105**	(0.964)	(6.897)
(1.000)	(0.462)	**0.978**	**1.135**
1.042	**0.910**	**0.994**	**0.859**

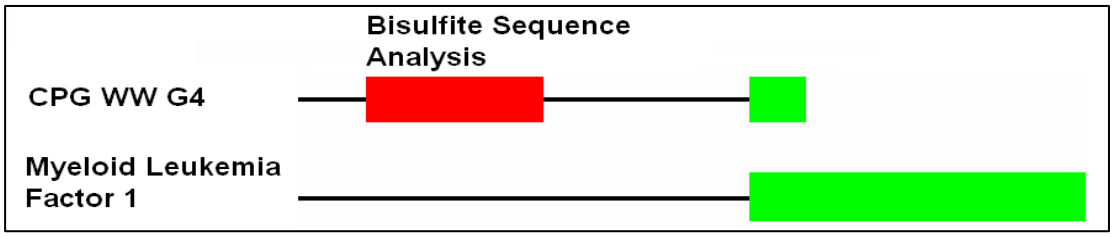

Figure 3.16. Bisulfite sequencing and BLAST analysis of WW G4 (myeloid leukemia factor 1) Bisulfite sequencing and BLAST analysis of WW G4. The region 184 bases upstream of the start site of myeloid leukemia factor 1 (MLF1) is hypomethylated in the sperm and egg relative to the liver.

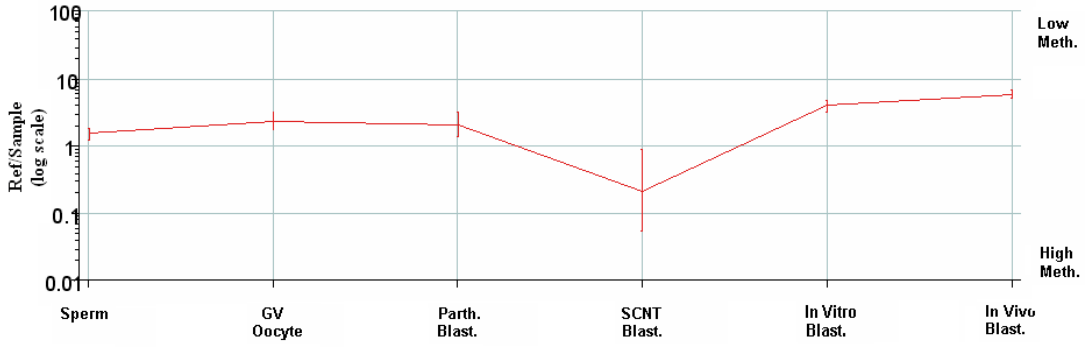

Figure 3.17. Methylation profile of WW G4 (myeloid leukemia factor 1) measured by using PDMH microarrays. The region was hypomethylated relative to the reference sample in all samples except for the SCNT-produced blastocysts where the region was hypermethylated. The region analyzed is 184 bases upstream of the start site of the myeloid leukemia factor 1 gene.

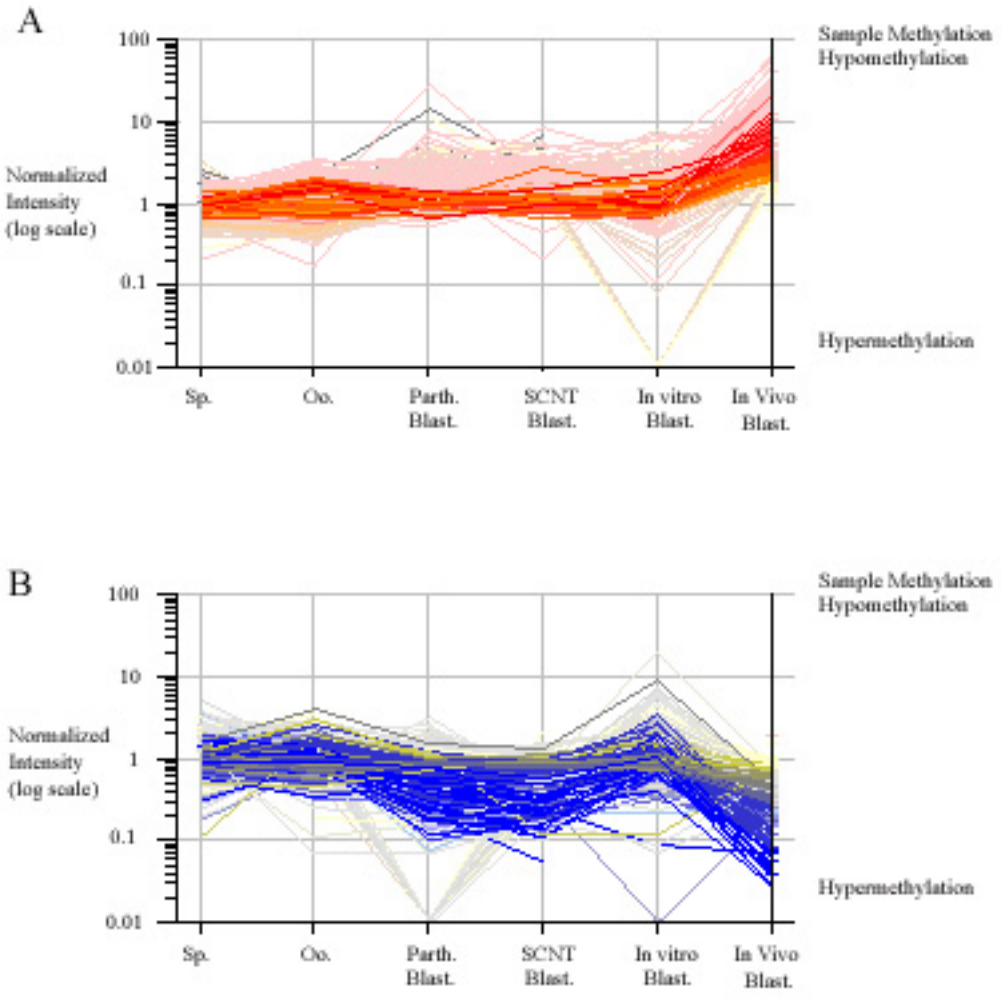

Figure 3.18. Clones grouped by similarity in the methylation status in the sperm, GV oocyte and blastocysts by using Self Organizing Map Analysis. A) The *in vivo*-produced blastocysts (In Vivo Blast) are hypomethylated relative to the sperm (Sp), GV oocytes (GV), SCNT-produced blastocysts (SCNT Blast), parthenogenetic-produced blastocysts (Parth Blast), and *in vitro*-produced blastocysts (In Vitro Blast). B) Extensive hypermethylation in the SCNT-produced blastocysts, parthenogenetic-produced blastocysts, and *in vivo*-produced blastocysts was not observed in the *in vitro*-produced blastocysts. BLAST analysis of the regions that were hypermethylated and hypomethylated in the *in vivo*-produced blastocysts relative to the liver are shown in Tables 3.5 and 3.6, respectively. Hypomethylated spots, as compared to the reference, are shown in red and hypermethylated are shown in blue.

Table 3.5. BLAST analysis of clones that were hypomethylated in the *in vivo*-produced blastocysts relative to the liver. A significant difference was detected by using ANOVA between the *in vivo*-produced blastocysts and the other samples ([a] - P<0.05, [b]- P<0.01, [c]-P<0.001, [d] -P<0.0001). There was a significant difference between the *in vivo*-produced blastocysts versus the other samples with 134/185 (72.43%) of the samples. n.d.-no data.

Clone ID	AA A1	B G2	B G5	BBB A12	BBB H7
Annotation	NS	NS	NS	Sus scrofa lutamate decarboxylase 2 (GAD2), mRNA	Multiple
Gene				GAD2	
IVF±SE	0.730±0.198 [c]	0.878±0.187 [b]	1.080±0.212 [a]	2.478±0.340 [b]	0.735±0.224 [b]
SCNT±SE	1.621±0.283 [b]	1.748±0.241 [c]	1.383±0.235 [a]	2.515±0.546 [d]	1.240±0.248 [c]
Parth±SE	1.753±0.370 [b]	0.992±0.203 [c]	1.390±0.360 [a]	1.333±0.224 [d]	1.107±0.197 [c]
Sperm±SE	1.184±0.169 [d]	0.960±0.172 [c]	0.853±0.191 [b]	1.027±0.175 [d]	0.887±0.183 [d]
Oocyte±SE	3.084±0.695 [b]	1.043±0.181 [c]	1.872±0.209 [a]	1.259±0.210 [d]	0.887±0.183 [d]
IVP±SE	12.490±1.608	21.521±11.047	21.885±25.810	15.517±1.937	10.564±2.250

Table 3.5 (continued)

CCC H7	D C10	D D10	D D6	EEE B7	EEE B9	EEE E3
Multiple	NS	NS	Multiple	Multiple	Multiple	only Bac matches
0.591±0.205	1.469±0.187 ^{a}	1.484±0.289 ^{c}	2.543±0.868 ^{c}	0.761±0.207 ^{b}	1.468±0.209 ^{b}	0.934±0.180 ^{c}
3.594	1.186±0.433	1.087±0.201 ^{b}	1.197±0.209 ^{b}	4.234±1.233 ^{b}	0.806±0.211 ^{a}	2.231±0.245 ^{b}
2.284±0.440	1.524±0.298	2.061±0.245 ^{b}	1.938±0.391 ^{b}	3.582±0.764 ^{b}	1.287±0.238 ^{a}	2.919±0.359 ^{b}
29.000±0.000	2.187±0.498	1.282±0.211 ^{c}	1.607±0.383 ^{c}	1.264±0.216 ^{c}	1.019±0.203 ^{a}	2.078±0.305 ^{c}
1.795±0.190	0.727±0.231 ^{b}	0.000±0.000	0.795±0.211 ^{c}	0.818±0.213 ^{c}	0.789±0.169 ^{b}	1.255±0.176 ^{d}
3.517±1.258	2.676±0.722	30.503±13.704	8.786±1.431	58.478±25.479	20.484±31.499	15.919±2.731

Table 3.5 (continued)

F E10	F F10	FF G1	G G10	GGG D4	II H10	III D1
Multiple	NS	only Bac matches	PREDICTED: Canis familiaris similar-DEAD (Asp-Glu-Ala-Asp) box	WNT8B gene	Multiple	Multiple
				WNT8B		
1.125±0.187 [b]	1.953±0.201	0.862±0.296	1.587±0.676	1.769±0.232 [a]	0.866±0.185	0.793±0.237 [d]
1.043±0.198 [a]	1.940±0.479	2.604±1.120	0.000±0.000	7.000±0.000	1.875±1.612	0.690±0.200 [b]
1.425±0.251 [a]	2.057±0.391	2.356±0.373	1.465±0.296	1.293±0.332	2.961±0.471	1.809±0.390 [b]
1.043±0.198 [b]	2.041±0.298	1.986±0.314	1.371±1.820	4.797±2.769 [a]	1.806±0.496	1.334±0.207 [c]
1.082±0.173 [b]	1.287±0.188 [b]	0.835±0.240 [a]	0.836±0.245 [a]	1.735±1.364	1.248±0.193 [b]	0.793±0.173 [d]
24.933±27.214	4.001±0.781	6.205±5.827	1.745±1.198	0.750±0.759	5.443±1.633	27.565±10.203

Table 3.5 (continued)

JJ B10	JJ D12	JJ E10	K G10	LL E4	NN G9	O D12
Bos taurus similar-Homeobox Protein SIX6 (Sine oculis homeobox homolog 6)	H.sapiens CpG island DNA	NS	Human cyclic AMP transcriptional regulator binding protein (CRE-BP1)	NS	NS	Multiple
			ATF2			
0.901±0.179 ^{c}	0.804±0.183 ^{c}	1.262±0.185 ^{b}	1.370±0.183 ^{b}	0.647±0.187 ^{d}	0.786±0.183 ^{a}	1.008±0.263 ^{b}
0.729±0.217 ^{c}	0.852±0.185 ^{b}	0.917±0.223 ^{b}	0.604±0.509 ^{a}	1.176±0.190 ^{d}	1.522±0.315	1.152±0.181 ^{a}
1.162±0.242 ^{c}	1.468±0.421 ^{b}	1.565±0.277 ^{b}	1.353±0.241 ^{a}	1.610±0.245 ^{d}	2.106±0.820	1.244±0.257 ^{a}
0.995±0.198 ^{c}	1.250±0.202 ^{b}	1.158±0.231 ^{b}	1.034±0.211 ^{b}	1.187±0.221 ^{d}	1.221±0.553	1.191±0.201 ^{b}
0.971±0.182 ^{c}	0.789±0.172 ^{c}	0.801±0.171 ^{c}	1.005±0.171 ^{c}	1.196±0.169 ^{d}	1.709±0.204 ^{a}	0.993±0.257 ^{b}
22.006±8.660	19.756±9.884	30.306±19.817	2.837±0.464	11.712±1.302	2.848±0.427	26.245±27.961

Table 3.5 (continued)

P F6	P H5	QQ A6	RR G5	UU C10	UU H3	X F12
Bos taurus similar-protoporphyrinogen oxidase,Last enzyme of heme synthesis	PREDICTED: Bos Taurussimilar-zinc finger, CSL domain	Multiple	H. sapiens genes for histones H2B.1 and H2A	NS	NS	Homo sapiens prostate antigen PARIS-1 mRNA, complete cds
	ZCSL2		HIST2H2BE			TBC1D2
0.770±0.204	n.d.	1.014±0.185 ^{b}	1.526±0.381 ^{b}	0.882±0.182 ^{b}	n.d.	1.203±0.185 ^{c}
1.228±0.818	1.135±0.272 ^{c}	0.943±0.205 ^{a}	0.902±0.292 ^{a}	0.010±0.000	1.412±0.466	0.723±0.204 ^{b}
1.582±0.372	1.124±0.371 ^{b}	1.632±0.247 ^{a}	2.115±0.370 ^{a}	1.066±0.325	1.767±0.350	1.554±0.246 ^{b}
2.094±0.586	1.042±0.347 ^{c}	1.133±0.205 ^{b}	2.118±0.446 ^{a}	2.993±0.859	0.838±0.251 ^{b}	1.431±0.325 ^{b}
0.873±0.347 ^{a}	2.245±0.778 ^{b}	0.926±0.169 ^{b}	1.007±0.198 ^{c}	0.697±0.258	1.096±0.210	0.847±0.185 ^{d}
2.328±0.674	34.769±19.815	29.106±36.958	12.783±5.020	1.458±0.862	1.723±0.653	8.198±1.664

Table 3.5 (continued)

X G2	XX H10	XX H12	Z D3
NS	NS	Homo sapiens splicing factor 3a, subunit 3, 60kDa (SF3A3), mRNA	NS
		SF3A3	
1.042±0.210 [b]	1.166±0.181 [b]	1.016±0.187 [c]	n.d.
0.938±0.322	0.961±0.213 [a]	0.775±0.194 [b]	0.812±0.255 [b]
2.129±0.313	2.382±0.781 [a]	1.642±0.405 [b]	1.485±0.253 [b]
1.240±0.328 [a]	1.450±0.245 [b]	1.089±0.213 [b]	1.226±0.205 [c]
1.251±0.203 [b]	0.979±0.178 [c]	1.102±0.169 [c]	0.877±0.189 [c]
3.596±0.692	15.403±5.999	30.199±16.716	22.118±9.730

Table 3.6. BLAST analysis of clones that were hypermethylated in the *in vivo*-produced blastocysts relative to the liver. A significant difference was detected by using ANOVA between the *in vivo*-produced blastocysts and the other samples ([a] - P<0.05, [b]- P<0.01, [c]-P<0.001, [d] -P<0.0001). There was a significant difference between the *in vivo*-produced blastocysts versus the other samples with 97/160 (58.78%) of the samples. n.d.- no data.

Clone ID	BLUE E3	CC C1	CCC B6	EE A11	EE A12	EE H2
Annotation	NS	NS	Multiple	mRNA; CpG island cluster	NS	Multiple
Gene						
IVF blast±SE	1.060±0.696 [a]	n.d.	0.723±0.000	1.232±0.199 [c]	0.935±0.386 [a]	0.966±0.301
SCNT blast±SE	0.237±0.285	0.324±0.293	0.808±0.297	0.476±0.241 [a]	0.264±0.248	0.334±0.235
Parth blast±SE	0.343±0.292	0.345±0.455	1.345±0.585	0.560±0.243 [b]	0.315±0.245	0.338±0.355
Sperm±SE	1.904±0.388 [c]	2.055±0.545 [b]	0.782±0.374	1.262±0.208 [c]	1.469±0.277	1.608±0.580
Oocyte±SE	1.323±0.456 [b]	1.450±0.607	1.386±0.459	0.876±0.201 [b]	1.072±0.207	1.131±0.284
IVP blast±E	0.206±0.371	0.375±0.565	0.632±0.898	0.173±0.354	0.146±0.361	0.173±0.409

Table 3.6 (continued)

EE H8	FF E4	G B8	HH A7	L E8	LL D3	N E12
Homo sapiens arylhydrocarbon receptor nuclear translocator	Multiple immune components	Canis amiliaris similar to Coatomer zeta-1 subunit	Myeloid leukemia Factor 1	CpG Island plus others	NS	Homo sapiens serine/threonine protein kinase Kp78 (ribosomal)
ARNT		COPZ1	MLF1			MARK3
0.963±0.443 [b]	0.864±0.181	n.d	4.885±1.886 [b]	0.721±0.446	n.d.	0.816±0.334
0.471±0.247	0.992±0.244	0.262±0.241	0.354±0.677	0.552±0.242	0.842±0.000	1.144±0.377
0.548±0.270 [a]	0.872±0.210	0.379±0.308	2.539±2.283 [a]	0.485±0.242	0.180±0.338	1.512±0.285
1.331±0.219 [c]	0.888±0.218	0.299±0.830	0.619±0.427	1.033±0.229 [a]	1.344±0.777	1.001±0.272
1.250±0.228 [b]	0.743±0.184	0.818±0.215 [b]	2.033±0.773 [a]	0.804±0.253	0.893±0.687	0.974±0.252
0.241±0.405	0.441±0.353	0.232±0.412	0.558±0.673	0.464±0.450	0.555±0.718	1.335±0.465

Table 3.6 (continued)

NN F4	PINK E2	PINK E9	PINK E10	PP C2	PP D6	PP E2
Mus musculus RIKEN cDNA 2810429O05 gene	NS	NS	Multiple	NS	NS	Multiple
n.d.	0.788±0.340 ^{c}	0.804±0.190 ^{d}	0.752±0.246 ^{c}	0.961±0.000 ^{a}	1.478±0.209 ^{b}	1.187±0.319 ^{c}
0.401±0.303	0.216±0.259 ^{a}	0.366±0.251 ^{b}	0.500±0.243 ^{c}	0.193±0.238	0.695±0.237 ^{a}	0.315±0.243 ^{a}
0.190±0.535	0.379±0.285 ^{a}	0.546±0.238 ^{c}	0.587±0.229 ^{c}	0.186±0.320	0.884±0.213 ^{b}	0.567±0.327
0.000±0.000	1.642±0.185 ^{d}	0.955±0.174 ^{d}	0.932±0.170 ^{d}	0.964±0.261 ^{c}	1.188±0.170 ^{c}	1.268±0.187 ^{d}
0.000±0.000	1.578±0.329 ^{c}	0.807±0.207 ^{c}	0.829±0.183 ^{d}	0.972±0.239 ^{b}	1.110±0.179 ^{b}	1.205±0.428 ^{b}
0.349±0.581	0.027±0.399	0.041±0.349	0.050±0.399	0.091±0.405	0.208±0.406	0.135±0.401

Table 3.6 (continued)

PP E4	PP E5	PP E6	PP G1	PP H6	Q A2	Q H5
PREDICTED: Bos taurus similar to malignant T cell amplified sequence 1	PREDICTED: Canis familiaris Similar to Methyltransferase-like	PREDICTED: Bos taurus similar to Paired box protein Pax-3	NS	Homo sapiens FRG1 (FRG1) gene, complete cds (multiple)	Homo sapiens serine/threonine protein kinase Kp78 (ribosomal)	PREDICTED: Bos taurus similar to Forkhead box protein J2
MCTS1		PAX3		FRG1	MARK3	FOXJ2
0.908±0.223	1.566±0.382 ^{b}	1.271±0.213 ^{b}	1.095±0.000 ^{b}	1.314±0.190 ^{c}	0.632±0.248	1.299±0.699 ^{c}
0.667±0.260	0.658±0.250 ^{a}	0.583±0.245 ^{a}	0.245±0.238 ^{a}	0.876±0.237 ^{b}	0.498±0.239	0.298±0.235 ^{b}
0.846±0.235	0.770±0.287 ^{a}	0.686±0.223 ^{a}	0.402±0.308 ^{a}	0.911±0.202 ^{c}	0.556±0.210	0.425±0.231 ^{c}
1.044±0.173 ^{a}	1.091±0.172 ^{c}	1.225±0.169 ^{c}	0.798±0.188 ^{d}	1.131±0.169 ^{c}	0.607±0.177 ^{a}	1.092±0.225 ^{c}
1.493±0.182 ^{b}	1.422±0.187 ^{c}	1.774±0.184 ^{c}	0.409±0.254 ^{c}	1.379±0.255 ^{c}	0.768±0.242 ^{a}	0.961±0.220 ^{c}
0.503±0.430	0.183±0.353	0.134±0.403	0.118±0.409	0.104±0.351	0.268±0.417	0.067±0.399

Table 3.6 (continued)

QQ D3	T A6	TT G8	W E3	W F1
NS	NS	790G17 on chromosome 1q21.1-21.3	NS	NS
2.111±0.912 [a]	n.d.	n.d.	2.809±0.000 [b]	1.990±0.191 [d]
0.663±0.234	1.206±0.781	0.235±0.511	0.322±0.241 [a]	0.340±0.236 [b]
0.738±0.234	0.772±0.000	0.289±0.523	0.444±0.285 [a]	0.760±0.250 [c]
0.632±0.176	1.485±0.873	3.954±0.000 [a]	1.134±0.229 [c]	1.147±0.178 [c]
0.740±0.258	1.347±0.000	0.737±0.613	1.470±0.262 [c]	1.162±0.219 [c]
0.642±0.366	0.217±0.890	0.440±0.661	0.147±0.405	0.038±0.349

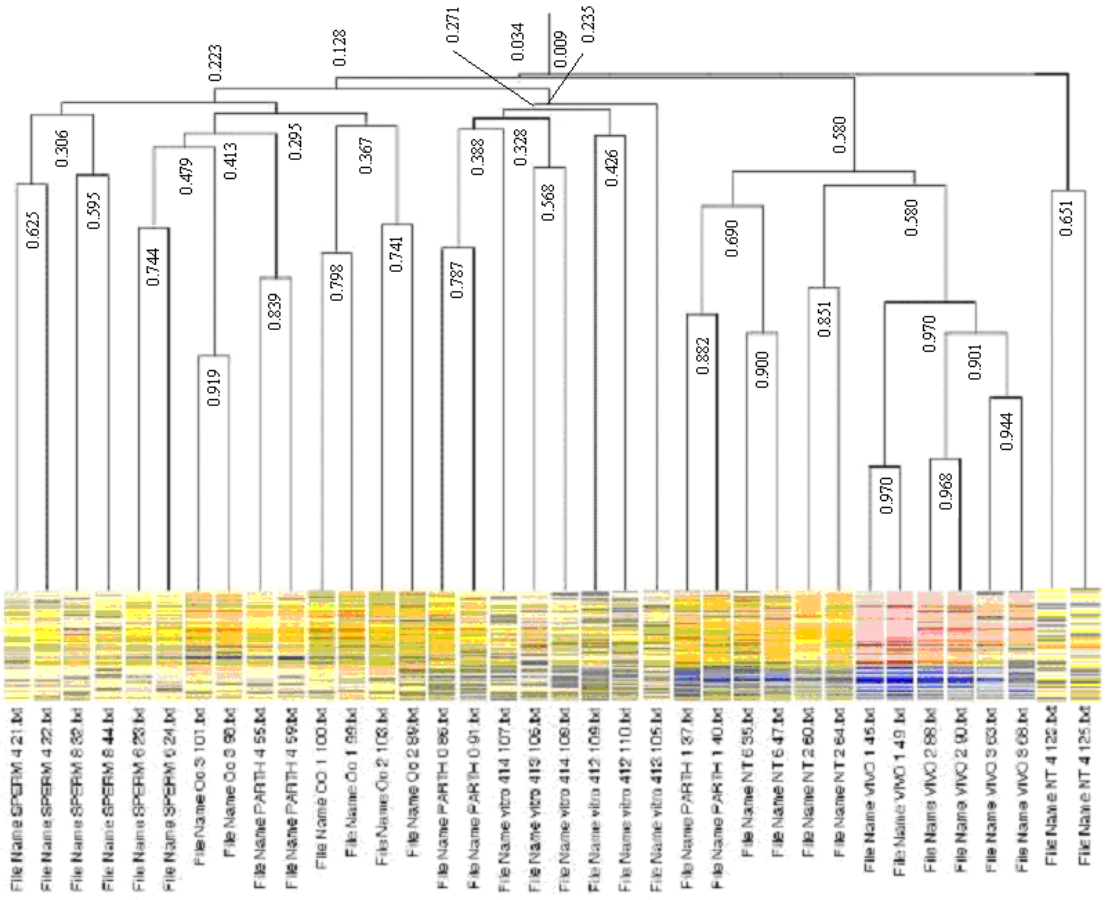

Figure 3.19. Sperm, GV oocytes, and *in vitro*-produced blastocysts had the highest similarity to each other by using hierarchical clustering analysis. The second group included SCNT-, and *in vivo*-produced blastocysts. Parthenogenetic-produced blastocysts did not cluster consistently with a specific sample or samples. This clustering pattern suggests that the methylation remodeling events of the SCNT-produced blastocysts more accurately mimics the remodeling of the *in vivo*-produced blastocysts than the remodeling that occurs in the *in vitro*-produced blastocysts. This condition tree only includes the data where there was at least one difference between the samples, thus the top branch shows a very low correlation.

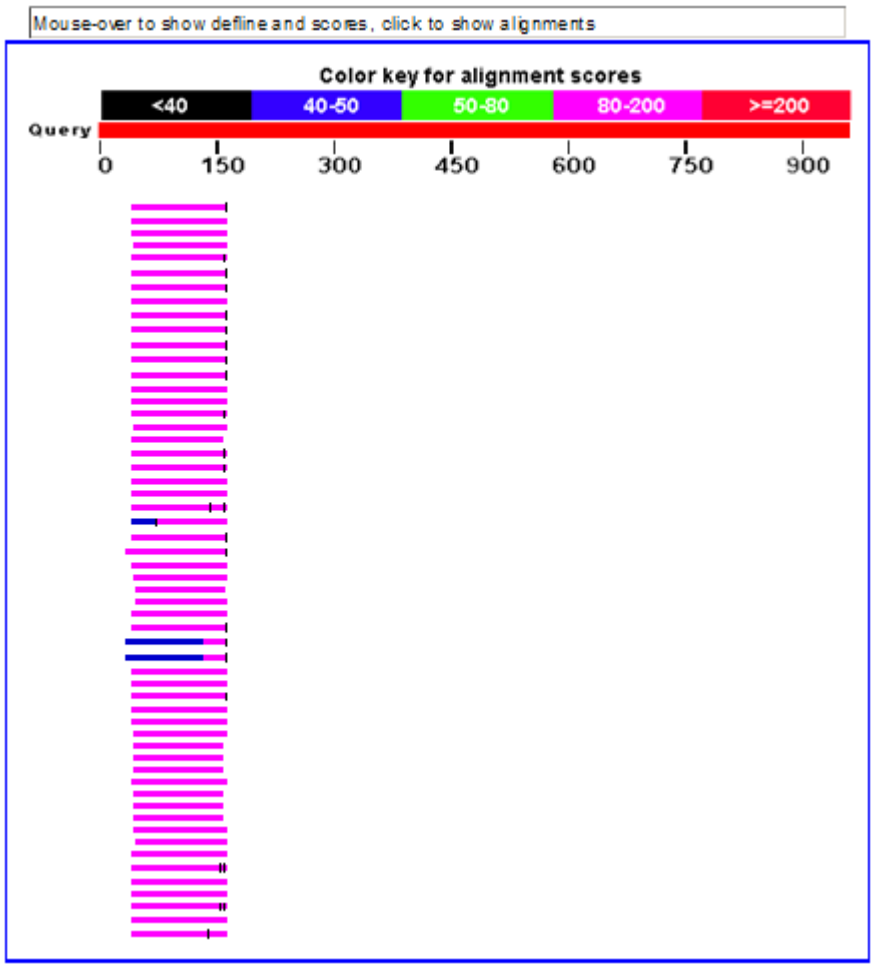

Figure 3.20. BLAST analysis identified multiple sequences as similar to sequence from the clone QQ A6. The multiple sequences identified includes sequences on porcine chromosomes 6, 7, 11, and 17 expressed in various tissues including the liver, thymus, trachea, uterus, and ovary including porcine genes including CKM, ASIP, and KIT. Hypermethylation of these genes may result in cross-hybridization in the microarray analysis but may not be detected by using bisulfite sequencing of the original sequence.

Table 3.7 Differential methylation in sperm and oocytes samples as measured by using PDMH microarray analysis. The normalized oocyte and sperm ratios (reference/sample) were analyzed by using ANOVA ($p<0.05$). A significant difference in methylation at the gamete stage identifies putatively imprinted genes.

Clone I.D.	P-value	OOCYTE±SE	SPERM±SE
B C3	0.040	1.937±0.188	1.0127±0.183
B G12	0.045	2.543±0.337	0.8317±0.197
B G7	0.037	0.555±0.265	1.156±0.257
BLUE D9	0.002	3.127±0.217	1.185±0.170
C B9	0.039	2.002±0.183	1.136±0.167
D E1	0.045	2.147±0.201	1.153±0.169
EE G10	0.039	1.457±0.229	0.560±0.180
EEE D4	0.006	2.825±0.223	1.074±0.191
F F3	0.045	0.935±0.236	2.500±0.244
III B12	0.012	1.097±0.236	0.403±0.232
JJ G10	0.037	0.805±0.186	1.060±0.173
NN E1	0.028	1.451±0.182	1.007±0.168
NN E2	0.039	0.664±0.185	0.870±0.167
NN G12	0.016	0.726±0.186	0.971±0.167
NN G6	0.005	1.474±0.187	0.759±0.173
NN G9	0.044	0.647±0.187	1.710±0.204
OO G8	0.028	0.664±0.210	0.927±0.167
PINK H4	0.001	2.644±0.191	1.164±0.170
PP D10	0.002	2.044±0.193	0.909±0.170
PP D9	0.039	1.155±0.187	0.738± 0.194
PP G3	0.000	1.871±0.227	0.116±0.166
PP H3	0.044	0.592±0.184	0.790±0.170
RR G7	0.044	2.12±0.190	0.788±0.195
S G2	0.011	1.465±0.185	0.843±0.202
T G6	0.012	2.291±0.188	1.039±0.170
TT G10	0.005	2.012±0.192	1.053±0.173
X G12	0.012	0.634±0.210	0.893±0.16)
X G9	0.039	0.604±0.186	1.562±0.172

Table 3.8. Differential methylation in the *in vivo*-produced blastocyst, sperm and GV oocyte as measured by using PDMH microarray analysis. The normalized *in vivo*-produced blastocyst and sperm ratios (reference/sample) and the normalized *in vivo*-produced blastocyst and oocyte ratios (reference/sample) were analyzed by using ANOVA. A significant difference was detected between the in vivo-produced blastocysts versus sperm and the in vivo-produced blastocysts and the GV-stage oocytes ([a] - P<0.05, [b]- P<0.01, [c]-P<0.001, [d] -P<0.0001). Putatively imprinted regions, identified in bold, are identified where the methylation status is retained from the sperm or GV-stage oocyte to the IVP-blastocyst.

Clone I.D.	IVP-blast±SE	OOCYTE±SE	SPERM±SE
B C3	4.083±1.165	**1.937±0.188**	1.0127±0.183 [b]
B G12	61.107±18.174	**2.543±0.337 [d]**	0.8317±0.197 [d]
B G7	2.281±0.994	0.555±0.265 [c]	1.156±0.257 [b]
BLUE D9	0.777±0.386	3.127±0.217 [c]	1.185±0.170
C B9	9.65±36.328	**2.002±0.183**	1.136±0.167
D E1	3.245±0.642	**2.147±0.201**	1.153±0.169 [b]
EE G10	1.025±0.449	1.457±0.229 [a]	0.560±0.180 [b]
EEE D4	32.965±20.055	**2.825±0.223 [b]**	1.074±0.191 [c]
F F3	2.134±0.540	0.935±0.236 [a]	**2.500±0.244**
III B12	0.604±0.491	1.097±0.236	**0.403±0.232**
JJ G10	5.116±1.021	0.805±0.186 [c]	1.060±0.173 [c]
NN E1	10.196 ±9.584	**1.451±0.182 [a]**	1.007±0.168 [b]
NN E2	3.052±5.912	0.664±0.185	0.870±0.167
NN G12	3.275±3.677	0.726±0.186	0.971±0.167
NN G6	3.554±0.553	**1.474±0.187 [b]**	0.759±0.173 [c]
NN G9	2.848±0.427	0.647±0.187 [c]	**1.710±0.204 [a]**
OO G8	4.616±4.788	0.664±0.210 [a]	0.927±0.167
PINK H4	0.275±0.423	2.644±0.191 [c]	1.164±0.170 [a]
PP D10	2.710±0.403	**2.044±0.193**	0.909±0.170 [c]
PP D9	0.249±0.409	1.155±0.187 [b]	**0.738±0.194 [a]**
PP G3	0.773±0.448	1.871±0.227	**0.116±0.166 [c]**
PP H3	0.599±0.040	0.592±0.184	0.790±0.170
RR G7	9.313±6.219	**2.120±0.190 [a]**	0.788±0.195 [b]
S G2	5.364±2.719	**1.465±0.185 [a]**	0.843±0.202 [b]
T G6	0.875±0.379	2.291±0.188 [b]	1.039±0.170
TT G10	3.032±0.713	**2.012±0.192**	1.053±0.173 [b]
X G12	1.383±0.610	0.634±0.210	0.893±0.169
X G9	5.713±1.560	0.604±0.186 [c]	**1.562±0.172 [b]**

CHAPTER IV

CORRELATION OF DEVELOPMENTAL DIFFERENCES TO THE METHYLATION PROFILES OF NUCLEAR TRANSFER DONOR CELLS

ABSTRACT

Methylation of DNA is the most commonly studied epigenetic mechanism of developmental competence and somatic cell nuclear transfer. Previous studies of epigenetics and the somatic cell nuclear transfer procedures have examined the effects of different culture media on donor cells and reconstructed embryos, and the methylation status of specific genes in the fetus or live offspring. Here, we used a microarray based approach to identify the methylation profiles of somatic cell nuclear transfer donor cells including three clonal porcine fetal fibroblast-like cell sublines and adult somatic cells selected from the kidney and mammary tissues. The methylation profiles of the donor cells were then analyzed with respect to the blastocyst rate after nuclear transfer. Clonal cell lines A2, A7, and A8 had blastocyst rates of 11.7%^{a}, 16.7%^{ab}, and 20%^{b}, respectively (^{ab} $P<0.05$). Adult somatic cells included kidney, mammary (large), and mammary(small) also had different blastocyst rates (^{ab} $P<0.05$) of 4.2% ^{a}, 10.7% ^{ab}, and 18.3% ^{b}, respectively. For the clonal donor cells and for the adult somatic cell groups, the donor cells with the highest blastocyst

rates also had methylation profiles with the lowest similarity to the methylation profiles of the *in vivo*-produced blastocysts. Conversely, the donor cells with the lowest blastocyst rates had methylation profiles with the highest similarity to the methylation profiles of the *in vivo*-produced blastocysts. Our findings show there is an inverse correlation to the similarity of the methylation profiles of the donor cells and the *in vivo*-produced embryos and to the blastocyst rates following somatic cell nuclear transfer.

INTRODUCTION

Somatic cell nuclear transfer has successfully produced live animals in many mammalian species including mice, pigs, sheep, goats, rats, cats, dogs, and bovine. Many cells types have been used as donor cells to produce live, fertile offspring. The somatic donor cells could be classified as either: 1) blastomeres from the early embryo, 2) embryonic stem cells, 3) somatic stem cells, or 4) fully differentiated somatic cells. The use of blastomeres as donor cells were successfully used to generate the first mammalian clones in the mouse (McGrath and Solter, 1984), pig (Prather et al., 1989), sheep (Willadsen, 1986), and bovine(Robl et al., 1987). Embryonic stem cells have been successfully used as donor cells in the mouse (Wakayama et al., 1999; Humpherys et al., 2001). Higher rates of development have been reported when using ES cells as the donor cells compared to somatic donor cells (Zhou et al., 2001; Eggan et al., 2002). Conversely, the use of inbred 129 ES

cells failed to produce any offspring that survived more than 1 day (Rideout III and Yanagimachi et al., 2000). Since ES cells have not been isolated from mammals other than mice cells, cultured somatic cells are the most common source of the donor karyoplasts in SCNT.

Adult somatic cells were first used as donor cells to produce the sheep, Dolly (Wilmut I. et al., 1997). Live offspring have been produced by using a donor cells from a wide variety of sources including fetal fibroblasts (Wilmut I. et al., 1997; Cibelli et al., 1998; Baguisi et al., 1999; Zhou et al., 2003), adult fibroblasts (Lanza et al., 2000; Galli et al., 2003), and adult cumulus cells (Polejaeva et al., 2000; Chesne et al., 2002). Recently, cloned mice were produced by using natural killer T (NKT) cells (Inoue et al., 2005). NKT cells were shown to have the same developmental potential as adult fibroblasts and cumulus cells (Wakayama et al., 2001) but greater than lymphocytes (Hochedlinger and Jaenisch, 2002). In general, the use of fetal and adult somatic cells in nuclear transfer is extremely inefficient in producing live offspring. These studies support the theory that the developmental potential is inversely correlated to the differentiation status of the donor cells (Hochedlinger et al., 2004). The highest developmental potential was observed when the donor nuclei are taken from the zygote and any additional development results in decreased developmental potential, presumably through a mechanism that increases resistance to epigenetic remodeling.

Previous studies have examined the methylation status and the expression of imprinted genes in the embryos and offspring derived by using SCNT. The specific

question we were interested in was to determine if the percent of development to the blastocyst of SCNT donor cells following nuclear transfer have methylation profiles, as determined by Porcine Differential Methylation Hybridization microarray analysis, that are more similar to the methylation profile of *in vivo*-produced blastocysts as compared to those that result in lower rates of development to the blastocyst stage. Our hypothesis is that there will be greater similarity between the methylation profiles of donor cells with high developmental potential and *in vivo*-produced blastocysts than between the methylation profiles of donor cells with low developmental potential and *in vivo*-produced blastocysts.

In this study, we examined the developmental potential of cultured clonal cells derived from a primary preparation of porcine fetal cells and of donor cells selected from kidney and mammary cells that were not cultured prior to SCNT. The methylation profiles of these donor cells were determined by using Porcine Differential Methylation Hybridization microarrays. The methylation profiles were then correlated to the developmental potential of the cells.

MATERIALS AND METHODS

Oocyte procurement and *in vitro* maturation

Cumulus-oocyte-complexes (COCs) were aspirated from ovaries from prepubertal gilts that were collected from a local abattoir. Germinal vesicle (**GV**)-stage oocytes were either collected for PDMH analysis or matured *in vitro* prior to in

vitro fertilization. The COCs were incubated in Tissue Culture Medium 199 (**TCM199**) (Gibco BRL, Grand Islands, NY) containing 0.1% (w/v) PVA, 10 ng/ml (w/v) epidermal growth Factor (**EGF**), 0.57 mM cysteine, 0.5 μg/ml (w/v) porcine follicle stimulating hormone (**FSH**) and 0.5 μg/ml (w/v) porcine lutenizing hormone (**LH**) (Abeydeera et al., 1998). The maturation media was pre-equilibrated in 5% CO_2 in air at 39ºC overnight. COCs were matured for 40-44 hours in 5% CO_2 at 39ºC prior to the removal of the cumulus cells by vortexing for three minutes in Hepes-buffered medium with 0.1% (w/v) hyaluronidase. Denuded oocytes were washed and held in modified Tris-buffered medium (mTBM) (Abeydeera and Day, 1997) prior to fertilization.

Nuclear transfer embryo production

Reconstructed embryos were produced using somatic cell nuclear transfer techniques as previously described (Lai and Prather, 2003). Porcine fetal fibroblast-like (**PFF**) cultures were established from a day 35 porcine fetus. After the second passage the cells were plated in to 96-well plates and cultured in Dubelcco's Modified Eagle Medium (**DMEM**) containing 15% (v/v) fetal calf serum (**FCS**), 75 μg/ml (w/v) penicillin G, and 50 μg/ml (w/v) streptomycin in 5% CO_2 at 39ºC. PFF colonies were harvested and transferred to 4-cell culture dishes (Nunc) for about 26-28 population doublings prior to freezing in DMEM/15% (v/v) FBS/10% (v/v) DMSO. The clonal cell sub-lines were used as donor cells in SCNT (Figure 4.1).

Seven replications were conducted with each of the donor cell lines used on the same day, thereby allowing direct comparison of the data.

Kidney and mammary tissues were collected from two full term sows and single cell suspensions were produced from the tissues after treatment with collagenase for 8 hours (Figure 4.2). The cells were frozen in 90% (v/v) FCS and 10% (v/v) DMSO. Small, round cells with smooth membranes were collected from the kidney cell preparation. Small round cells with smooth membranes and large cells with rough, asymmetrical membranes were selected from the mammary cell preparation. Six replications for mammary-small (MS) and mammary-large (ML) cells and five replications for kidney cells (K) were conducted on the same day thereby allowing direct comparison of the data.

Kidney and Mammary Donor Cell Culture

The kidney and mammary donor cells were selected and transferred to culture media in order to characterize the *in vitro* growth and morphological characteristics. Donor cells (n=150) from kidney and mammary tissues were selected and cultured under oil in 200µl DMEM/20% (v/v) FCS/Pen-Strep in 5% CO_2 at 37ºC for 7 days. As a positive control, the original single-cell preparations were left in culture medium and cultured along with the selected donor cells. This procedure was repeated twice.

Statistical Analysis

Treatment means and cell number data were analyzed with the SAS General Linear Models Procedure (SAS Institute, Inc., Cary, NC) by using Duncan's multiple range test.

Porcine Differential Methylation Hybridization

Porcine CpG island clones from a Porcine CpG Island Library (**PCGIL**) (United Kingdom Human Genome Mapping Project, Hinxton, Cambridge, United Kingdom) were cultured in 96 well plates. The cloned inserts were amplified by polymerase chain reaction (**PCR**) using the library specific primers 3558 (5'- CGG CCG CCT GCA GGT CGA CCT TAA) and 3559 (5'- AAC GCG TTG GGA GCT CTC CCT TAA). The PCR reaction was performed in a 10 µl reaction containing 1X Deep Vent DNA Polymerase Buffer, 10% DMSO, 400 pM of each primer, 100 pm each dATP, dTTP, dCTP, and dGTP , and .018 units Deep Vent Polymerase (New England Biolabs, Beverly, MA). The PCR program consisted of a denaturation step at 98ºC for 4 minutes followed by 30 cycles of denaturation at 95ºC for 30 seconds, annealing at 55ºC for 30 seconds and extension for 72ºC for 1 minute. A final extension at 72ºC completed the program. PCR products were stored at -20ºC until needed. Restriction digestion with *Bstu I* was performed using 1.5µl of the PCR reaction in 1X NEB 2 and 0.4 units *Bstu I* at 60ºC for at least 1 hour. The digested and undigested PCR products were run on a 1.5% 0.5X TBE agarose gel. *Bstu I* positive clones where the PCR product was cut, indicating the presence of a *Bstu I*

site (CGCG) in the insert, were reracked and recultured in 96 well plates. Plates with *Bstu I* positive clones were PCR amplified in a 50µl reaction and purified in Millipore 96 well PCR Purification plates in preparation for printing. The purified PCR products were dried and resuspended in 10µl 50% DMSO/1% CHAPS (Rickman et al., 2003) .

The resuspended PCR products were printed on Gold Seal glass microscope slides (Fisher Scientific, Hampton, NH) that were coated with 0.02% (w/v) poly-L-lysine (Sigma, St. Louis, MO) in 0.5X PBS (Eisen and Brown, 1999). The slides were stored for 3 weeks at room temperature under desiccation before printing with a pick and place robot. The printed slides were cross linked at 120 mJ/cm^2 for 20s (Spectrolinker; Spectronics Corp., Westbury, NY) prior to blocking in 0.018% (w/v) succinic anhydride (Sigma, St. Louis, MO) and 0.043 M sodium borate (Sigma, St. Louis, MO) in 1-methyl-2-pyrrolidinone (Sigma, St. Louis, MO) (Eisen and Brown, 1999). The slides were stored under desiccation and at room temperature until hybridization.

DNA Isolation

The DNA was isolated from the donor cells by adding H_2O to a final volume of 25 µl and incubating at 98ºC for 15 minutes.

Amplicon Generation, Labeling and Hybridization

Amplicons were produced by digesting the donor cell DNA with the restriction enzyme *Mse I* (50 units) in 1X NEB 2, and 1X BSA at 37ºC overnight as recommended by the supplier (New England Biolabs (NEB), Beverly, MA). The restricted DNA was ligated to PCR linkers produced by mixing oligomers (H-24, 5'-AGG CAA CTG TGC TAT CCG AGG GAT and H-12, 5'-TAA TCC CTC GGA), heating to 65ºC, and cooling to room temperature. The DNA was digested with the methylation sensitive restriction enzyme *Bstu I* (NEB) as recommended. The intact DNA fragments were amplified by PCR using H-24 as the linker specific primer. The PCR program consisted of a denaturation step at 98ºC for 5 minutes followed by 40 cycles of denaturation at 95ºC for 1 minute, annealing at 55ºC for 1 minute and extension for 72ºC for 1 minute. A final extension of 72ºC for 10 minutes completed the program.

The PCR products were labeled with amino allyl-dUTP using the BioPrime labeling system with modifications. The PCR products were purified with a Qiagen PCR Purification Kit and resuspended in 29 µl H_2O, mixed with 1X Bioprime buffer, dNTPs (2:3 dUTP:dTTP, dATP, dGTP, dCTP), 40 units Klenow, and incubated for 60 minutes at 37ºC. Amino allyl-dUTP incorporated PCR products were purified with the Qiaquick columns using PB buffer, phosphate washing buffer (5mM KPO_4, 80% E to H, pH 8.0) and phosphate elution buffer (4 mM KPO_4, pH 8.5). The samples were dried and resuspended in 0.1 M sodium carbonate buffer (pH 9.0) and mixed with Cy3 for the donor cell DNA or mixed with Cy5 for the liver reference sample.

The samples were incubated for 60 minutes at room temperature. The labeling reactions were purified with Qiaquick columns by using PB buffer, PE buffer, and EB buffer. The labeling efficiency was then analyzed spectrophotometrically by using the Nanodrop ND-1000 (Nanodrop, Wilmington, DE). Comparable amounts of labeled test sample and liver reference sample, based on the incorporation of the Cy 3 and Cy5 dyes, were mixed together. The combined samples were purified, dried, and resuspended in 26 µl hybridization buffer (50% formamide, 5X SSC, 0.1% SDS). The samples were denatured at 95ºC for 3 minutes and immediately transferred to ice before being applied to a microarray slide with a coverslip. The microarrays were incubated at 42ºC for 8-12 hours before removing the coverslip in Wash I (1X SSC/0.2% (w/v) SDS), and washing in Wash II (1X SSC/0.2% (w/v) SDS), Wash III (0.1X SSC/0.2% (w/v) SDS), Wash IV (0.1X SSC), and Wash V (H_2O). The slides were immediately dried by using centrifugation at 1500xg for 5 minutes, and scanned with an Axon 4000B scanner.

Microarray Analysis

Microarray images were analyzed with GenePix 4.0 and spots with intensities where at least 25% of the pixels were greater than 1 standard deviation from the background in either the Cy3 or Cy5 channel were further analyzed with Gene Spring version 7.2. The LOWESS normalized data was analyzed by ANOVA assuming all variances to be equal, $P<0.05$ using the Benjamini and Hochberg False Discovery Rate for multiple testing. Specific clones were selected for sequencing based on the

similarity or significant difference in the methylation profiles of sperm and *in vivo*-produced blastocysts (Bonk et al., 2006). Donor cell methylation profiles and bisulfite sequencing results were compared to the liver and *in vivo*-produced blastocyst methylation profiles and bisulfite sequencing results previously described (Bonk et al., 2006).

Bisulfite Sequencing Analysis

The DNA from the clonal donor cells was treated with bisulfite by using the EZ DNA Methylation-Gold Kit (Zymo Research, Orange, CA) according to the vendor's recommendations. Primers (Table 4.1) were designed for bisulfite treated DNA by using the MethPrimer software (Li et al., 2002). PCR was performed as shown below:

H_2O	32.5 µl
DNTP	1.3 µl
10X Buffer (TagGold)	5 µl
$MgCl_2$	5 µl
Forward Primer (10 µM)	2 µl
Reverse Primer (10 µM)	2 µl
DNA (Bisulfite treated)	2 µl
AmpliTaq Gold (5u/µl)	0.25 µl
Total	50 µl

The PCR program consisted of a denaturation step at 98ºC for 3 minutes followed by 50 cycles of denaturation at 95ºC for 15 seconds, annealing at 55ºC for 30 seconds and extension for 72ºC for 30 seconds. A final extension of 72ºC for 5 minutes completed the program.

The PCR reaction was purified by using the Qiaquick columns as described by the vendor. The PCR products were cloned by using the pGEM T-Easy Kit (Promega, Madison, WI) according to the vendor's recommendations. The vectors were transformed in to DH10B cells (Invitrogen) and grown on LB/IPTG/X-Gal/Ampicillin agar plates. Recombinant colonies were selected for sequencing based on the blue/white screening criteria. The cytosines of the CpG sites were identified as methylated or unmethylated if a C or T was present in the sequence, respectively. The percent methylation was calculated for the respective sequence and the methylation status of the microarrays and bisulfite sequencing were compared. A ratio of liver:donor cell methylation was calculated by using the following formula,

$$Rm = (100 - M_S) / (100 - M_L)$$

Where: M_S is the average % CpG methylation for a sequence in the sample

M_L is the average % CpG methylation for a sequence in the liver reference

The use of this formula provides a means to calculate a ratio that indicates the relative levels of methylation in a given sequence when one of the samples lacks methylated CpG dinucleotides. 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.

RESULTS

Development of Reconstructed Embryos after Somatic Cell Nuclear Transfer

The blastocyst rate for the clonal cell lines were significantly higher ($p<0.05$) for A8 (20.0%) than for A2 (11.7%) (Table 4.2). Significant differences were not observed for the fusion rates, cleavage rates or the mean cell numbers for the clonal cell lines. The blastocyst rate for the MS donor cells (18.3%) was significantly higher ($P<0.05$) than the K (4.2%) donor cells (Table 4.3). The cleavage rate was significantly higher ($P<0.05$) for the ML and MS donor cells (46.7% and 45.9%) than for the K donor cells (26.2%). Mean cell numbers of the blastocysts for the K, MS, and ML cells were 22.8, 23.6, and 26.3, respectively. Significant differences were not observed for the fusion rates or the mean cell numbers for the ML, MS, and K donor cells.

Differential Methylation in the Donor Cells and Blastocysts

Microarray data was analyzed by using the GeneSpring 7.2 software to perform an ANOVA assuming all variances to be equal, $P<0.01$ using the Benjamini and Hochberg False Discovery Rate for multiple testing. Of the 2,445 clones that were analyzed, 380 (15.5%) were found to be significantly different in at least one of the biological conditions of donor cells and *in vivo*-produced blastocysts.

A condition tree was generated based on the methylation profiles of the clonal cell lines, kidney cells, mammary cells and the *in vivo*-produced blastocysts by using the GeneSpring 7.2 software (Figure 4.3). Bootstrap analysis by using the TIGR Multiple Array Viewer software was used to validate the condition tree generated by using the GeneSpring 7.2 software (Fig. A.4). Samples with high levels of similarity in the overall methylation profiles are grouped together in the condition tree. Increasing disparity in the methylation profiles results in the samples being located farther apart in the condition tree. Conversely, the donor cells with the highest blastocysts rates after SCNT (A8 (16.7%), MS (18.3%), and A7 (20.0%)) grouped away from the *in vivo*-produced blastocysts. These results show that the similarities of the donor cell and *in vivo*-produced blastocyst methylation profiles appear to be inversely correlated with the developmental potential of the donor cells after SCNT.

Additional validation of the hierarchical clustering was performed by using bootstrap analysis. These results are presented in Figure A.4 in the Appendix. Unfortunately, the TIGR Multiple Array Viewer software used to do the bootstrap analysis does not include the same correlation analysis that is used by the GeneSpring

software. Specifically, the Standard Correlation used in the GeneSpring software is commonly referred to as Pearson correlation around zero. The TIGR Multiple Array Viewer does not contain this correlation procedure so the Pearson Correlation analysis was substituted. Therefore, caution should be used in attempting to extrapolate the bootstrapping results to the clustering generated by using GeneSpring. The strongest support was provided for the clustering of the MS and *in vivo*-blastocysts.

Donor Cell Culture

After 7 days in culture, no cells were observed in the culture drops for the K or the MS cells. Conversely, cells were observed to be growing from the ML donor cell group. These cells had a spindle shape associated with fibroblast-like cells or a "cobblestone" shape associated with endothelial cells.

Bisulfite Sequencing Analysis

Spots previously identified as differentially methylated in the donor cells and the blastocyst groups were selected for additional analysis by using bisulfite modified PCR (Bonk et al., 2006). The methylation status for 5 clones (B G2, HH A7, K D3, S E3, and X G2) was determined in the clonal cell lines A2 or A7 (Figure 4.4-4.8). The methylation liver/donor cell methylation ratios were calculated for 5 clones using DNA from the clonal cell lines A2 and A7. The ratios calculated from the percent methylation of the reference and test samples based on bisulfite PCR analysis were

similar for the five samples that were tested (Table 4.4). 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 the relative methylation levels 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 and the microarray analysis data are in agreement for all of the samples. Differential methylation hybridization microarray analysis of the methylation levels of the donor cells was validated by the bisulfite modification PCR analysis and depicted graphically (Figure 4.9).

Similarity of Donor Cells and In Vivo-Produced Blastocyst Methylation Profiles

The similarity of methylation profiles in the donor cells and the *in vivo*-produced blastocysts were analyzed by using Self Organizing Map analysis. Clones that had similar hypomethylation and hypermethylation to in *vivo*-produced blastocysts clustered together are shown in Figure 4.10. Hierarchical clustering with bootstrapping was performed by using the TIGR Multiple Array Viewer software to validate the clustering generated with the GeneSpring software (Figure A.4). The complete Self Organizing Map analysis is shown in Figure A.5 and the BLAST analysis of the sequenced genes is shown in Table A.3. The Cy5/Cy3 ratios of the normalized ratios are shown where the reference sample was labeled with Cy5 and

the test sample was labeled with Cy3. The Cy5/Cy3 ratio of one indicates equivalent hybrization levels are calculated for the normalized ratio. A Cy5/Cy3 ratio greater than one indicates there is less methylation in the test sample than in the reference sample. Conversely, a Cy5/Cy3 ratio less than one indicate that the test sample is more methylated than the reference sample. Of particular interest are those groups that show a general trend that correlates to the blastocyst rate. These results suggest that the genes associated with the clones in these groupings may be important in the regulation of developmentally relevant genes. Alternatively, these clones may represent regions that are resistant to epigenetic remodeling during early development and this resistance results in lower development rates following SCNT.

DISCUSSION

To date, studies of epigenetics and SCNT typically involved the identification of factors related to culture conditions that affect the methylation of the donor cells and the reconstructed embryos, or to the identification of errors in epigenomic reprogramming at some point after the SCNT procedure. The objective of this study was to correlate the methylation profiles of various donor cells prior to SCNT to the developmental potential of the respective reconstructed embryos following SCNT. Our hypothesis was that the donor cells with the highest blastocyst rate, measured by the blastocyst rate after *in vitro* culture, would have methylation profiles that were the most similar to the methylation profiles of *in vivo*-produced blastocysts. The results

presented here show that there are subpopulations of somatic cells in tissues that have differential potential to direct the development of reconstructed embryos. This differential potential to direct development occurs with donor cells that have undergone extended culture as well as with donor cells that have not been cultured. The significantly lower cleavage rate and blastocyst rate observed in the kidney donor cells imply an intrinsic resistance of the epigenome to reprogramming following SCNT.

We used a microarray based approach to characterize the methylation profiles of the donor cells from adult somatic tissues and fetal tissues and the methylation profiles of *in vivo*-produced blastocysts (Bonk et al., 2006). The similarities of the donor cell methylation profiles and the methylation profiles of the *in vivo*-produced embryos were inversely correlated. Specifically, donor cells from kidney tissues were found to have methylation profiles with the highest similarity to in *vivo*-produced embryos and the lowest blastocyst rate following SCNT of all the donor cells. Conversely, the methylation profiles of the small mammary cells and the clonal cell lines A7 and A8 were found to be the most dissimilar to the *in vivo*-produced blastocyst, yet these donor cells yielded the highest rate of blastocyst development. It should be noted that the entire blastocyst, including the inner cell mass and the trophoblast cells, was included in generating the methylation profile. The effect of analyzing this mixed population of cell types is not known since the differences in the global methylation status of the *in vivo* inner cell mass and trophectoderm has not been extensively studied.

One reason mouse ES cells have been used in SCNT is because it is thought that the epigenetic programming is limited in such a way that there will be minimal reprogramming needed to mimic that of the early blastomere thereby resulting in higher rates of development. Somatic cells are used as donor cells in all other animals since true ES cells have yet to be identified. Here we showed that the lowest rates of blastocyst development were shown to be associated with cells that were the most similar to the methylation profile of the *in vivo*-produced blastocyst.

The donor cells used in the current study were either clonally derived or were selected, based on morphology, from kidney or mammary cells that were not cultured *in vitro*. The goal of both strategies was to start with a homogenous population of donor cells thereby minimizing the likelihood that only a small, select population of donor cells in a primary culture are primarily responsible for the development of the reconstructed embryos. Cells were identified in the clonally derived cells and also the uncultured cells that resulted in high, medium, and low rates of blastocyst development. These results support the idea that successful nuclear transfer requires reprogramming of the donor cells. This basic idea had been intuitively accepted without the presence of a comprehensive study that would control for the technical problems that may affect development. Hiiragi and Solter (2005) recently demonstrated that the developmental rate of reconstructed embryos is inversely correlated to the stage of the embryos from which the donor blastomeres were collected. These results indirectly supports other studies where the rates of blastocyst development and the production of live offspring following SCNT decreases when

donor cells are collected from progressively later stages of the early mouse embryo (Cheong et al., 1993; Heyman et al., 2002; Hiiragi and Solter, 2005) and the early cattle embryo (Heyman et al., 2002).

Most nuclear transfer experiments have used donor cells that were cultured *in vitro*. In this experiment, we used adult somatic cells that had not been extensively cultured *in vitro*. These groups were included to assess the effect of minimizing the effect that culture medium and extended culture times have on the methylation status and blastocyst rates following SCNT. The large disparity we observed in the blastocyst rates of the kidney cells and the small mammary cells was not expected. This difference in developmental potential seems to indicate that the epigenetic status of the selected kidney cells is significantly more resistant to reprogramming and remodeling than the other donor cells.

The inability of the kidney cells and the small mammary cells to grow in standard culture medium suggests that there is a selection process when primary cultures are established from fetal or somatic tissues. These cells may be a form of stem cells that requires stem cell specific media that contains growth factors such as LIF, EGF, PDGF, bFGF, ECGF, and insulin (Kues et al., 2005). The presence of cells in the adult mammary tissues that result in high blastocyst rates following SCNT, but are likely excluded from most primary cell culture preparations, presents a potential source of a stem cell-like cell population that is highly abundant in mammary tissues and probably most other tissues. Optimization of the selection procedure and of the culture media for these cells could create a readily available population of cells with

the developmental potential that is functionally similar to isogenic embryonic stem cells. Potentially homologous subpopulations of multitotent cells in the adult mouse testis have been found to contribute to the multiple organs after injection in to the early blastocyst (Guan et al., 2006). *In vitro* culture in the appropriate media resulted in the development of these cells to derivatives of the three germ layers.

In conclusion, this study shows that a wide range of developmental potential is present in donor cells regardless of whether the cells were in extended *in vitro* culture. Also, the similarity of the methylation profiles of the donor cells to the *in vivo*-produced blastocyst shows an inverse correlation to blastocyst rate following nuclear transfer. Therefore, the epigenetic condition of some donor cells is resistant to the detrimental effects of extended culture on donor cells, and there are subpopulations in somatic cells that show variable resistance to epigenetic remodeling following SCNT.

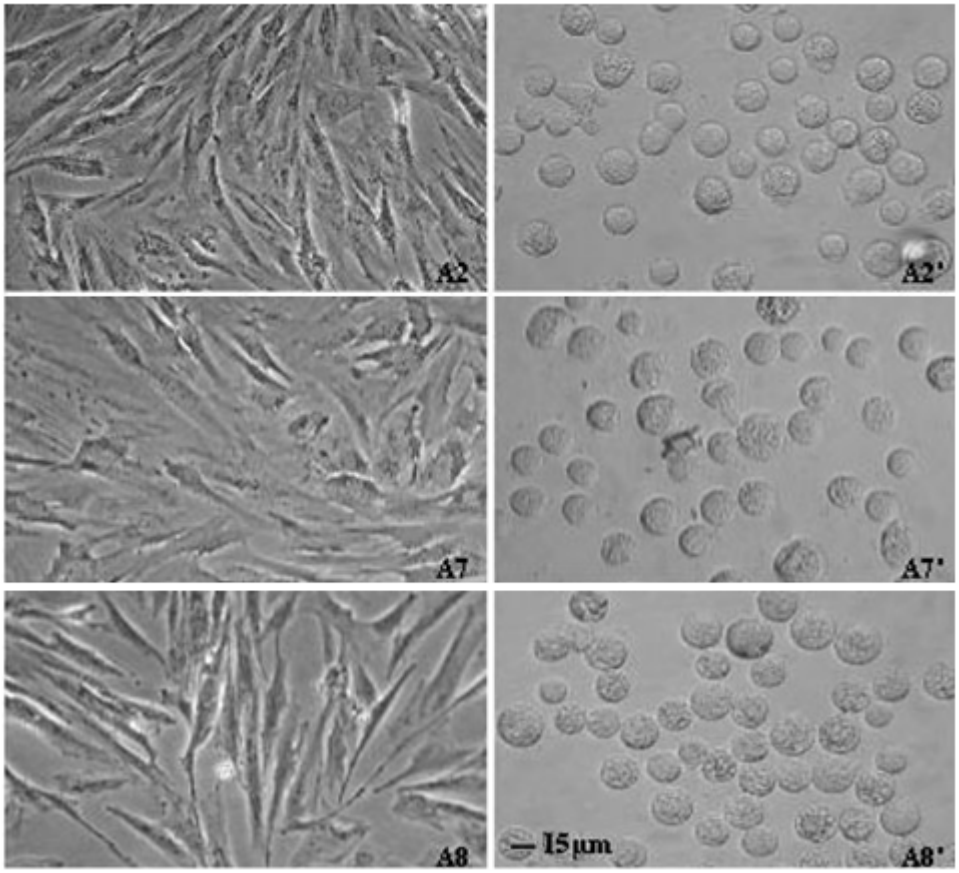

Figure 4.1. Clonal cell lines cultured (A2-A8) and trypsinized (A2'-A8')

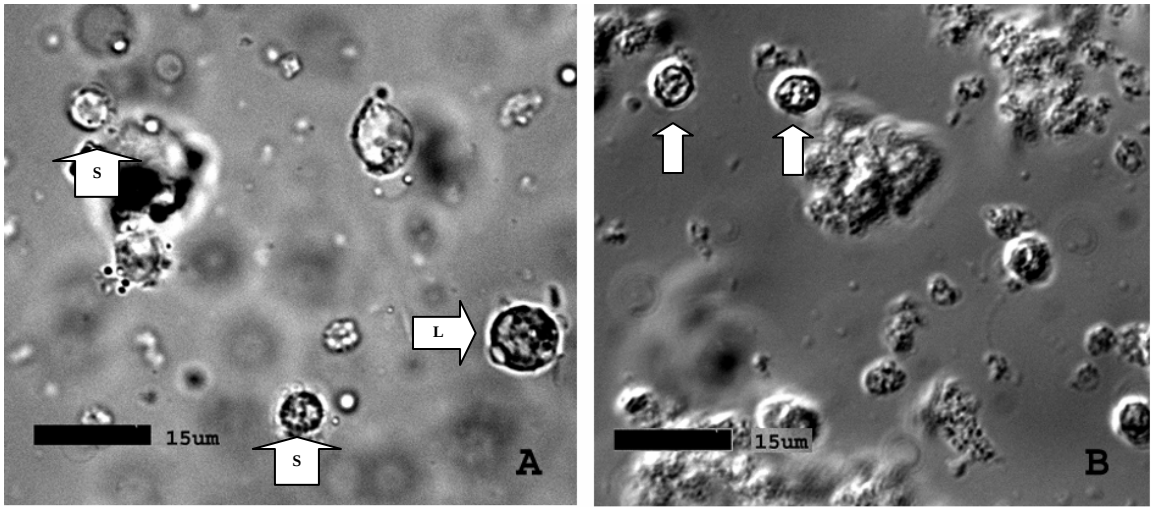

Figure 4.2. Somatic donor cells from mammary (A) and kidney (B) tissues. Arrows indicate the large (L) and small (S) cells that were selected from the mammary cells.

Table 4.1. 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 4.2. *In vitro* development of SCNT-produced embryos derived from porcine fetal fibroblast-like clonal cell lines. The blastocyst rates for the clonal cell lines A2, A7, and A8 were significantly different (a,b in the same column, $P<0.05$) after SCNT. A significant difference was not observed for the fusion rate, cleavage rate, or blastocyst cell number.

Cell-line	No. (%) of fused/manipulated	No. (%) of cleaved	No. (%) of blastocyst	# Cell (mean±SE) in blastocysts (range)
A2	94/139 (67.6)	66 (70.2)	11 (11.7)[a]	25.5±2.0 (17-42)
A7	84/133 (63.2)	63 (75.0)	14 (16.7)[ab]	24.3±1.6 (18-40)
A8	95/131 (72.5)	67 (70.5)	19 (20.0)[b]	25.7±1.4 (19-37)

Table 4.3 *In vitro* development of SCNT-produced embryos derived from porcine adult mammary and kidney tissue. The blastocyst rates for the donor cells Kidney, Mammary-Large, and Mammary-Small were significantly different (a,b in the same column, $P<0.05$) after SCNT. A significant difference was not observed for the fusion rate, cleavage rate, or blastocyst cell number.

Donor cell	No. (%) of fused/manipulated	No. (%) of cleaved	No. (%) of blastocyst	# Cell (mean±SE) in blastocysts (range)
Kidney	103/224 (46.0)	27 (26.2)[a]	4 (4.2)[a]	22.5± 2.2 (16-27)
Mammary-Large	77/179 (37.5)	35 (46.7)[b]	8 (10.7)[ab]	23.6± 1.3 (21-25
Mammary- Small	77/179 (43.0)	34 (45.9)[b]	22 (18.3)[b]	26.5±2.6 (19-44)

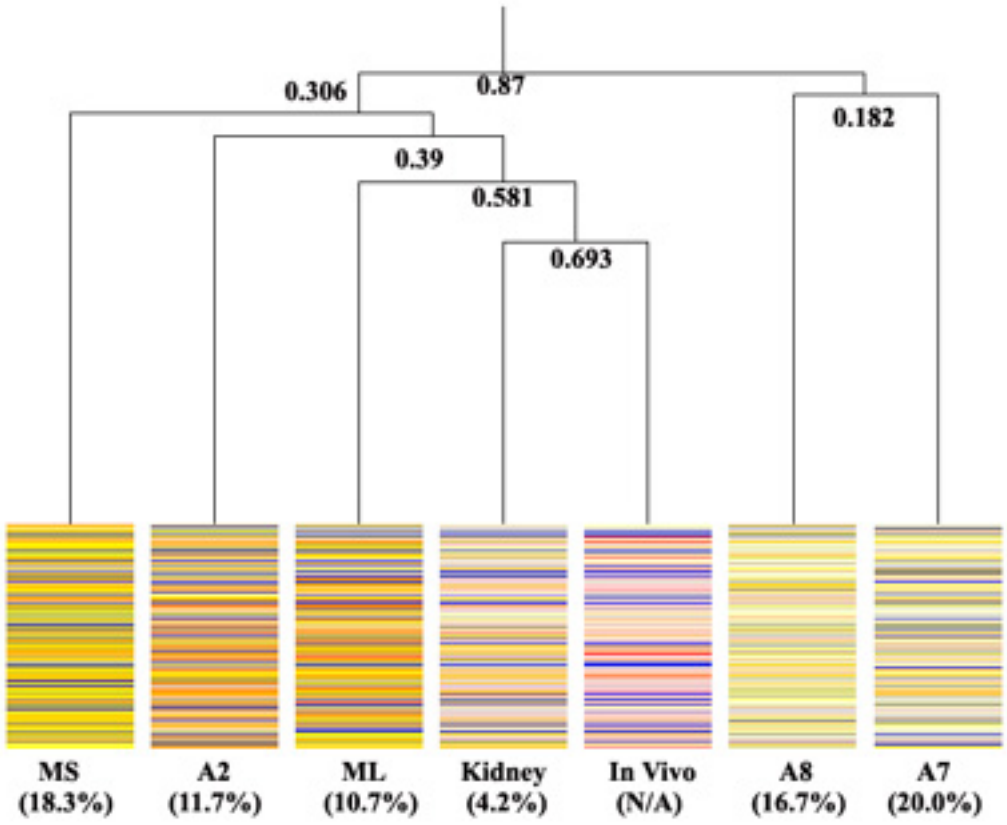

Figure 4.3. Hierarchical clustering of the methylation profiles of the clonal donor cells (A2, A7, and A8,), somatic cells (kidney, (K), Mammary-Large (ML), and Mammary-Small (MS)), and *in vivo*-produced blastocysts. Developmental potential is negatively correlated to similarity to the *in vivo*-produced blastocyst methylation profile. Donor cells that with the lowest blastocyst rates after SCNT had the most similar methylation profiles while donor cells with higher blastocyst rate did not cluster with the *in vivo*-produced blastocysts. The blastocyst rate after SCNT is shown in parentheses below each of the donor cell types.

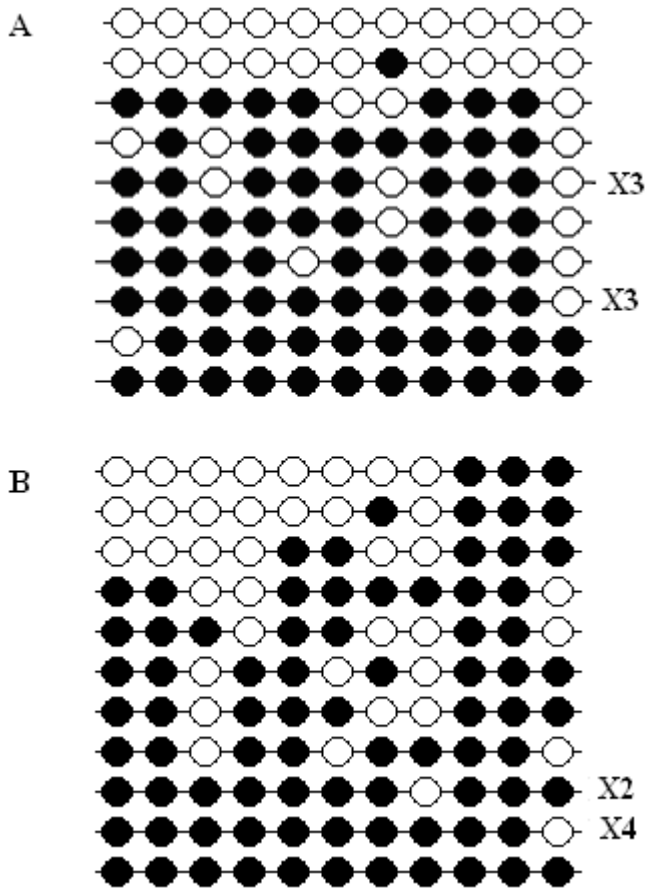

Figure 4.4. Methylation status of the Clone B G2 in the liver (A) and clonal cell line A2 (B) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. The liver and donor cell epigenomes show similar levels of methylation at this region.

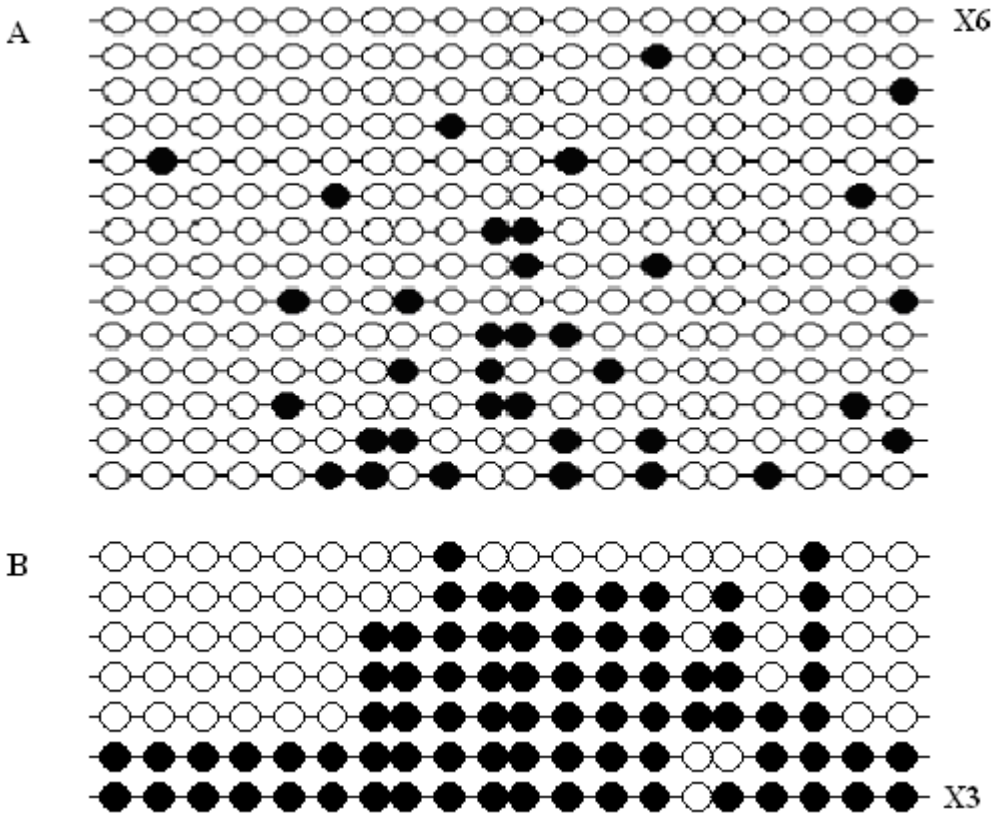

Figure 4.5. Methylation status of the Clone HH A7 in the liver (A) and clonal cell line A7 (B) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. The donor cell epigenome shows higher methylation than the liver genome at this region.

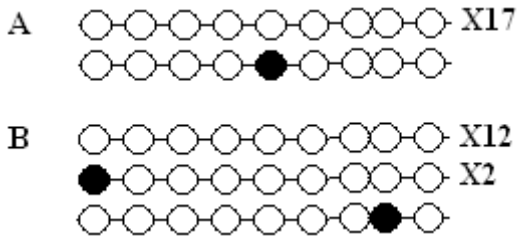

Figure 4.6. Methylation status of the Clone K D3 in the liver (A) and clonal cell line A2 (B) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. The liver and donor cell epigenomes are essentially unmethylated at this region.

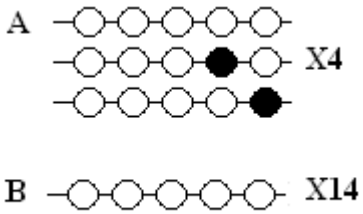

Figure 4.7. Methylation status of the Clone S E3 in the liver (A) and clonal cell line A2 (B) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. The liver and donor cell epigenomes are essentially unmethylated at this region.

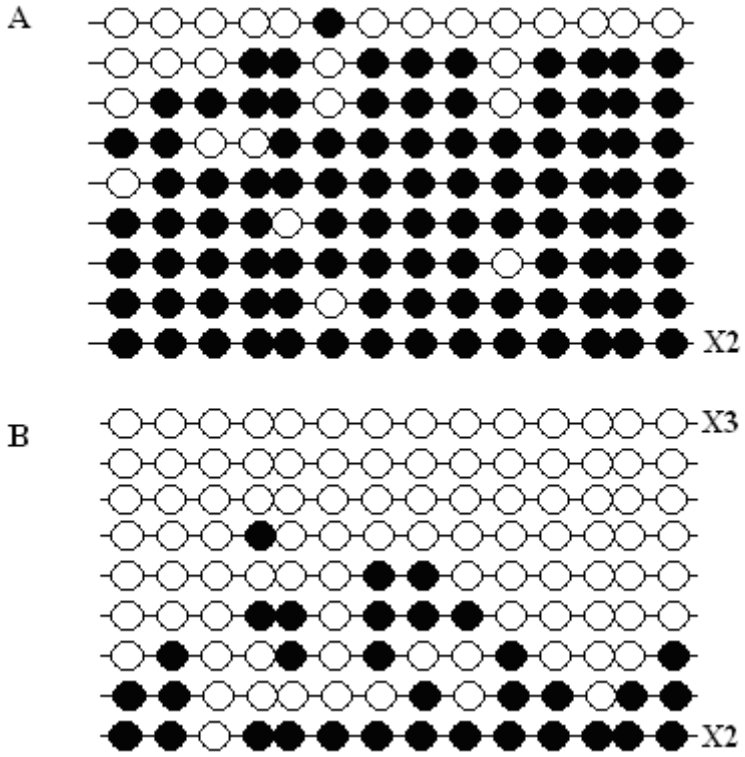

Figure 4.8. Methylation status of the Clone X G2 in the liver (A) and clonal cell line A7 (B) detected by using bisulfite sequencing. Closed circles identify methylated cytosines and open circles identify unmethylated cytosines in the sequenced clones. The number of clones with the same methylation pattern is shown to the right of the sequence. The liver is hypermethylated relative to the donor cell epigenome.

Table 4.4. 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

	Bisulfite Analysis	
CPG Clone	Liver	Donor Cell
B G2 (A7)	69.2%	73.9%
HH A7 (A7)	9.2%	65.6%
K D3 (A2)	0.7%	1.5%
S E3 (A7)	6.9%	0.0%
X G2 (A2)	80.7%	16.3%

B

Reference/Sample	
Bisulfite	Microarray
0.847	1.180
0.379	0.530
0.992	0.610
1.074	1.310
4.337	2.640

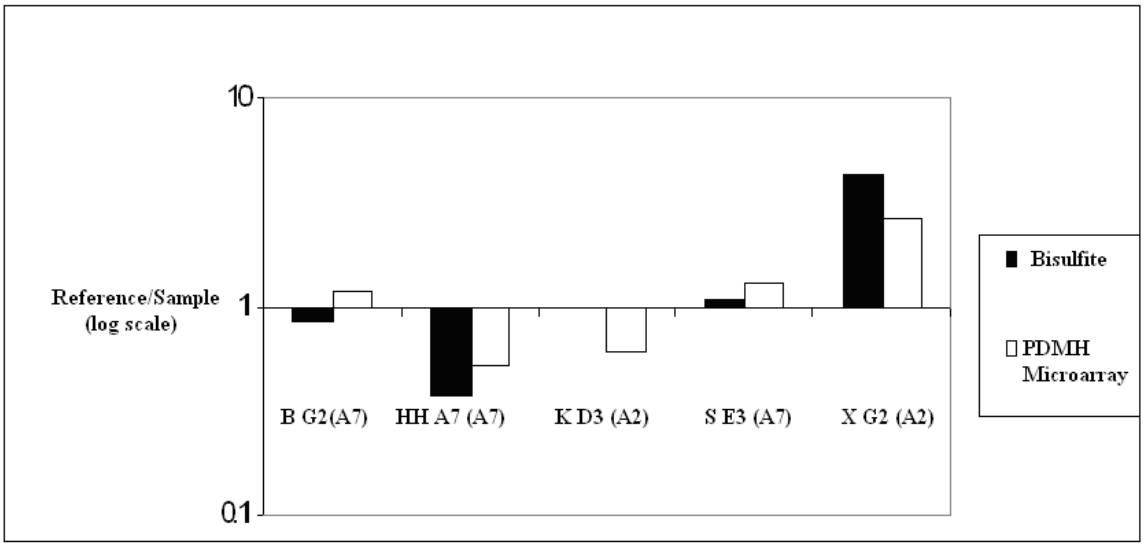

Figure 4.9. Methylation status of CpG sites of five regions were analyzed by using PDMH microarrays and bisulfite modification PCR sequencing. Similar ratios were obtained when methylation was analyzed by either method thereby validating the microarray data.

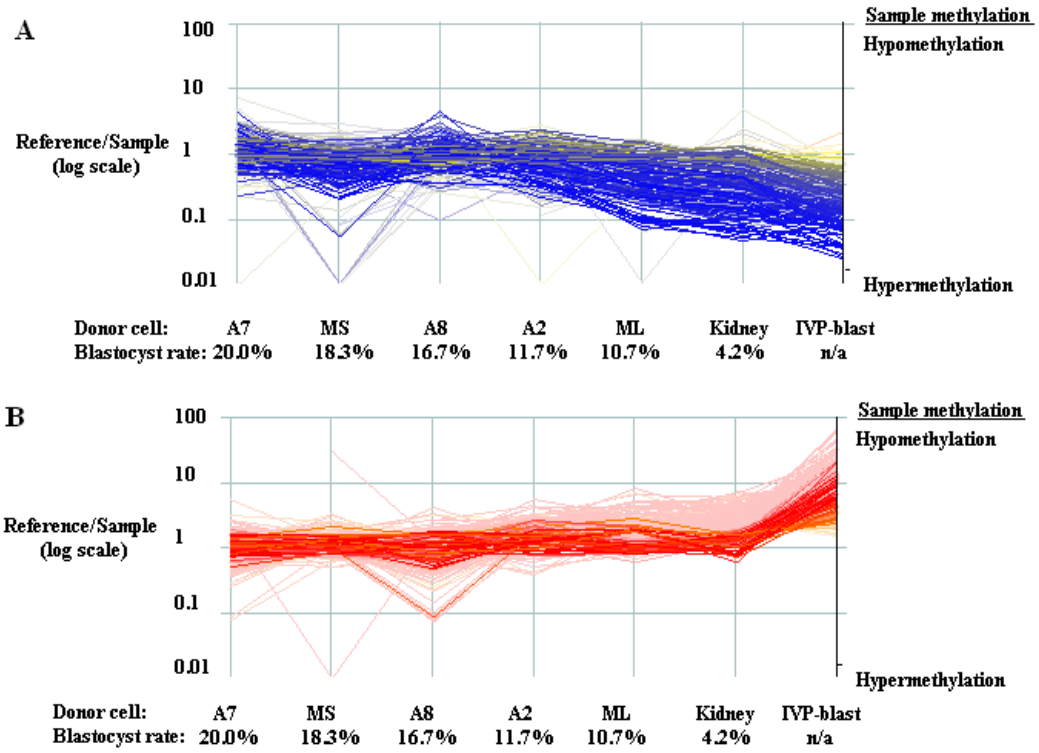

Figure 4.10. Clones with similar methylation profiles in the donor cells and the *in vivo*-produced blastocysts were clustered by using Self Organizing Map analysis. Hypermethylation (A) and hypomethylation (B) of the donor cells are correlated with lower blastocyst rates after SCNT. Those shown above were found to be different ($P<0.01$) in at least one of the biological samples of donor cells.

CHAPTER V

SUMMARY

The purpose of this research is to study the dynamics of DNA methylation in porcine gametes and in early embryos produced by using *in vivo*, *in vitro*, parthenogenetic, and SCNT procedures. An additional purpose of this research is to assess the methylation profiles of clonal cell lines and adult somatic cells with respect to developmental potential following SCNT. Using Differential Methylation Hybridization microarrays and bisulfite sequencing analysis, we showed that blastocysts produced by using *in vitro* techniques fail to be epigenetically remodeled compared to *in vivo*-produced blastocysts. The remodeling of *in vitro*-produced blastocysts shared the least similarity to the *in vivo*-produced blastocysts despite having higher developmental potential after embryo transfer to a recipient than the parthenogenetic- and SCNT-produced blastocysts. Furthermore, the similarity of the methylation profiles of the donor cells to the *in vivo*-produced blastocyst shows an inverse correlation to blastocyst rate following SCNT. Specifically, the epigenetic condition of some donor cells is resistant to the detrimental effects of extended culture on donor cells, and there are subpopulations in somatic cells that show variable resistance to epigenetic remodeling following SCNT.

These studies show that donor cells with methylation profiles that are similar to the *in vivo*-produced blastocyst, including the trophectoderm and the inner cell

mass, are not as developmentally competent following SCNT when compared to donor cells that have methylation profiles which are less similar to the *in vivo*-produced blastocyst. These results are consistent with previous studies where blastomeres could not be used successfully as donor cells in SCNT (McGrath and Solter, 1984; Wakayama et al., 2000). Apparently, a substantial epigenetic change occurs in the murine cells of the ICM to the generation of embryonic stem cells (ESC) to make the ESCs competent to produce live offspring following SCNT. It follows that previous attempts to generate ESCs, with the exceptions of humans and mice, induces epigenetic changes such that differentiation is unavoidable with the culture media tested to date.

Hiiragi and Solter (2005) demonstrated that the earliest stage of blastomeres resulted in the highest rate of blastocyst development. Recent successes in establishing pluripotent stem cells from somatic tissues (Guan et al., 2006) demonstrates a potential alternative to the ethical challenges of embryonic stem cells. This study demonstrates that PDMH microarray analysis is an effective procedure for the identification of cells with high developmental potential following SCNT. The most appropriate follow-up studies will identify the methylation profiles of cells with the highest developmental potential (i.e. early zygote). The resulting methylation profile can then be used to identify populations of somatic stem cells that have high developmental potential following SCNT. Identification of the appropriate *in vitro* culture media could allow for the selection of a population of pluripotent cells from many different adult tissues.

The putative cross-hybridization observed with the microarray analysis appears to result from an interaction of the relatively large size of the sequences that were spotted on the arrays and the common regions of multiple sequences. Specifically, hypermethylation of these similar sequences may result in cross-hybridization in the microarray analysis resulting in a false positive signal for a given sequence as shown by using bisulfite sequencing. This issue could be rectified by spotting shorter sequences on the microarrays. Optimally, the spotted sequences would be shorter (170 base pairs instead of 500 base pairs) and would match the sequences analyzed with the validation procedure (e.g. bisulfite analysis or pyrosequencing). The putatively imprinted genes (Table 3.8) are a reasonable listing of sequences to include in the next generation of PDMH microarrays.

The selection of donor cells for SCNT from a heterogeneous population of cells will likely result in the selection of numerous cell types. Accordingly, the developmental potential, as measured by the blastocyst rate after SCNT, will reflect an average of the developmental potential of the donor cells that were selected. Likewise, the effect of a specific treatment of the donor cells prior to SCNT will reflect the average effect on the change in development of the various cell types rather than the effect on a unique cell population. Therefore, in the future it is critical that procedures are developed to ensure that a homogenous population of cells is selected for SCNT studies thereby allowing for the real effect of a treatment on developmental potential to be identified.

APPENDIX

Table A.1. PDMH analysis identified spots (n=921) with significant differences (P<0.01) in the methylation in the gametes and blastocysts

Systematic Name	A A4	A B11	A C10	A C11
In vivo Blast t-test p-value	0.0933	0.279	0.00359	0.000204
In vivo Blast normalized	1.401 (0.99 -2.932)	1.351 (0.988 -3.484)	2.271 (1.245 -3.244)	5.945 (4.557 -9.744)
In vitro Blast t-test p-value	0.0044	No reps.	0.123	0.00134
In vitro Blast normalized	0.504 (0.399 -0.61)	0.01*	0.668 (0.341 -1.263)	0.636 (0.498 -0.793)
NT Blas t t-test p-value	0.0962	0.749	0.301	0.0735
NT Blast normalized	1.735 (0.933 -4.291)	1.053 (0.679 -1.895)	1.19 (0.887 -2.346)	1.235 (1.032 -1.673)
Parth Blast t-test p-value	0.7	0.964	0.257	0.485
Parth Blast normalized	0.915 (0.638 -2.601)	1.023 (0.558 -2.432)	0.883 (0.637 -1.267)	0.934 (0.734 -1.211)
GV Oocyte t-test p-value	0.0192	0.659	0.731	0.0249
GV Oocyte normalized	0.594 (0.332 -0.87)	0.869 (0.511 -1.781)	0.881 (0.196 -1.578)	0.861 (0.717 -1.025)
Sperm t-test p-value	0.704	0.887	0.0739	0.122
Sperm normalized	1.072 (0.575 -1.949)	0.984 (0.603 -1.263)	1.202 (0.974 -1.581)	0.803 (0.454 -0.971)

Table A.1 (continued)

A C12	A C3	A C6	A C8	A D3	A D8
0.00627	0.00159	0.0348	0.00912	0.00495	0.000857
2.945 (1.229 -7.311)	3.931 (1.559 -7.722)	2.867 (1.667 -4.983)	2.877 (1.276 -8.33)	13.45 (3.478 -104.1)	5.75 (1.914 -9.167)
0.601	0.0162	0.00392	0.596	0.021	0.937
1.135 (0.719 -1.76)	0.784 (0.631 -0.961)	0.73 (0.604 -0.899)	0.821 (0.333 -3.541)	0.571 (0.355 -0.855)	0.987 (0.646 -1.729)
0.000462	0.0359	0.875	0.278	0.418	0.334
1.777 (1.497 -2.224)	1.413 (0.995 -1.983)	0.978 (0.707 -1.428)	0.855 (0.477 -1.151)	1.184 (0.693 -2.332)	1.082 (0.88 -1.433)
0.127	0.359	0.542	0.537	0.773	0.655
1.619 (0.838 -3.58)	1.123 (0.784 -1.524)	0.967 (0.778 -1.1)	0.903 (0.509 -1.344)	0.961 (0.568 -1.309)	1.048 (0.802 -1.511)
0.721	0.189	0.0447	0.00291	0.0182	0.00961
0.979 (0.826 -1.108)	1.193 (0.815 -1.55)	0.91 (0.824 -1.023)	2.128 (1.195 -2.943)	0.749 (0.53 -0.9)	0.564 (0.324 -0.726)
0.178	0.663	0.177	0.137	0.00548	0.103
0.814 (0.432 -1.07)	0.924 (0.469 -1.29)	0.874 (0.63 -1.182)	0.725 (0.394 -1.198)	0.895 (0.829 -0.967)	0.789 (0.575 -1.271)

Table A.1 (continued)

A D9	A E5	A F10	A F2	A G1	A H10
0.00814	No reps.	6	0.0557	0.00952	0.000291
4.114 (2.072 -9.441)	2.152	6.825 (3.975 -15.44)	4.504 (1.31 -41.59)	1.681 (1.096 -2.344)	7.457 (4.724 -11.27)
No reps.	No reps.	0.205	0.948	No reps.	0.256
0.936	5.53	0.771 (0.36 -1.244)	1.011 (0.523 -1.499)	0.01*	0.856 (0.516 -1.149)
0.00935	0.299	0.00466	0.317	0.349	0.00352
2.302 (1.222 -5.622)	1.294 (0.698 -1.607)	1.497 (1.058 -1.78)	1.107 (0.907 -1.505)	1.255 (0.621 -2.572)	1.537 (1.109 -1.961)
0.414	No reps.	0.575	0.675	0.414	0.907
1.16 (0.613 -1.73)	1.77	1.045 (0.853 -1.386)	0.969 (0.737 -1.254)	1.362 (0.623 -2.368)	0.986 (0.673 -1.371)
0.783	0.418	0.305	0.905	0.128	0.745
0.975 (0.742 -1.229)	1.101 (0.849 -1.443)	1.166 (0.875 -1.659)	0.994 (0.862 -1.148)	0.735 (0.363 -1.26)	0.965 (0.755 -1.36)
0.223	0.00988	0.106	0.0297	0.777	0.0771
1.386 (0.862 -2.378)	0.878 (0.789 -0.963)	0.928 (0.828 -1.034)	1.174 (1.018 -1.412)	0.974 (0.728 -1.316)	0.806 (0.567 -1.124)

Table A.1 (continued)

AA A1	AA C11	AA D10	AA D12	AA D2	AA D6
3.68E -05	0.705	4.81E -05	3.52E -05	0.0609	0.0277
12.49 (7.937 -16.69)	0.889 (0.323 -2.212)	2.749 (2.05 -3.627)	47.82 (23.4 -124.5)	4.259 (1.329 -41.02)	0.565 (0.434 -0.76)
0.013	No reps.	0.196	0.0702	0.0257	No reps.
0.73 (0.568 -0.933)	0.01*	1.597 (1.083 -3.699)	0.738 (0.395 -0.944)	0.657 (0.392 -0.97)	2.444
0.00417	0.173	0.0477	0.0371	0.448	0.0698
1.621 (1.398 -2.235)	0.814 (0.531 -0.994)	1.376 (0.894 -1.931)	1.323 (0.938 -1.595)	1.159 (0.718 -1.997)	1.56 (1.269 -2.5)
0.0489	No reps.	0.972	0.0205	0.648	NO DATA
1.753 (0.89 -2.656)	0.785	1.017 (0.265 -3.846)	1.386 (1.028 -1.892)	1.053 (0.788 -1.585)	NO DATA
0.00611	0.0119	0.00201	0.00129	0.0126	0.644
3.084 (1.84 -5.356)	1.71 (1.141 -2.25)	0.384 (0.24 -0.623)	2.702 (1.756 -4.186)	0.742 (0.593 -0.897)	0.951 (0.796 -1.082)
0.0424	0.369	0.000265	No reps.	0.000278	0.114
1.184 (1.029 -1.558)	1.395 (0.894 -3.561)	0.391 (0.276 -0.534)	0.958	0.67 (0.589 -0.813)	1.192 (1.155 -1.23)

Table A.1 (continued)

AA D7	AA E10	AA E12	AA F11	AA F7	AA G5
0.0129	No reps.	3.88E -05	0.205	0.00286	0.00589
3.608 (2.807 -4.671)	11	3.665 (2.939 -5.611)	1.315 (0.98 -1.569)	11.2 (6.699 -20)	6.574 (2.057 -18.24)
No reps.	No reps.	0.503	NO DATA	NO DATA	0.0634
0.463	3.5	0.83 (0.568 -1.702)	NO DATA	NO DATA	0.74 (0.437 -0.943)
0.00193	0.000707	0.00262	0.307	0.00079	0.375
2.109 (1.616 -2.706)	2.907 (2.063 -4.193)	1.817 (1.363 -2.753)	1.187 (0.804 -1.562)	2.096 (1.703 -2.521)	1.136 (0.821 -2.048)
0.0468	0.00924	0.169	No reps.	0.0849	0.472
2.016 (1.319 -5.283)	2.366 (1.596 -3.093)	1.251 (0.696 -1.745)	7.619	2.046 (1.382 -2.992)	0.935 (0.731 -1.326)
0.0333	0.607	0.624	0.301	0.00118	0.00274
1.493 (1.069 -2.018)	1.163 (0.378 -2.536)	1.085 (0.633 -1.773)	1.227 (0.913 -1.442)	3.601 (1.966 -4.708)	0.851 (0.759 -0.93)
0.998	0.758	0.282	0.376	0.78	0.133
1 (0.631 -2.007)	0.956 (0.675 -1.656)	1.079 (0.816 -1.226)	1.171 (0.791 -1.604)	0.936 (0.466 -1.815)	0.775 (0.495 -1.008)

Table A.1 (continued)

AA G7	AA H11	AA H5	B A1	B A5	B A8
0.0427	0.00042	7.02E -06	0.00381	0.000125	0.984
10.29 (4.81 -16.24)	30.4 (15.16 -134.5)	33.62 (20.45 -73.36)	4.949 (3.349 -14.09)	6.923 (5.315 -10.63)	1.011 (0.44 -4)
0.0678	0.00445	0.203	0.516	0.2	No reps.
2.233 (0.978 -4.4)	0.819 (0.709 -0.942)	0.793 (0.434 -1.214)	1.147 (0.852 -1.983)	1.578 (0.828 -3.054)	0.01*
0.00286	0.0956	0.0462	0.00465	0.0101	0.00487
2.916 (1.392 -4.52)	1.456 (0.813 -2.871)	1.482 (0.933 -2.618)	1.99 (1.306 -3.043)	2.922 (1.512 -7.96)	2.243 (1.064 -3.154)
0.122	0.0835	0.0152	0.305	0.0138	0.421
1.52 (0.91 -3.012)	1.226 (0.977 -1.672)	1.648 (1.315 -2.506)	1.26 (0.583 -2.447)	2.138 (1.106 -3.162)	1.655 (0.701 -7.406)
0.0279	0.936	0.0018	0.531	0.249	0.527
1.347 (1.042 -1.865)	0.98 (0.559 -2.339)	2.777 (1.666 -4.361)	1.059 (0.813 -1.502)	1.204 (0.848 -1.804)	0.662 (0.423 -1.037)
0.29	0.00492	0.0725	0.607	0.847	0.787
0.847 (0.436 -1.065)	0.807 (0.682 -0.917)	1.205 (0.929 -1.46)	1.099 (0.796 -2.062)	1.023 (0.808 -1.679)	0.899 (0.515 -1.684)

Table A.1 (continued)

B B1	B B2	B B6	B C1	B C11	B C12
0.000227	0.000173	0.028	0.0299	0.000324	0.00833
7.433 (3.881 -14.85)	7.037 (3.903 -11.23)	2.244 (1.808 -2.902)	5.278 (1.661 -12.49)	32.15 (4.4 -126)	20.59 (4.574 -452.3)
0.516	0.223	No reps.	0.0677	0.652	0.126
0.398 (0.01 -2.475)	0.801 (0.443 -1.035)	0.01*	0.631 (0.427 -1.096)	1.072 (0.745 -1.701)	1.293 (0.834 -1.795)
0.00127	0.0609	0.811	0.00132	0.00838	0.0846
1.825 (1.488 -2.172)	1.216 (0.84 -1.417)	0.903 (0.352 -3)	1.833 (1.328 -2.454)	1.618 (1.252 -2.462)	1.23 (0.846 -1.629)
0.223	0.739	No reps.	0.0225	0.00169	0.329
1.212 (0.871 -1.911)	1.034 (0.742 -1.482)	0.975	1.433 (0.985 -2.009)	1.192 (1.159 -1.308)	1.426 (0.357 -2.856)
0.142	0.0879	0.674	0.648	0.0272	0.00254
0.897 (0.736 -1.113)	0.772 (0.48 -1.174)	0.739 (0.432 -1.265)	0.956 (0.69 -1.138)	1.119 (0.948 -1.238)	2.027 (1.134 -2.703)
0.112	0.0436	0.583	0.554	0.601	0.29
0.862 (0.632 -1.078)	0.855 (0.781 -1.012)	0.857 (0.46 -1.324)	0.928 (0.656 -1.272)	0.937 (0.596 -1.226)	0.786 (0.314 -1.24)

Table A.1 (continued)

B C2	B C3	B C4	B C6	B C8	B C9
0.0126	0.134	0.00256	0.00983	0.0127	0.00407
4.372 (1.25 -9.805)	4.083 (0.087 -12.43)	6.162 (2.717 -16.84)	13.12 (4.467 -237.1)	3.227 (2.273 -5.784)	3.915 (3.105 -6.429)
0.0808	0.00655	0.142	0.795	0.812	0.00835
0.734 (0.622 -0.859)	1.423 (1.065 -1.907)	1.255 (0.795 -1.82)	0.944 (0.369 -1.516)	1.094 (0.562 -1.559)	2.664 (2.63 -2.698)
0.0002	0.866	0.607	0.00596	0.659	0.0604
1.711 (1.548 -1.868)	1.012 (0.768 -1.247)	1.058 (0.703 -1.449)	1.225 (1.075 -1.443)	1.091 (0.567 -1.669)	1.803 (1.226 -5.957)
0.835	0.0356	0.307	0.304	0.0561	0.000102
1.033 (0.765 -1.792)	1.31 (1.002 -1.678)	1.311 (0.425 -2.388)	1.3 (0.701 -2.681)	1.473 (0.954 -2.098)	2.324 (2.286 -2.351)
0.274	5.28E -05	0.0112	0.000494	0.498	0.918
1.086 (0.92 -1.278)	1.937 (1.543 -2.234)	1.71 (0.923 -2.365)	1.569 (1.373 -1.983)	0.919 (0.633 -1.503)	1.015 (0.66 -1.4)
0.608	0.909	0.257	0.0511	0.193	0.182
0.93 (0.612 -1.466)	1.013 (0.631 -1.355)	0.818 (0.44 -1.188)	1.132 (0.951 -1.31)	1.154 (0.925 -1.809)	0.819 (0.513 -1.309)

Table A.1 (continued)

B D2	B D5	B D6	B D7	B E11	B G1
0.0169	0.0231	0.00967	0.0608	0.0111	0.00231
0.814 (0.713 - 0.974)	4.192 (1.16 -18.02)	2.141 (1.397 -5.13)	2.048 (0.957 -5.041)	4.853 (3.469 -5.831)	30.75 (11.06 -108.5)
No reps.	0.446	0.401	0.879	No reps.	0.0628
1.725	1.13 (0.881 -1.364)	1.75 (1.164 -2.631)	0.945 (0.703 -1.27)	3.596	0.685 (0.498 -1.035)
0.397	0.000515	0.00199	0.0177	0.000217	0.000109
1.191 (0.783 -1.979)	2.399 (1.417 -2.981)	1.793 (1.104 -2.137)	1.355 (0.95 -1.591)	2.465 (1.981 -3.599)	1.286 (1.218 -1.335)
No reps.	0.189	0.234	0.267	0.00307	0.597
0.01*	1.361 (0.849 -2.491)	1.534 (0.686 -2.689)	1.322 (0.647 -2.493)	2.154 (1.929 -2.786)	1.095 (0.576 -1.71)
0.821	1	0.000887	0.21	0.11	0.948
0.933 (0.327 - 1.583)	1 (0.714 -1.282)	0.409 (0.295 -0.529)	0.46 (0.166 -1.078)	1.515 (0.869 -2.679)	0.996 (0.828 -1.223)
0.964	0.96	0.276	0.000485	0.64	0.572
0.967 (0.391 - 3.422)	1.006 (0.726 - 1.37)	0.684 (0.463 -1.108)	0.555 (0.45 -0.743)	1.05 (0.859 -1.484)	0.963 (0.761 -1.159)

Table A.1 (continued)

B G10	B G12	B G2	B G4	B G5	B G6
0.0123	2.07E -05	0.000582	0.00198	0.00391	0.000158
6.405 (3.464 -13.42)	61.11 (36.02 -160)	21.52 (9.229 -82.5)	2.08 (1.42 -3.22)	21.89 (6.526 -423)	3.433 (2.002 -4.393)
No reps.	0.0587	0.414	0.181	0.653	0.105
1.175	1.482 (0.924 -2.017)	0.878 (0.465 -1.248)	1.23 (0.784 -1.795)	1.08 (0.695 -1.987)	1.3 (0.942 -1.609)
0.00491	0.00623	0.000413	0.789	0.00206	0.00161
2.086 (1.361 -3.097)	1.908 (1.281 -3.523)	1.748 (1.462 -2.297)	1.021 (0.792 -1.257)	1.383 (1.13 -1.666)	1.815 (1.23 -2.482)
0.459	0.0101	0.959	0.14	0.224	0.612
1.18 (0.539 -1.634)	1.552 (1.149 -2.476)	0.992 (0.61 -1.848)	1.14 (0.865 -1.407)	1.39 (0.616 -2.906)	1.188 (0.669 -3.769)
0.0757	0.000791	0.635	0.0233	0.00126	0.000161
1.261 (0.983 -1.426)	2.543 (1.648 -3.522)	1.043 (0.782 -1.348)	1.534 (0.818 -2.101)	1.872 (1.214 -2.35)	1.495 (1.231 -1.588)
0.578	0.32	0.501	0.309	0.423	0.0352
1.066 (0.888 -1.35)	0.832 (0.392 -1.216)	0.96 (0.806 -1.177)	0.869 (0.525 -1.135)	0.853 (0.433 -1.191)	1.211 (0.929 -1.464)

Table A.1 (continued)

B G7	B H3	B H7	BBB A12	BBB B12	BBB B4
0.00662	0.000104	5.86E -05	3.25E -06	0.0352	0.0192
2.281 (1.994 -2.458)	5.654 (4.136 -10.32)	11.23 (5.67 -21.62)	15.52 (11.59 -23.04)	1.853 (1.228 -3.518)	7.476 (2.592 -20.4)
0.814	0.404	0.802	0.00104	NO DATA	0.423
1.154 (0.718 -1.853)	0.804 (0.431 -1.636)	1.061 (0.636 -1.535)	2.478 (1.708 -4.006)	NO DATA	1.52 (1.095 -2.11)
0.0702	0.00766	0.018	0.00624	0.000102	0.000811
2.748 (1.103 -5)	1.547 (1.096 -2.169)	2.217 (1.194 -5.434)	2.515 (1.612 -5.523)	1.438 (1.369 -1.525)	2.103 (1.556 -3.124)
0.046	0.681	0.0432	0.0883	0.184	0.00582
1.529 (1.483 -1.576)	1.063 (0.741 -1.789)	1.448 (1.014 -2.069)	1.333 (0.831 -2)	1.982 (1.617 -2.43)	2.134 (1.677 -2.633)
0.00048	0.121	0.00864	0.0123	0.0638	0.826
0.555 (0.516 -0.606)	0.752 (0.392 -1.133)	1.335 (1.006 -1.665)	1.259 (1.035 -1.385)	0.417 (0.216 -0.9)	0.949 (0.43 -1.723)
0.163	0.0519	0.0189	0.698	0.00732	0.823
1.156 (0.955 -1.466)	0.751 (0.463 -1.036)	1.264 (1.076 -1.66)	1.027 (0.753 -1.162)	0.723 (0.575 -0.812)	0.992 (0.871 -1.092)

Table A.1 (continued)

BBB B5	BBB D12	BBB E12	BBB E4	BBB G11	BBB G2
0.00334	0.049	0.00421	2.88E -05	0.053	0.00116
6.441 (4.925 -12.27)	9.031 (1.558 -36.5)	12.66 (8.484 -33)	3.521 (2.666 -5.107)	8.624 (3.982 -23)	4.331 (1.607 -6.928)
NO DATA	0.25	0.698	0.69	0.519	0.128
NO DATA	1.208 (1 -1.779)	1.073 (0.935 -1.232)	1.144 (0.549 -2.723)	1.21 (0.988 -1.481)	1.251 (1.03 -1.961)
0.0318	0.00244	1.85E -05	0.000292	0.0149	0.00422
2.899 (1.162 -8.182)	1.562 (1.218 -2.206)	2.2 (1.95 -2.372)	1.463 (1.268 -1.653)	2.493 (1.191 -5.472)	2.378 (1.483 -3.601)
0.252	0.0818	0.0161	0.472	0.26	0.108
5.54 (2.707 -11.34)	1.527 (1.07 -3.075)	1.783 (1.067 -2.907)	1.081 (0.85 -1.545)	2.765 (1.325 -10.17)	1.438 (0.762 -2.197)
0.0596	0.898	0.381	0.876	0.717	0.18
1.258 (1.011 -1.556)	1.016 (0.723 -1.469)	1.206 (0.702 -2.653)	1.027 (0.589 -1.708)	0.94 (0.558 -1.631)	1.192 (0.913 -1.713)
0.404	0.737	0.0266	0.115	0.991	0.39
0.899 (0.717 -1.494)	1.047 (0.608 -1.38)	1.204 (1.043 -1.553)	0.763 (0.427 -1.246)	0.998 (0.646 -1.809)	0.873 (0.457 -1.296)

Table A.1 (continued)

BBB H7	BLUE A9	BLUE C1	BLUE C10	BLUE C12	BLUE C5
7.78E -05	0.041	0.0895	1.81E -05	0.00384	0.000198
10.56 (5.676 -21.98)	2.267 (0.901 -4.867)	1.974 (1.05 -5.271)	6.499 (4.353 -8.754)	2.471 (1.586 -3.996)	2.99 (1.986 -4.381)
0.117	0.791	0.156	0.362	0.697	0.602
0.735 (0.453 -1.06)	1.039 (0.679 -1.549)	0.913 (0.714 -1.062)	0.911 (0.713 -1.3)	0.711 (0.367 -1.379)	1.146 (0.597 -1.821)
0.258	0.655	0.247	0.0023	0.00015	0.0238
1.24 (0.763 -2.345)	1.045 (0.85 -1.438)	1.124 (0.946 -1.713)	2.535 (1.786 -4.786)	1.541 (1.3 -1.716)	1.525 (0.964 -2.247)
0.389	0.134	0.147	0.00162	0.377	0.00459
1.107 (0.817 -1.57)	0.925 (0.781 -1.033)	1.104 (0.871 -1.333)	1.62 (1.236 -2.202)	1.311 (0.773 -1.76)	1.367 (1.119 -1.674)
0.024	0.000162	0.00839	0.908	0.164	0.384
0.887 (0.784 -0.975)	0.82 (0.773 -0.883)	0.78 (0.644 -0.908)	1.01 (0.77 -1.26)	0.734 (0.458 -1.633)	0.907 (0.602 -1.214)
0.903	0.216	0.0315	0.521	0.0415	0.579
1.009 (0.793 -1.258)	0.911 (0.726 -1.038)	0.719 (0.543 -1.093)	0.938 (0.655 -1.228)	0.831 (0.686 -0.977)	1.078 (0.776 -1.83)

Table A.1 (continued)

BLUE C7	BLUE C8	BLUE D10	BLUE D11	BLUE D5	BLUE D6
0.00434	0.00548	0.000485	0.000646	0.000323	3.65E -05
0.083 (0.0461 -0.156)	0.349 (0.257 -0.491)	0.0976 (0.0532 -0.16)	0.162 (0.114 -0.193)	2.417 (1.765 -3.199)	0.39 (0.285 -0.452)
No reps.	NO DATA	0.104	NO DATA	0.49	No reps.
1.725	NO DATA	0.677 (0.635 -0.722)	NO DATA	0.763 (0.294 -1.383)	1.28
0.0682	0.00495	0.00033	0.00166	0.654	0.0029
0.336 (0.101 -0.847)	0.427 (0.238 -0.525)	0.241 (0.11 -0.308)	0.263 (0.118 -0.414)	1.032 (0.878 -1.209)	0.497 (0.339 -0.594)
0.0656	No reps.	0.0207	0.0632	0.84	0.24
0.352 (0.167 -0.705)	0.481	0.269 (0.0869 -0.542)	0.222 (0.123 -0.472)	1.028 (0.706 -1.398)	0.302 (0.188 -0.486)
0.0621	0.822	0.0117	0.543	0.288	0.496
0.788 (0.543 -1.01)	0.918 (0.475 -1.367)	0.697 (0.565 -0.876)	0.87 (0.447 -1.544)	0.885 (0.594 -1.234)	1.108 (0.85 -1.47)
0.865	0.0619	0.911	0.181	0.298	0.173
1.042 (0.676 -1.941)	2.307 (1.359 -4.453)	1.035 (0.724 -1.78)	3.618 (1.822 -12.91)	0.916 (0.772 -1.277)	1.515 (0.659 -3.565)

Table A.1 (continued)

BLUE D7	BLUE D8	BLUE D9	BLUE E11	BLUE E2	BLUE E3
0.0193	0.0144	0.248	0.000165	0.000284	0.000294
1.968 (1.281 -3.291)	0.667 (0.46 -0.857)	0.777 (0.422 -1.283)	0.0862 (0.0478 -0.116)	0.0753 (0.0349 -0.12)	0.206 (0.106 -0.376)
0.359	0.997	0.703	No reps.	0.0755	0.933
0.902 (0.754 -1.158)	1 (0.812 -1.31)	0.949 (0.605 -1.507)	0.636	0.626 (0.455 -0.971)	1.06 (0.607 -1.852)
4.65E -05	0.94	0.121	0.00593	0.0144	0.0052
1.835 (1.634 -2.248)	0.995 (0.879 -1.309)	0.8 (0.484 -1.046)	0.194 (0.0617 -0.522)	0.112 (0.01 -0.457)	0.237 (0.107 -0.427)
0.105	0.687	0.759	0.0503	0.0255	0.0444
2.418 (1.372 -4.005)	1.02 (0.888 -1.257)	1.088 (0.471 -2.046)	0.336 (0.0737 -0.696)	0.189 (0.0547 -0.442)	0.343 (0.0744 -0.72)
0.913	0.0128	1.48E -05	0.202	0.235	0.435
1.018 (0.586 -1.499)	0.87 (0.776 -0.976)	3.127 (2.407 -3.652)	1.334 (0.937 -1.843)	1.287 (0.827 -2.541)	1.323 (0.529 -2.01)
0.0336	0.0616	0.0635	0.937	0.315	0.0464
0.809 (0.713 -1.155)	0.882 (0.777 -1.055)	1.185 (1.016 -1.597)	0.984 (0.387 -1.37)	1.398 (0.623 -2.145)	1.904 (1.081 -2.679)

Table A.1 (continued)

BLUE E6	BLUE E8	BLUE F11	BLUE F2	BLUE F3	BLUE F8
0.0123	0.000193	0.0528	0.00394	0.00103	8.59E -05
3.813 (2.39 -7.122)	0.121 (0.0706 -0.192)	0.654 (0.46 -1.106)	0.522 (0.341 -0.861)	0.441 (0.349 -0.559)	0.205 (0.134 -0.282)
0.381	0.381	NO DATA	No reps.	NO DATA	0.151
1.44 (0.782 -2.392)	1.778 (1.041 -5)	NO DATA	0.788	NO DATA	1.418 (1.304 -1.543)
0.000586	0.00523	0.118	0.0365	0.276	0.00809
2.317 (1.739 -3.774)	0.24 (0.0881 -0.364)	0.682 (0.248 -0.846)	0.672 (0.406 -0.933)	0.733 (0.369 -1.039)	0.385 (0.129 -0.563)
0.00533	0.455	0.624	0.39	0.19	0.152
2.437 (2.196 -2.748)	0.642 (0.191 -6.168)	1.104 (0.662 -1.558)	0.824 (0.537 -1.191)	0.456 (0.358 -0.58)	0.555 (0.302 -1.296)
0.0514	0.106	0.0379	0.0182	0.278	0.901
1.411 (0.91 -2.107)	1.544 (0.828 -2.784)	1.944 (0.897 -3.027)	1.476 (1.024 -2.259)	2.403 (1.595 -3.621)	1.038 (0.511 -1.628)
0.0328	0.0299	0.0953	0.134	0.00707	0.143
1.173 (0.964 -1.37)	1.444 (1.152 -1.728)	1.7 (1.02 -2.627)	0.804 (0.577 -1.104)	2.374 (2.053 -2.591)	0.803 (0.495 -1.056)

Table A.1 (continued)

BLUE G3	BLUE G4	BLUE G8	BLUE H10	BLUE H11	BLUE H2
0.00343	0.000515	6.84E -06	4.39E -05	1.09E -05	0.00858
0.342 (0.25 -0.666)	0.202 (0.128 -0.284)	0.296 (0.248 -0.371)	0.122 (0.0688 -0.199)	2.892 (2.305 -3.303)	3.448 (2.27 -5.722)
NO DATA	NO DATA	NO DATA	0.441	0.616	0.591
NO DATA	NO DATA	NO DATA	1.468 (1.068 -2.019)	1.243 (0.574 -3.11)	1.075 (0.858 -1.235)
0.122	0.00397	0.0212	0.00246	0.0735	0.00125
0.706 (0.468 -1.671)	0.257 (0.111 -0.499)	0.444 (0.164 -0.972)	0.222 (0.118 -0.348)	1.494 (0.956 -2.199)	2.418 (1.621 -4.238)
0.306	0.171	0.0362	0.0749	0.766	0.00492
0.548 (0.242 -1.098)	0.38 (0.114 -1.182)	0.428 (0.277 -0.806)	0.242 (0.0919 -0.667)	1.136 (0.321 -4.444)	2.938 (1.728 -8.042)
0.342	0.762	0.424	0.84	7.61E -05	0.683
1.17 (0.799 -1.799)	1.123 (0.68 -2.117)	1.204 (0.785 -2.582)	0.899 (0.595 -1.361)	0.477 (0.383 -0.564)	0.937 (0.733 -1.672)
0.184	0.0136	0.385	0.648	0.00024	0.97
0.894 (0.709 -1.126)	1.983 (1.344 -2.375)	1.2 (0.613 -1.915)	0.845 (0.474 -1.411)	0.518 (0.412 -0.647)	1.006 (0.697 -1.632)

Table A.1 (continued)

BLUE H5	BLUE H8	BLUE H9	C A10	C A2	C A6
0.0226	0.00295	8.46E -06	0.00016	0.00022	0.0013
2.586 (1.935 -2.992)	0.148 (0.0693 -0.245)	3.633 (3.07 -4.832)	5.981 (3.276 -10.62)	5.845 (2.733 -8.546)	14.58 (6.761 -35)
NO DATA	0.0389	0.307	0.837	0.495	0.211
NO DATA	1.261 (1.012 -1.552)	0.828 (0.411 -1.184)	0.962 (0.579 -1.35)	0.839 (0.493 -1.448)	0.795 (0.425 -1.124)
0.0278	0.00334	0.00266	0.197	0.0375	0.000632
2.577 (1.348 -4.172)	0.91 (0.847 -0.96)	1.447 (1.21 -1.649)	1.537 (0.511 -2.303)	1.457 (0.862 -1.857)	2.167 (1.715 -2.995)
0.153	0.631	0.253	0.332	0.0379	0.0548
1.583 (1.414 -1.771)	0.989 (0.926 -1.037)	1.165 (0.933 -1.765)	1.368 (0.524 -3.655)	1.14 (0.963 -1.315)	1.481 (1.026 -2.534)
0.743	0.353	0.926	0.402	0.0638	0.819
1.057 (0.607 -1.397)	0.958 (0.854 -1.105)	1.012 (0.769 -1.618)	0.916 (0.672 -1.186)	0.837 (0.641 -1.093)	1.036 (0.76 -1.67)
0.607	0.517	0.122	0.305	0.599	0.829
0.915 (0.627 -1.429)	1.055 (0.869 -1.334)	0.879 (0.693 -1.057)	1.123 (0.773 -1.509)	0.934 (0.618 -1.479)	1.032 (0.554 -1.521)

Table A.1 (continued)

C B2	C B4	C B8	C B9	C D3	C E2
9.77E -05	0.0231	0.00264	0.0465	1.50E -05	0.0195
3.072 (2.096 -4.259)	1.741 (0.95 -2.578)	5 (2.227 -10.91)	9.65 (1.584 -302.5)	6.796 (5.175 -9.128)	4.467 (4.266 -4.677)
No reps.	0.0126	0.331	0.0825	0.342	NO DATA
0.652	0.514 (0.339 -0.923)	2.037 (0.712 -4.777)	0.659 (0.352 -1.354)	0.9 (0.651 -1.152)	NO DATA
0.00247	0.717	0.00532	0.0992	0.0105	0.00826
1.906 (1.428 -2.34)	0.968 (0.697 -1.172)	1.82 (1.139 -2.955)	0.774 (0.496 -1.097)	1.967 (1.059 -3.739)	2.088 (1.009 -3.056)
0.324	0.272	0.0203	0.632	0.0679	0.389
1.698 (0.831 -3.41)	0.781 (0.446 -1.422)	1.516 (0.957 -2.342)	0.92 (0.545 -1.415)	1.157 (0.929 -1.431)	1.478 (1.125 -1.941)
0.149	0.00566	0.0296	0.000317	0.463	0.469
1.251 (0.759 -1.475)	1.972 (1.31 -3.193)	1.292 (0.975 -1.799)	2.002 (1.547 -2.559)	1.083 (0.841 -1.486)	0.836 (0.591 -1.57)
0.985	0.281	0.375	0.0972	0.0856	0.283
1.003 (0.713 -1.667)	0.815 (0.421 -1.227)	0.88 (0.488 -1.233)	1.136 (1.01 -1.532)	1.243 (0.895 -1.818)	0.771 (0.415 -1.589)

Table A.1 (continued)

C E4	C E6	C E8	C E9	C F5	C F6
0.345	5.47E -05	0.00295	0.00012	0.125	0.0198
1.552 (0.831 -2.876)	3.798 (2.782 -5.315)	5.182 (1.441 -11.45)	5.087 (3.376 -8.877)	0.762 (0.417 -1.156)	6.555 (3.861 -9.221)
NO DATA	0.386	No reps.	0.51	No reps.	0.849
NO DATA	0.888 (0.574 -1.251)	1.525	1.327 (0.207 -2.633)	0.01*	0.912 (0.621 -1.338)
0.00463	0.0218	0.00923	0.000145	0.0492	0.0874
2.043 (1.944 -2.253)	1.422 (1.057 -2.004)	2.223 (0.897 -3.26)	2.235 (1.543 -2.588)	0.933 (0.88 -1.048)	1.5 (0.928 -1.852)
No reps.	0.973	0.00523	0.00935	0.357	0.354
0.01*	0.997 (0.711 -1.238)	1.981 (1.457 -2.678)	1.701 (1.079 -2.717)	0.658 (0.172 -2.306)	1.973 (1.293 -3.01)
0.494	0.681	0.276	0.0145	0.325	0.00168
0.83 (0.617 -1.287)	0.921 (0.498 -1.604)	1.161 (0.659 -1.507)	1.543 (1.017 -2.082)	0.886 (0.629 -1.317)	1.361 (1.229 -1.723)
0.535	0.00372	0.382	0.00629	0.117	0.0286
0.906 (0.696 -1.076)	1.168 (1.058 -1.285)	1.102 (0.82 -1.527)	1.437 (1.108 -1.956)	0.65 (0.352 -1.112)	1.752 (1.233 -4.153)

Table A.1 (continued)

C G10	C G11	C G3	C G9	C H10	C H2
0.0117	0.000162	0.000877	0.000126	0.0879	0.00195
1.768 (1.229 -2.812)	3.238 (2.219 -4.429)	0.334 (0.285 -0.407)	1.622 (1.353 -1.846)	3.749 (1.406 -21.22)	5.318 (1.82 -14.31)
0.735	0.599	0.706	0.763	0.551	0.0761
0.931 (0.487 -1.45)	1.061 (0.745 -1.371)	1.153 (0.396 -3.445)	1.071 (0.636 -1.538)	0.895 (0.41 -1.407)	1.384 (1.063 -1.874)
0.721	0.0171	3.05E -05	0.000981	0.724	0.132
0.966 (0.757 -1.254)	1.374 (1.037 -1.899)	0.393 (0.332 -0.5)	1.872 (1.417 -2.555)	1.032 (0.8 -1.38)	3.093 (1.913 -7.714)
0.0683	0.0505	0.0995	0.912	0.918	0.0392
0.862 (0.67 -1.004)	1.136 (0.931 -1.302)	0.438 (0.252 -0.637)	1.027 (0.692 -1.739)	0.991 (0.749 -1.222)	2.228 (0.864 -4.329)
0.564	0.0288	0.632	0.0463	0.652	0.01
0.94 (0.677 -1.242)	1.273 (0.97 -1.641)	0.9 (0.642 -1.992)	0.473 (0.296 -1.23)	0.972 (0.781 -1.145)	1.447 (1.015 -1.867)
0.0602	0.723	0.561	0.0243	0.00665	0.00919
1.113 (0.988 -1.265)	0.962 (0.622 -1.29)	0.904 (0.61 -1.538)	0.587 (0.396 -1.239)	1.181 (1.049 -1.317)	1.561 (1.238 -2.478)

Table A.1 (continued)

CC A5	CC B3	CC C1	CC F2	CC F3	CC G8
0.00109	7.75E -05	0.0056	0.00579	0.00417	0.00229
22.23 (3.638 -87.69)	0.225 (0.148 -0.308)	0.375 (0.335 -0.431)	0.491 (0.302 -0.608)	0.539 (0.398 -0.962)	0.41 (0.276 -0.548)
0.34	NO DATA	NO DATA	No reps.	NO DATA	NO DATA
0.847 (0.496 -1.391)	NO DATA	NO DATA	5.347	NO DATA	NO DATA
0.168	0.00586	0.00228	0.0184	0.00817	0.00512
1.53 (0.511 -2.788)	0.252 (0.0754 -0.461)	0.324 (0.222 -0.734)	0.381 (0.13 -0.711)	0.423 (0.233 -0.693)	0.579 (0.475 -0.758)
0.705	0.182	0.191	0.217	0.488	0.176
1.046 (0.848 -1.407)	0.144 (0.0816 -0.254)	0.345 (0.199 -1.03)	0.637 (0.262 -1.401)	1.372 (0.918 -2.904)	0.355 (0.265 -0.476)
0.0037	0.418	0.383	0.353	0.132	0.0441
3.036 (2.015 -5.357)	0.802 (0.361 -1.287)	1.45 (0.487 -2.249)	1.199 (0.75 -1.556)	1.857 (1.63 -2.116)	0.509 (0.28 -0.664)
0.014	0.446	0.041	0.456	0.0511	0.339
1.331 (1.11 -1.759)	1.261 (0.907 -2.784)	2.055 (1.638 -2.733)	1.231 (0.437 -2.388)	1.712 (1.203 -3.543)	2.107 (1.358 -3.27)

Table A.1 (continued)

CC H3	CCC A8	CCC B8	CCC C4	CCC D12	CCC D2	CCC D4
0.0704	0.00566	0.0269	2.60E -05	0.00178	0.00679	0.107
2.456 (1.022 -4.654)	6.319 (2.886 -17.5)	6.7 (2.334 -57.53)	0.461 (0.392 -0.535)	4.043 (2.42 -9.312)	0.647 (0.477 -0.974)	1.499 (0.759 -2.187)
No reps.	0.357	0.705	No reps.	0.0348	0.0364	No reps.
52.5	0.881 (0.813 -0.954)	1.063 (0.661 -1.874)	3	0.757 (0.745 -0.769)	3.777 (3.5 -4.075)	0.01*
0.138	0.000512	0.0785	7.24E -06	0.0381	0.362	No reps.
1.43 (0.927 -2.831)	2.593 (2.068 -4.533)	1.214 (0.911 -1.597)	0.665 (0.61 -0.715)	1.474 (1.163 -2.882)	0.867 (0.586 -1.292)	1.705
0.155	0.0935	0.56	0.355	0.167	0.817	0.00333
4.051 (1.885 -14)	2.07 (1.543 -3.328)	1.052 (0.904 -1.365)	0.613 (0.357 -1.369)	1.809 (0.51 -4.108)	0.882 (0.377 -2.858)	1.611 (1.525 -1.659)
0.0342	0.172	0.000982	0.0475	0.408	0.00289	0.289
1.797 (1.003 -3.208)	1.317 (0.686 -2.026)	0.804 (0.71 -0.866)	1.513 (1.339 -1.818)	1.152 (0.733 -1.827)	1.666 (1.195 -2.194)	1.226 (0.679 -1.779)
0.0152	0.0111	0.019	0.0024	0.307	0.086	0.0429
1.983 (1.376 -3.363)	1.33 (0.941 -1.523)	0.783 (0.579 -0.898)	1.967 (1.57 -2.615)	1.137 (0.813 -1.389)	1.806 (0.749 -3.626)	1.482 (1.171 -2.044)

Table A.1 (continued)

CCC D9	CCC E11	CCC E7	CCC E9	CCC F11	CCC F12
0.00339	0.097	9.15E -05	3.96E -06	0.0486	0.00652
7.078 (3.51 -20.99)	0.543 (0.391 -0.793)	4.917 (3.281 -8.317)	3.485 (2.921 -4.373)	0.705 (0.452 -1.212)	0.448 (0.299 -0.692)
0.214	NO DATA	0.0373	0.269	No reps.	No reps.
0.726 (0.281 -1.385)	NO DATA	1.48 (0.955 -2.323)	1.233 (0.732 -1.625)	5.5	2.12
0.268	0.708	0.000731	0.00145	0.163	0.0217
1.267 (0.815 -2.326)	1.077 (0.723 -1.748)	1.578 (1.359 -2.01)	1.567 (1.214 -1.827)	0.641 (0.247 -1.061)	0.637 (0.474 -0.756)
0.379	No reps.	0.0638	0.142	No reps.	NO DATA
1.104 (0.776 -1.4)	0.01*	1.216 (1.011 -1.53)	1.319 (0.767 -1.983)	0.37	NO DATA
0.0943	0.0188	0.000905	0.00811	0.316	NO DATA
0.836 (0.575 -1.009)	1.56 (1.174 -2.186)	1.295 (1.111 -1.403)	1.503 (1.1 -1.747)	1.185 (0.82 -1.635)	NO DATA
0.465	0.0496	0.000973	0.00153	0.0937	0.334
0.947 (0.71 -1.134)	1.662 (1.315 -1.901)	1.413 (1.228 -1.617)	1.673 (1.511 -2.176)	1.507 (1.037 -2.015)	1.31 (1 -2)

Table A.1 (continued)

CCC F6	CCC F7	CCC G10	CCC G2	CCC H1	CCC H10
0.145	0.000645	0.0124	3.50E -06	0.024	1.46E -06
1.282 (0.799 -1.702)	4.397 (3.02 -7.443)	0.457 (0.328 -0.794)	0.295 (0.237 -0.342)	2.82 (1.652 -4.852)	0.179 (0.151 -0.216)
0.738	0.097	NO DATA	0.671	0.241	0.583
0.948 (0.631 -1.386)	1.285 (0.997 -2.089)	NO DATA	1.718 (0.664 -4.443)	0.734 (0.369 -1.438)	1.849 (0.83 -4.116)
0.757	1.96E -05	0.00548	0.00196	0.477	0.00139
0.989 (0.869 -1.089)	1.681 (1.477 -1.841)	0.589 (0.374 -0.855)	0.394 (0.193 -0.519)	1.097 (0.817 -1.523)	0.302 (0.173 -0.519)
0.84	0.122	No reps.	0.0776	0.313	0.0782
0.966 (0.606 -1.623)	1.304 (0.869 -2.14)	0.474	0.457 (0.257 -0.817)	0.955 (0.87 -1.084)	0.142 (0.112 -0.181)
0.000614	0.00119	0.279	0.658	0.0963	0.0896
1.591 (1.312 -1.979)	1.456 (1.192 -1.792)	1.898 (1.405 -2.564)	0.929 (0.618 -1.589)	0.878 (0.723 -1.083)	1.24 (0.996 -1.639)
0.000827	0.099	0.111	0.251	0.706	0.433
1.673 (1.316 -2.058)	1.234 (0.738 -1.43)	1.873 (0.92 -3.5)	0.83 (0.621 -1.216)	1.026 (0.795 -1.191)	1.477 (0.662 -2.239)

Table A.1 (continued)

CCC H12	CCC H2	CCC H7	D B12	D B4	D B7
0.00155	2.15E -06	0.0059	0.000185	0.00394	0.0807
28.44 (8.761 -157)	3.43 (2.787 -3.87)	3.517 (1.214 -8.501)	6.515 (4.378 -15.86)	2.552 (1.553 -5.593)	3.798 (1.224 -23.06)
0.0699	0.397	No reps.	0.78	No reps.	0.249
0.73 (0.484 -1.058)	1.154 (0.789 -1.573)	3.594	1.055 (0.627 -1.84)	2.128	0.824 (0.543 -1.29)
0.378	0.000538	0.0208	0.0756	0.000856	0.208
1.214 (0.837 -2.158)	1.534 (1.361 -1.926)	2.284 (1.749 -3.838)	1.618 (0.82 -2.571)	1.862 (1.42 -2.523)	0.898 (0.774 -1.258)
0.451	0.513	No reps.	0.00241	0.0427	0.212
1.129 (0.718 -1.652)	1.129 (0.701 -1.677)	29	1.887 (1.224 -2.62)	1.434 (1.07 -2.242)	0.921 (0.773 -1.128)
0.861	0.00476	0.978	0.196	0.54	0.00733
0.992 (0.884 -1.18)	1.513 (1.17 -2.216)	0.994 (0.603 -1.502)	2.496 (0.134 -4.328)	0.92 (0.598 -1.236)	0.761 (0.579 -0.896)
0.431	0.000499	0.000125	0.231	0.579	0.711
0.969 (0.874 -1.097)	1.964 (1.619 -2.318)	1.795 (1.555 -2.219)	1.155 (1.055 -1.369)	1.071 (0.799 -1.574)	1.029 (0.85 -1.324)

Table A.1 (continued)

D B8	D C10	D C5	D C7	D D10	D D12
0.143	0.00135	0.000978	0.0283	0.00023	0.00303
1.141 (0.999 -1.504)	2.676 (1.905 -5.012)	3.976 (2.998 -6.855)	1.801 (1.003 -2.991)	30.5 (13.14 -100)	7.955 (3.323 -26.73)
0.0128	0.465	0.64	0.338	0.067	0.00542
0.83 (0.827 -0.833)	1.186 (0.819 -1.544)	1.059 (0.894 -1.283)	0.717 (0.309 -1.167)	1.087 (0.982 -1.162)	1.492 (1.253 -1.955)
0.249	0.0657	0.00452	0.00428	0.00106	0.491
0.908 (0.731 -1.236)	1.524 (1.043 -3.156)	1.648 (1.245 -2.154)	1.846 (1.355 -3.2)	2.061 (1.37 -3.09)	1.076 (0.824 -1.559)
0.044	0.00829	0.397	0.388	0.0293	0.155
1.493 (1.079 -1.825)	2.187 (1.32 -3.611)	1.157 (0.795 -1.896)	0.899 (0.67 -1.066)	1.282 (0.974 -1.522)	1.463 (0.691 -2.817)
0.0591	0.0857	0.134	0.0748	0.0271	0.01
0.766 (0.612 -1.033)	0.591 (0.229 -1.203)	0.767 (0.515 -1.101)	0.775 (0.555 -1.03)	1.469 (0.995 -2.208)	1.744 (1.075 -2.294)
0.0493	0.232	0.0215	0.244	NO DATA	0.027
0.82 (0.807 -0.832)	0.727 (0.241 -1.201)	0.777 (0.629 -0.928)	0.838 (0.612 -1.416)	NO DATA	1.259 (0.891 -1.447)

Table A.1 (continued)

D D3	D D4	D D6	D D7	D D8	D D9
0.00537	0.000106	3.52E -05	0.000668	2.45E -05	4.01E -05
18.83 (7.053 -87)	4.276 (3.083 -7.805)	8.786 (6.29 -18.2)	4.983 (4.12 -6.087)	16.56 (10.75 -33.4)	5.55 (3.801 -8.584)
0.489	0.2	0.0118	0.297	0.938	0.0681
0.805 (0.236 -2.12)	0.516 (0.344 -1.038)	1.197 (1.091 -1.261)	1.339 (0.739 -2.077)	1.015 (0.582 -1.674)	0.697 (0.367 -1.121)
0.0124	0.00895	0.0178	0.353	0.438	0.00103
1.769 (1.093 -3.219)	1.981 (1.314 -3.009)	1.938 (0.881 -3.096)	1.718 (0.495 -3.813)	1.312 (0.554 -2.317)	1.953 (1.317 -2.548)
0.944	0.414	0.0157	0.0241	0.00138	0.0115
0.991 (0.724 -1.558)	1.335 (0.552 -2.16)	1.607 (0.971 -2.266)	2.324 (1.187 -7.217)	2.126 (1.588 -3.278)	1.565 (0.888 -1.903)
0.229	0.0496	0.127	0.016	0.021	0.396
0.82 (0.546 -1.144)	1.203 (1.006 -1.636)	1.484 (0.945 -2.226)	1.431 (1.12 -2.071)	1.321 (0.983 -1.712)	1.145 (0.617 -1.582)
0.0526	0.0129	0.323	0.345	0.0396	0.175
0.833 (0.692 -1.123)	0.742 (0.641 -0.953)	0.795 (0.378 -1.532)	0.855 (0.479 -1.302)	1.266 (1.086 -1.874)	1.117 (0.905 -1.431)

Table A.1 (continued)

D E1	D E10	D E11	D E3	D E5	D F12
0.0015	0.00082	0.00936	0.000938	4.01E -06	0.125
3.245 (1.822 -6.148)	20.14 (6.637 -82.65)	2.954 (1.263 -6.343)	8.917 (4.57 -20.46)	15.55 (11.71 -28.63)	1.931 (1.032 -4.486)
0.0538	0.796	0.626	0.0241	0.0926	NO DATA
1.387 (1.109 -2.545)	0.979 (0.742 -1.194)	1.121 (0.753 -1.427)	2.035 (1.026 -4.816)	2.059 (0.934 -6.224)	NO DATA
0.68	0.00815	0.339	0.577	0.0829	0.684
1.039 (0.777 -1.455)	1.67 (1.207 -2.352)	1.035 (0.938 -1.188)	1.393 (0.16 -3.239)	1.724 (0.523 -2.891)	1.069 (0.663 -1.474)
0.19	0.237	0.461	0.0033	0.0764	No reps.
1.291 (0.8 -2.07)	1.134 (0.924 -1.545)	0.957 (0.829 -1.183)	2.08 (1.512 -2.978)	2.987 (1.173 -8.695)	0.01*
0.000422	0.398	0.882	0.00477	0.0069	0.0425
2.147 (1.41 -2.652)	0.883 (0.633 -1.269)	0.961 (0.4 -2.244)	1.731 (1.143 -2.589)	1.53 (1.093 -2.112)	0.545 (0.524 -0.568)
0.104	0.583	0.00372	0.81	0.00203	0.683
1.153 (0.927 -1.481)	1.012 (0.95 -1.09)	0.613 (0.422 -0.81)	0.969 (0.663 -1.386)	1.974 (1.285 -2.721)	0.871 (0.622 -1.558)

Table A.1 (continued)

D F3	D H11	DDD B3	DDD C11	DDD C4	DDD D6
0.00128	0.000267	0.0638	0.642	0.365	0.00367
2.328 (1.626 -3.765)	4.438 (2.408 -7.071)	2.689 (1.23 -8.32)	0.877 (0.642 -1.712)	0.913 (0.732 -1.184)	4.947 (2.886 -11.76)
0.233	0.609	0.459	NO DATA	No reps.	0.54
1.26 (0.831 -1.965)	1.149 (0.476 -2.092)	1.099 (0.696 -1.513)	NO DATA	9	1.337 (0.752 -2.861)
0.729	0.283	0.781	0.304	0.517	0.00545
1.043 (0.862 -1.771)	1.134 (0.763 -1.44)	1.018 (0.802 -1.217)	1.26 (0.646 -2.926)	1.121 (0.889 -2.105)	2.781 (1.881 -4.81)
0.0777	0.203	0.831	0.204	0.145	0.0654
1.517 (0.743 -2.856)	0.843 (0.672 -1.277)	0.99 (0.895 -1.213)	8.196 (4.071 -16.5)	4.115 (1.675 -5.283)	2.541 (1.06 -4.255)
0.377	0.429	0.0426	0.68	No reps.	0.0144
0.78 (0.372 -1.555)	1.2 (0.504 -2.202)	0.86 (0.687 -1.005)	1.111 (0.597 -1.605)	0.933	1.504 (1.02 -1.702)
0.0282	0.0141	0.23	0.247	0.394	0.182
0.651 (0.422 -0.997)	0.559 (0.348 -0.833)	0.841 (0.451 -1.005)	1.213 (0.816 -1.861)	1.322 (0.844 -2.071)	1.182 (0.989 -1.737)

Table A.1 (continued)

DDD D9	DDD E10	DDD E4	DDD E8	DDD F5	DDD H5
0.0126	3.82E -05	0.000361	0.0337	0.00158	5.10E -05
1.68 (1.093 -2.435)	6.102 (3.648 -9.136)	5.786 (3.75 -8.648)	2.652 (1.665 -4.767)	3.26 (2.189 -4.729)	2.76 (2.033 -3.272)
NO DATA	0.24	0.163	NO DATA	0.207	0.492
NO DATA	1.509 (0.553 -4.893)	1.094 (1.04 -1.188)	NO DATA	1.508 (1.313 -1.731)	0.783 (0.34 -1.549)
0.00662	0.000435	0.00823	0.000505	0.00312	0.564
1.646 (1.152 -2.063)	1.858 (1.433 -2.54)	1.654 (0.984 -2.413)	2.022 (1.461 -2.534)	1.684 (1.154 -2.196)	1.093 (0.782 -1.454)
0.194	0.00891	0.0178	No reps.	0.0208	0.812
2.056 (0.999 -7.203)	2.087 (1.19 -3.924)	1.355 (0.916 -1.719)	3.025	1.629 (1.357 -2.201)	0.893 (0.22 -3.544)
0.000761	0.0255	0.396	0.0471	0.00113	0.00753
0.512 (0.438 -0.621)	1.355 (1.081 -1.908)	1.129 (0.909 -1.813)	1.236 (0.995 -1.567)	1.546 (1.299 -1.77)	0.405 (0.205 -0.676)
0.769	0.508	0.583	0.241	0.00505	0.00339
0.955 (0.735 -1.363)	0.934 (0.611 -1.12)	1.049 (0.768 -1.351)	1.161 (0.769 -1.568)	1.457 (1.17 -1.988)	0.606 (0.519 -0.81)

Table A.1 (continued)

E A11	E A12	E A6	E B4	E C11	E C3
0.0195	0.00151	0.0015	0.105	0.00117	0.7
0.637 (0.412 -0.856)	23.76 (4.804 -164)	2.656 (1.39 -3.846)	1.981 (1.767 -2.221)	4.661 (1.789 -8.564)	0.928 (0.636 -1.474)
0.188	0.904	0.253	No reps.	0.0155	No reps.
1.211 (0.784 -1.593)	1.011 (0.741 -1.225)	1.277 (1.003 -1.698)	0.01*	1.261 (1.092 -1.462)	5.273
0.0139	0.0749	0.00993	0.0776	0.627	0.00841
0.915 (0.856 -1.015)	1.161 (0.984 -1.581)	1.712 (1.155 -2.756)	1.613 (0.723 -2.103)	1.132 (0.697 -2.735)	1.615 (1.178 -2.58)
0.26	0.428	0.824	0.0294	0.301	0.0729
0.835 (0.603 -1.23)	1.146 (0.689 -1.563)	1.104 (0.267 -3.866)	3.256 (2.5 -4.894)	1.116 (0.878 -1.495)	1.476 (0.983 -1.932)
0.0684	0.000969	0.00143	0.251	0.0353	0.147
1.259 (0.937 -1.585)	1.868 (1.382 -2.493)	0.468 (0.342 -0.673)	0.68 (0.302 -0.944)	1.152 (1.035 -1.341)	0.814 (0.694 -1.277)
0.000771	0.145	0.0117	0.422	0.844	0.153
1.439 (1.227 -1.668)	1.118 (0.951 -1.457)	0.657 (0.496 -0.97)	1.232 (0.655 -2.499)	0.972 (0.64 -1.537)	0.725 (0.383 -1.362)

Table A.1 (continued)

E C4	E C6	E D1	E D10	E D8	E E12
0.0995	0.0132	7.09E -05	1.79E -06	0.000658	0.000104
1.599 (0.958 -4.448)	0.763 (0.708 -0.882)	5.553 (3.609 -8.851)	4.45 (3.725 -5.148)	44.38 (22.72 -129)	10.48 (6.601 -22.8)
NO DATA	No reps.	0.00315	0.036	0.29	0.444
NO DATA	0.01*	0.513 (0.408 -0.563)	0.73 (0.551 -0.993)	1.151 (0.995 -1.822)	0.919 (0.647 -1.199)
0.0451	0.749	0.00846	0.0152	0.0138	0.00787
0.896 (0.842 -1.1)	0.894 (0.516 -1.77)	1.775 (1.146 -2.601)	1.663 (0.888 -2.304)	1.865 (1.024 -3.328)	1.681 (1.194 -2.67)
0.567	0.385	0.212	0.0861	0.0991	0.126
0.9 (0.443 -1.294)	1.557 (0.791 -3.178)	1.338 (0.825 -2.7)	1.358 (0.849 -2.079)	1.542 (0.586 -2.818)	1.145 (0.862 -1.386)
0.0488	0.779	0.139	0.934	0.000707	0.517
0.527 (0.225 -1.009)	0.948 (0.537 -1.992)	0.818 (0.649 -1.169)	0.986 (0.636 -1.59)	2.061 (1.513 -2.639)	0.902 (0.587 -1.344)
0.00996	0.703	0.227	0.0517	0.435	0.322
0.534 (0.347 -0.995)	1.074 (0.598 -1.476)	0.916 (0.746 -1.03)	1.217 (0.913 -1.536)	1.103 (0.779 -1.621)	1.102 (0.794 -1.539)

Table A.1 (continued)

E E2	E E6	E E9	E F1	E G2	E G4
0.00048	0.122	0.0343	0.0121	0.00172	0.371
10.47 (7.391 -23.78)	1.582 (0.964 -2.687)	2.09 (0.921 -4.712)	0.602 (0.44 -1.018)	2.651 (1.415 -4.498)	1.148 (0.843 -1.555)
0.0049	No reps.	0.0544	NO DATA	0.335	No reps.
0.66 (0.55 -0.859)	6.5	0.618 (0.499 -0.974)	NO DATA	0.805 (0.401 -1.28)	2.068
0.0054	0.00905	0.733	0.0592	0.00829	0.00643
1.929 (1.358 -2.883)	1.542 (1.367 -1.891)	0.967 (0.68 -1.287)	0.621 (0.323 -0.988)	1.747 (1.299 -3.107)	1.478 (1.175 -2.089)
0.295	No reps.	0.195	No reps.	0.106	0.171
1.2 (0.764 -2.063)	0.01*	0.874 (0.619 -1.203)	0.01*	1.405 (0.979 -2.03)	1.689 (0.756 -3)
0.882	0.24	0.0515	0.0451	0.429	0.0305
0.988 (0.829 -1.339)	0.676 (0.578 -0.789)	0.595 (0.339 -0.902)	0.627 (0.606 -0.648)	0.878 (0.637 -1.276)	0.598 (0.396 -0.968)
0.0785	0.261	0.00687	0.915	0.357	0.348
0.809 (0.627 -1.064)	1.184 (1 -1.452)	0.523 (0.348 -0.775)	0.983 (0.731 -1.746)	0.921 (0.649 -1.195)	1.134 (0.722 -1.797)

Table A.1 (continued)

E G7	E H12	E H3	EE A11	EE A12	EE A8
0.466	0.703	2.56E -05	0.00045	0.00237	0.000471
0.672 (0.261 -1.893)	1.05 (0.805 -1.488)	3.918 (2.667 -5.057)	0.173 (0.0922 -0.383)	0.146 (0.0624 -0.61)	0.558 (0.442 -0.715)
0.102	No reps.	0.51	0.035	0.771	0.07
3.296 (2.717 -3.998)	0.795	0.754 (0.0493 -1.592)	1.232 (0.948 -1.489)	0.935 (0.628 -1.23)	1.519 (1 -2)
0.00061	0.64	0.119	0.000942	0.000971	0.00918
0.463 (0.321 -0.635)	0.901 (0.629 -1.581)	1.351 (0.945 -2.245)	0.476 (0.372 -0.645)	0.264 (0.137 -0.547)	0.487 (0.27 -0.617)
No reps.	No reps.	0.968	0.000504	0.000996	0.0579
0.804	0.01*	1.009 (0.501 -1.945)	0.56 (0.443 -0.701)	0.315 (0.169 -0.545)	0.441 (0.204 -1.115)
0.252	No reps.	0.0122	0.564	0.655	0.0621
1.426 (0.976 -2.109)	1.125	0.692 (0.484 -0.889)	0.876 (0.463 -1.719)	1.072 (0.807 -1.582)	1.388 (0.843 -2.168)
0.759	0.669	0.000272	0.206	0.0849	0.0314
0.926 (0.647 -1.812)	1.708 (0.672 -4.342)	0.581 (0.509 -0.762)	1.262 (0.72 -1.688)	1.469 (0.988 -2.626)	1.66 (1.114 -3.358)

Table A.1 (continued)

EE A9	EE B1	EE B3	EE B6	EE B7	EE B9
0.0171	0.000207	0.0165	0.0605	0.00148	4.42E -05
0.643 (0.488 -0.955)	12.24 (5.919 -27.79)	0.458 (0.218 -0.718)	0.434 (0.141 -0.914)	0.386 (0.303 -0.441)	0.287 (0.193 -0.392)
0.621	0.263	0.0546	0.563	NO DATA	0.663
0.693 (0.01 -1.503)	0.768 (0.336 -1.275)	0.78 (0.669 -1.094)	0.92 (0.51 -1.296)	NO DATA	1.257 (0.85 -1.857)
0.00293	0.0205	0.00721	0.0587	0.0123	0.0022
0.503 (0.27 -0.643)	1.609 (1.233 -2.663)	0.787 (0.685 -0.942)	0.816 (0.562 -0.971)	0.396 (0.18 -0.639)	0.382 (0.192 -0.534)
0.0051	0.397	0.128	0.274	0.0732	0.071
0.425 (0.25 -0.735)	1.12 (0.702 -1.552)	0.718 (0.427 -1.114)	0.909 (0.677 -1.185)	0.458 (0.234 -0.857)	0.407 (0.287 -0.666)
0.541	0.433	0.0129	0.0561	0.902	0.305
1.106 (0.7 -1.803)	0.886 (0.641 -1.237)	1.362 (1.084 -1.66)	1.213 (0.974 -1.556)	0.982 (0.618 -1.326)	0.821 (0.5 -1.393)
0.00578	0.372	0.197	0.0194	0.282	0.134
1.708 (1.074 -2.513)	1.065 (0.825 -1.338)	1.346 (0.65 -2.028)	1.172 (0.985 -1.36)	1.427 (0.761 -3.606)	1.403 (0.845 -2.604)

Table A.1 (continued)

EE C10	EE C2	EE C4	EE C8	EE C9	EE D10
0.0013	1.96E -05	0.00609	0.037	8.43E -05	4.78E -06
0.448 (0.329 -0.733)	0.321 (0.236 -0.377)	0.383 (0.26 -0.745)	3.603 (1.29 -17.82)	0.378 (0.25 -0.424)	0.171 (0.12 -0.212)
0.953	No reps.	0.923	0.0552	0.63	NO DATA
1.016 (0.634 -1.399)	1.957	0.972 (0.377 -1.812)	1.316 (0.914 -1.866)	0.874 (0.335 -1.474)	NO DATA
0.806	5.74E -06	0.000433	0.311	0.0383	0.000263
0.965 (0.734 -1.488)	0.363 (0.319 -0.438)	0.405 (0.34 -0.503)	1.126 (0.945 -1.729)	0.646 (0.38 -1.229)	0.603 (0.536 -0.737)
0.00605	0.172	0.00118	0.724	0.208	0.0723
0.61 (0.507 -0.698)	0.675 (0.453 -1.087)	0.619 (0.559 -0.664)	1.131 (0.272 -2.659)	0.777 (0.349 -1.135)	0.603 (0.417 -1.318)
0.257	0.376	0.826	0.554	0.109	0.04
1.131 (0.819 -1.403)	0.768 (0.385 -1.566)	0.977 (0.756 -1.203)	1.039 (0.846 -1.235)	1.157 (0.841 -1.356)	0.776 (0.629 -0.874)
0.897	0.768	0.0316	0.516	0.000708	0.447
1.017 (0.742 -1.711)	1.015 (0.918 -1.171)	1.743 (0.977 -2.423)	0.909 (0.52 -1.276)	1.6 (1.244 -1.853)	0.84 (0.439 -1.915)

Table A.1 (continued)

EE D11	EE D5	EE D9	EE E11	EE E3	EE E9
0.00369	0.0291	1.29E -05	1.44E -05	0.000324	0.00149
0.184 (0.088 -0.421)	0.555 (0.234 -0.856)	0.247 (0.203 -0.332)	4.143 (2.947 -5.313)	1.877 (1.426 -2.259)	0.463 (0.282 -0.608)
NO DATA	NO DATA	NO DATA	0.032	0.182	0.18
NO DATA	NO DATA	NO DATA	1.437 (1.192 -1.84)	0.801 (0.463 -0.94)	1.77 (1.5 -2.088)
0.00114	0.00361	0.000205	0.0152	0.0123	0.00717
0.332 (0.225 -0.45)	1.354 (1.149 -1.485)	0.455 (0.305 -0.526)	1.621 (0.913 -2.228)	1.289 (1.096 -1.7)	0.448 (0.232 -0.723)
0.00714	0.127	0.201	0.267	0.396	0.19
0.319 (0.204 -0.472)	1.305 (0.944 -1.73)	0.537 (0.189 -1.06)	1.194 (0.611 -1.67)	1.267 (0.521 -2.106)	0.552 (0.197 -0.907)
0.5	0.819	0.174	0.169	0.0374	0.253
0.787 (0.455 -1.62)	1.037 (0.748 -1.515)	0.783 (0.442 -1.002)	1.373 (0.683 -1.969)	1.347 (0.976 -1.785)	1.21 (0.826 -2.091)
0.0592	0.0667	0.076	0.0181	0.000599	0.00969
1.129 (1.116 -1.142)	1.681 (0.807 -2.685)	1.181 (1.106 -1.299)	1.597 (1.16 -2.963)	1.814 (1.442 -2.431)	1.49 (1.084 -2.033)

Table A.1 (continued)

EE F9	EE G1	EE G11	EE H10	EE H2	EE H3
0.0555	0.00731	4.05E -05	0.12	3.53E -05	0.000284
0.819 (0.635 -1.114)	3.55 (1.51 -12.94)	0.248 (0.219 -0.297)	0.703 (0.38 -1.081)	0.173 (0.148 -0.235)	0.393 (0.251 -0.51)
No reps.	0.302	No reps.	0.0181	0.878	No reps.
0.01*	0.856 (0.513 -1.229)	0.588	1.83 (1.558 -2.047)	0.966 (0.576 -1.738)	2
0.16	0.139	0.00203	0.00156	9.59E -05	0.113
0.812 (0.515 -1.058)	1.213 (0.899 -1.774)	0.316 (0.162 -0.456)	0.738 (0.619 -0.885)	0.334 (0.27 -0.458)	0.796 (0.58 -1.142)
No reps.	0.848	0.0158	0.412	0.00839	0.206
1.055	1.009 (0.908 -1.176)	0.558 (0.409 -0.96)	1.225 (0.645 -2.282)	0.338 (0.187 -0.621)	0.735 (0.435 -1.022)
0.329	0.0385	0.478	0.0349	0.564	0.653
1.237 (0.748 -2.062)	0.732 (0.563 -0.828)	0.949 (0.788 -1.134)	1.606 (1.099 -1.894)	1.131 (0.575 -1.818)	0.918 (0.587 -1.726)
0.158	0.0692	0.492	0.0149	0.208	0.648
1.815 (1.07 -2.591)	0.857 (0.696 -1.025)	0.868 (0.439 -1.253)	1.395 (1.155 -1.795)	1.608 (0.684 -3.552)	1.096 (0.647 -1.729)

Table A.1 (continued)

EE H4	EE H7	EE H8	EE H9	EEE B3	EEE B7
0.188	9.16E -05	0.000765	0.00701	0.0246	8.98E -05
0.756 (0.519 -1.354)	6.425 (4.997 -9.43)	0.241 (0.153 -0.347)	1.972 (1.403 -3.684)	7.312 (1.681 -83.48)	58.48 (19.48 -151)
No reps.	0.957	0.866	0.0612	0.034	0.00559
4.271	1.014 (0.408 -2.088)	0.963 (0.583 -1.516)	1.334 (1.003 -1.578)	0.802 (0.633 -0.998)	4.234 (2.375 -10.98)
0.0934	0.122	0.00117	0.00164	0.392	0.00142
0.865 (0.678 -1.058)	1.345 (1.02 -2.761)	0.471 (0.317 -0.634)	2.165 (1.42 -3.507)	1.091 (0.885 -1.48)	3.582 (2.149 -6.598)
0.642	0.812	0.0163	0.114	0.459	0.208
1.091 (0.888 -1.5)	0.969 (0.678 -1.438)	0.548 (0.373 -0.851)	1.287 (0.897 -1.703)	0.959 (0.809 -1.166)	1.264 (0.875 -2.234)
0.0616	0.839	0.192	0.00442	0.0275	0.0256
1.417 (0.932 -2.08)	1.026 (0.713 -1.583)	1.25 (1.026 -1.802)	1.63 (1.092 -2.117)	0.86 (0.763 -0.958)	2.543 (1.118 -6.315)
0.0257	0.835	0.0586	0.442	0.14	0.367
1.624 (1.053 -2.229)	0.981 (0.677 -1.246)	1.331 (1.015 -1.735)	0.844 (0.388 -1.665)	0.859 (0.574 -1.009)	0.818 (0.458 -1.296)

Table A.1 (continued)

EEE B9	EEE D4	EEE D6	EEE E3	EEE E8	EEE F8
0.00649	0.00158	0.0432	7.53E -05	0.00657	3.23E -05
20.48 (5.424 -169)	32.96 (5.45 -64.5)	1.949 (1.105 -4.583)	15.92 (9.497 -24.2)	6.543 (3.508 -13.37)	9.866 (6.946 -19.22)
0.234	0.0501	0.0917	0.000687	0.454	0.995
0.806 (0.496 -1.186)	1.463 (1.054 -2.195)	1.119 (0.868 -1.232)	2.231 (1.696 -3.192)	0.786 (0.639 -0.968)	1.003 (0.718 -1.401)
0.0968	0.022	0.215	0.000299	0.00441	0.0304
1.287 (0.967 -1.998)	2.544 (1.212 -4.591)	1.152 (0.84 -1.63)	2.919 (2.14 -4.393)	2.732 (1.75 -5.435)	2.005 (0.726 -3.541)
0.88	0.000952	0.64	0.00367	0.0514	0.26
1.019 (0.814 -1.781)	3.381 (2.44 -5.388)	1.037 (0.827 -1.314)	2.078 (1.3 -3.604)	1.605 (1.197 -2.088)	1.524 (0.488 -3.015)
0.0178	4.35E -05	0.000413	0.0393	0.601	0.00932
0.761 (0.596 -0.898)	2.825 (2.349 -3.675)	0.818 (0.762 -0.909)	1.468 (0.881 -2.3)	1.13 (0.516 -1.93)	1.819 (1.122 -2.739)
0.0151	0.419	0.657	0.0927	0.371	0.384
0.789 (0.618 -1.012)	1.074 (0.919 -1.44)	0.954 (0.669 -1.213)	1.255 (0.934 -2.022)	0.907 (0.626 -1.344)	0.934 (0.698 -1.106)

Table A.1 (continued)

EEE G9	EEE H11	F A6	F C4	F E10	F F10
0.0113	No reps.	0.000348	0.00166	0.00285	0.00116
2.612 (2.142 -3.029)	1.07	12.64 (7.868 -27)	3.689 (1.844 -8.757)	24.93 (4.727 -167)	4.001 (2.813 -6.481)
No reps.	0.459	0.278	No reps.	0.5	0.0333
1.129	1.078 (0.872 -1.444)	0.766 (0.363 -1.589)	2.335	1.091 (0.807 -1.818)	1.94 (1.696 -2.485)
0.0536	No reps.	0.000979	1.42E -05	0.0539	0.0105
2.106 (0.789 -4.516)	1.554	2.089 (1.324 -2.829)	2.56 (2.472 -2.686)	1.425 (1.052 -2.487)	2.057 (0.942 -3.497)
0.913	No reps.	0.122	0.0802	0.456	0.00174
0.931 (0.554 -1.565)	5	1.582 (0.802 -3.47)	1.455 (1.037 -2.119)	1.043 (0.885 -1.265)	2.041 (1.481 -2.459)
0.00895	0.000443	0.736	0.301	0.425	0.162
0.37 (0.143 -0.646)	0.508 (0.446 -0.628)	0.942 (0.624 -1.725)	0.887 (0.637 -1.132)	0.934 (0.763 -1.186)	1.125 (0.917 -1.371)
0.0931	No reps.	0.142	0.94	0.233	0.0652
0.665 (0.476 -1.354)	1.82	0.798 (0.489 -1.157)	1.014 (0.481 -1.801)	1.082 (0.937 -1.38)	1.287 (0.946 -1.985)

Table A.1 (continued)

F F3	F G6	F H11	FF A1	FF A10	FF A2
0.00396	0.168	0.0512	2.87E -06	0.000286	0.000535
2.134 (1.404 -4.059)	2.165 (1.482 -4.488)	3.003 (2.749 -3.282)	9.683 (7.095 -12.58)	5.389 (4.19 -6.057)	3.957 (1.989 -6.247)
NO DATA	No reps.	NO DATA	0.0109	0.527	0.00658
NO DATA	0.01*	NO DATA	1.605 (1.032 -2.417)	0.11 (0.01 -1.213)	0.868 (0.785 -0.964)
0.738	0.000947	0.0747	0.0123	0.00164	0.1
0.98 (0.801 -1.155)	1.189 (1.143 -1.207)	1.329 (1.001 -1.885)	1.891 (1.301 -3.802)	2.506 (1.866 -3.294)	1.428 (0.89 -2.386)
0.823	0.0485	No reps.	0.00343	0.0672	0.658
1.043 (0.521 -1.779)	1.744 (1.671 -1.819)	2.363	1.542 (1.165 -1.891)	1.862 (0.832 -3.463)	0.96 (0.678 -1.27)
0.69	0.231	0.0104	0.084	0.717	0.791
0.935 (0.564 -1.386)	0.735 (0.47 -1.348)	0.522 (0.516 -0.528)	1.378 (0.938 -2.235)	0.931 (0.681 -1.799)	0.957 (0.604 -1.687)
0.000216	0.367	0.0254	0.00464	0.000367	0.756
2.5 (1.709 -3.326)	0.766 (0.484 -1.522)	2.056 (1.664 -2.493)	1.41 (1.187 -1.658)	1.487 (1.31 -1.61)	0.979 (0.767 -1.188)

Table A.1 (continued)

FF B7	FF B8	FF C4	FF C5	FF E12	FF E4
0.00371	0.0675	0.0103	0.00793	0.00105	0.000192
19.35 (3.852 -119)	6.919 (5.632 -8.5)	10.84 (1.923 -36.5)	2.365 (1.067 -4.199)	4.816 (2.761 -13.6)	0.441 (0.31 -0.537)
0.761	0.392	0.93	0.553	0.66	0.464
1.03 (0.727 -1.309)	0.849 (0.655 -1.374)	1.018 (0.866 -1.196)	0.433 (0.161 -1.166)	1.302 (0.426 -5)	0.864 (0.502 -1.433)
0.071	0.364	0.0108	0.0848	0.00979	0.927
1.417 (1.027 -2.28)	2.732 (1.432 -5.213)	2.52 (1.663 -5.362)	1.363 (0.955 -2.512)	2.093 (1.442 -3.272)	0.992 (0.808 -1.377)
0.105	0.0159	0.00255	0.0417	0.0335	0.088
1.212 (1.038 -1.941)	2.127 (1.858 -2.563)	2.499 (2.09 -3.287)	1.399 (0.934 -2.149)	1.588 (0.914 -2.024)	0.872 (0.698 -1.087)
0.663	0.451	0.952	0.473	0.61	0.00648
0.948 (0.673 -1.484)	0.874 (0.558 -1.323)	0.993 (0.671 -1.561)	0.888 (0.618 -1.592)	0.929 (0.58 -1.267)	0.743 (0.55 -0.868)
0.555	0.667	0.0529	0.24	0.573	0.399
1.048 (0.762 -1.334)	1.082 (0.582 -1.442)	0.816 (0.646 -0.983)	0.933 (0.846 -1.067)	1.079 (0.749 -1.456)	0.888 (0.69 -1.11)

Table A.1 (continued)

FF E9	FF F10	FF G1	FF G9	FF H7	G A10
0.000465	0.000618	0.109	0.00639	0.00129	0.00406
0.161 (0.0991 -0.292)	22.67 (11.04 -50.5)	6.205 (1.777 -16.41)	0.549 (0.517 -0.604)	7.767 (3.296 -15.31)	0.499 (0.337 -0.664)
No reps.	0.786	0.195	NO DATA	0.127	0.859
1.951	1.072 (0.564 -1.66)	2.604 (1.924 -3.524)	NO DATA	1.364 (1.08 -1.636)	0.955 (0.451 -2.079)
0.00149	0.00544	0.0025	0.00478	0.0104	0.0324
0.37 (0.292 -0.539)	2.12 (1.165 -3.626)	2.356 (1.411 -3.521)	0.454 (0.231 -0.713)	4.103 (1.549 -20)	0.741 (0.578 -1.021)
0.0217	0.139	0.00955	No reps.	0.0361	0.00339
0.291 (0.121 -0.671)	1.511 (0.795 -4.043)	1.986 (1.396 -3.042)	1.009	1.794 (1.127 -2.888)	0.603 (0.435 -0.772)
0.358	0.109	4.26E -05	0.511	0.00689	0.0399
0.851 (0.489 -1.335)	1.684 (0.773 -4.22)	1.953 (1.692 -2.296)	1.851 (0.979 -3.5)	1.302 (1.021 -1.49)	1.461 (0.904 -2.024)
0.658	0.754	0.409	0.0793	0.00721	0.00358
1.101 (0.569 -1.709)	0.96 (0.671 -1.124)	0.835 (0.417 -1.208)	3.412 (2.926 -3.979)	1.465 (1.071 -1.853)	1.602 (1.251 -2.359)

Table A.1 (continued)

G A12	G B1	G B7	G C1	G C10	G C8
0.00105	0.768	3.18E -05	0.00101	5.64E -05	0.0763
16.18 (8.521 -22.72)	0.92 (0.559 -1.216)	0.117 (0.0888 -0.166)	3.106 (1.421 -4.213)	2.521 (2 -3.046)	4.51 (1.628 -34.24)
0.599	NO DATA	NO DATA	0.423	0.683	0.487
0.946 (0.732 -1.278)	NO DATA	NO DATA	0.0755 (0.01 -0.57)	1.068 (0.593 -1.826)	0.834 (0.313 -1.638)
0.0254	0.0577	0.015	0.000937	0.114	0.481
1.632 (0.911 -2.882)	1.334 (1.124 -1.749)	0.222 (0.0603 -0.442)	2.004 (1.665 -2.437)	1.357 (0.881 -2.027)	0.926 (0.697 -1.242)
0.0049	No reps.	0.0998	No reps.	0.633	0.777
1.457 (1.139 -1.88)	0.01*	0.115 (0.0332 -0.429)	1.854	1.268 (0.292 -4.351)	0.918 (0.328 -1.773)
0.0259	NO DATA	0.375	0.412	0.00918	0.0719
2.249 (0.915 -3.213)	NO DATA	0.816 (0.42 -1.784)	0.716 (0.239 -1.823)	0.418 (0.227 -0.728)	0.911 (0.797 -1.06)
0.00511	No reps.	0.813	0.124	0.000354	0.062
1.248 (1.021 -1.431)	1.221	0.907 (0.544 -2.751)	0.631 (0.324 -1.27)	0.48 (0.413 -0.56)	0.744 (0.48 -1.027)

Table A.1 (continued)

G D4	G E1	G E10	G E11	G E3	G E9
0.0188	0.00366	0.0202	0.00145	0.729	0.0142
0.753 (0.647 -1.104)	2.613 (1.897 -4.492)	3.079 (1.391 -12.12)	5.363 (2.439 -15.05)	1.123 (0.833 -2.017)	1.551 (1.135 -2.637)
No reps.	0.251	0.701	0.545	NO DATA	0.949
1	0.637 (0.528 -0.768)	1.049 (0.695 -1.429)	0.869 (0.448 -1.825)	NO DATA	1.011 (0.601 -1.588)
0.259	0.102	0.135	0.00886	0.127	0.00632
0.878 (0.704 -1.112)	1.329 (1.105 -2.228)	1.181 (0.93 -1.63)	1.616 (1.132 -2.115)	1.498 (1.123 -1.948)	1.466 (1.302 -1.921)
No reps.	0.22	0.0546	0.0441	No reps.	0.0937
0.493	1.521 (0.976 -3.343)	0.908 (0.838 -1.063)	1.254 (0.907 -1.707)	7.795	1.577 (1.046 -2.347)
0.77	0.941	0.486	0.984	0.252	0.228
1.032 (0.877 -1.376)	0.995 (0.845 -1.197)	0.964 (0.768 -1.065)	0.997 (0.61 -1.557)	1.18 (0.908 -1.78)	0.734 (0.417 -1.099)
0.0405	0.081	0.986	0.243	0.0436	0.0367
1.767 (1.479 -2.21)	0.702 (0.446 -1.148)	0.999 (0.811 -1.332)	0.875 (0.675 -1.33)	1.359 (0.922 -1.832)	0.703 (0.591 -0.931)

Table A.1 (continued)

G F10	G F11	G G11	G G12	G G2	G G5
0.0163	0.0514	0.00805	0.00812	0.000711	0.0278
10.87 (2.218 -119.4)	1.717 (1.028 -2.981)	1.787 (1.237 -2.764)	4.05 (1.529 -8.802)	2.085 (1.366 -2.835)	3.722 (2.329 -9.572)
0.0896	0.317	0.374	0.36	No reps.	0.67
0.704 (0.421 -1.171)	1.196 (0.939 -1.5)	1.312 (0.901 -2.794)	0.913 (0.754 -1.34)	0.01*	0.883 (0.392 -2.179)
0.591	0.0106	0.659	0.063	0.0627	1.18E -05
1.046 (0.912 -1.37)	1.756 (1.166 -3.287)	0.932 (0.514 -1.256)	1.36 (1 -2.285)	1.465 (0.901 -2.948)	2.103 (1.876 -2.407)
0.0417	0.0151	0.5	0.755	0.104	0.0343
0.852 (0.767 -1.126)	1.725 (1.025 -2.198)	1.067 (0.763 -1.5)	0.968 (0.748 -1.371)	1.486 (1.392 -1.586)	2.131 (1.2 -3.06)
0.081	0.0401	0.0031	0.863	0.495	0.985
0.798 (0.56 -0.994)	0.781 (0.575 -0.945)	0.655 (0.501 -0.826)	1.01 (0.892 -1.289)	0.837 (0.596 -2.136)	1.003 (0.568 -1.533)
0.0823	0.0179	0.0223	0.361	0.838	0.91
0.834 (0.612 -1.156)	0.597 (0.373 -0.913)	0.68 (0.459 -0.855)	1.072 (0.968 -1.509)	0.946 (0.458 -1.334)	1.019 (0.49 -1.461)

Table A.1 (continued)

G H3	G H5	G H8	GGG E8	GGG F2	GGG G1
0.00505	8.50E -06	0.00137	0.000506	0.0959	0.035
1.879 (1.387 -2.909)	11.19 (7.452 -19.63)	6.488 (4.118 -14.67)	2.78 (1.604 -4.178)	11.68 (1.634 -391)	11.71 (4.854 -24.5)
0.567	0.0194	0.618	0.922	0.855	0.517
1.303 (0.94 -1.808)	0.728 (0.539 -0.943)	1.075 (0.636 -1.3)	0.942 (0.58 -1.531)	0.973 (0.604 -1.536)	0.762 (0.396 -1.305)
0.00211	0.00479	0.0101	0.276	0.196	0.00351
1.788 (1.263 -2.633)	1.747 (1.136 -2.568)	1.899 (1.153 -3.222)	1.418 (0.831 -2.832)	1.132 (0.882 -1.641)	1.92 (1.245 -3.011)
0.332	0.306	0.0053	0.746	0.194	0.00155
1.322 (0.855 -1.73)	1.102 (0.884 -1.325)	1.411 (1.153 -1.748)	1.215 (0.26 -10.34)	1.114 (0.888 -1.405)	1.973 (1.531 -3.246)
0.833	0.793	0.89	0.0049	0.00943	0.336
1.017 (0.772 -1.226)	1.019 (0.857 -1.291)	0.983 (0.613 -1.402)	0.439 (0.253 -0.736)	0.757 (0.564 -0.901)	0.873 (0.591 -1.308)
0.304	0.0933	0.314	0.00119	0.167	0.686
1.071 (0.851 -1.307)	0.851 (0.641 -1.08)	1.092 (0.934 -1.241)	0.507 (0.382 -0.707)	0.824 (0.484 -1.165)	0.929 (0.507 -1.225)

Table A.1 (continued)

GGG G5	HH A12	HH A8	HH B10	HH C9	HH E8
0.768	0.00135	0.00108	0.0016	0.00865	0.00213
1.051 (0.589 -1.47)	66.62 (21.44 -252)	0.566 (0.436 -0.641)	11.2 (4.231 -41.09)	9.729 (4.116 -20.24)	23.03 (4.317 -50.5)
No reps.	0.527	NO DATA	0.0814	0.365	0.38
0.01*	1.066 (0.822 -1.427)	NO DATA	0.709 (0.431 -1.056)	0.718 (0.318 -1.286)	2.588 (0.508 -9)
0.18	0.186	0.296	0.533	0.0309	0.00704
0.811 (0.613 -1.375)	1.22 (0.707 -1.729)	1.212 (0.947 -1.89)	1.088 (0.818 -1.581)	1.989 (1.14 -4.387)	5.342 (0.82 -10.36)
0.0839	0.425	0.587	0.255	0.0233	0.475
0.788 (0.584 -1.047)	1.149 (0.688 -1.618)	0.852 (0.579 -1.36)	0.873 (0.716 -1.426)	1.825 (1.165 -3.58)	1.49 (0.183 -8.941)
0.0274	0.000598	0.0635	0.106	0.586	0.0168
0.437 (0.233 -0.807)	2.563 (1.534 -3.541)	1.296 (1.064 -1.607)	0.83 (0.603 -1.041)	0.87 (0.557 -1.615)	2.248 (1.15 -4.321)
0.0073	0.000139	0.0137	0.692	0.119	0.275
0.517 (0.262 -0.71)	1.557 (1.287 -1.709)	2.233 (2.194 -2.272)	0.962 (0.74 -1.265)	0.73 (0.384 -1.305)	1.234 (0.699 -1.654)

Table A.1 (continued)

II B5	II C4	II C6	II E5	II E7	II F5
0.00364	0.0118	0.0235	0.0955	0.00209	0.00862
0.466 (0.294 -0.614)	0.358 (0.182 -0.694)	1.624 (1.234 -3.333)	0.69 (0.509 -1.001)	9.42 (3.155 -42.45)	0.238 (0.148 -0.362)
NO DATA	NO DATA	0.335	NO DATA	0.743	NO DATA
NO DATA	NO DATA	0.78 (0.435 -1.224)	NO DATA	0.938 (0.762 -1.317)	NO DATA
0.155	0.000117	0.884	0.241	3.68E -05	0.00637
0.605 (0.22 -1.007)	0.375 (0.307 -0.509)	0.985 (0.791 -1.426)	1.253 (0.84 -1.786)	2.174 (1.87 -2.624)	0.485 (0.259 -0.742)
No reps.	0.293	0.294	No reps.	0.0017	0.0174
0.01*	0.263 (0.01 -0.561)	0.924 (0.761 -1.133)	0.01*	1.652 (1.256 -2.231)	0.154 (0.146 -0.162)
NO DATA	0.188	0.00142	0.554	0.926	No reps.
NO DATA	2.271 (0.991 -3.736)	0.624 (0.476 -0.791)	1.121 (0.913 -1.542)	1.015 (0.593 -1.675)	2.064
No reps.	0.0885	0.00772	No reps.	0.0136	0.5
1.562	3.215 (1.75 -6.327)	0.73 (0.605 -0.971)	0.909	1.386 (1.158 -2.031)	0.991 (0.983 -1)

Table A.1 (continued)

II G11	II G12	II H10	III A6	III B12	III C10
0.156	7.70E -05	0.00147	0.00137	0.0787	0.292
3.108 (0.0993 -5.202)	3.196 (2.11 -4.062)	5.443 (1.715 -12.21)	2.553 (1.31 -3.558)	0.604 (0.305 -1)	0.735 (0.522 -1.486)
0.159	0.0309	0.522	0.452	No reps.	NO DATA
1.452 (0.77 -3.508)	1.62 (1.004 -3.089)	1.875 (0.956 -3.677)	1.806 (1.087 -3)	1	NO DATA
0.00947	0.0697	0.00216	0.0237	0.155	0.409
1.475 (1.105 -1.917)	2.736 (0.99 -10.45)	2.961 (1.62 -3.845)	1.743 (1.021 -2.562)	1.347 (0.792 -2.658)	0.828 (0.406 -1.24)
0.509	0.922	0.0638	0.627	0.14	No reps.
1.036 (0.91 -1.158)	1.026 (0.482 -1.883)	1.806 (0.851 -3.883)	1.402 (0.292 -8.861)	0.537 (0.259 -1.185)	1.5
0.0195	0.464	0.00489	0.000181	0.413	0.375
1.347 (1.143 -1.786)	1.106 (0.75 -1.857)	1.769 (1.126 -2.497)	0.405 (0.296 -0.498)	1.097 (0.86 -1.478)	1.536 (1.153 -2.047)
0.645	0.114	0.0643	0.000505	0.000198	No reps.
0.914 (0.446 -1.385)	0.821 (0.611 -1.103)	1.248 (0.907 -1.714)	0.473 (0.372 -0.719)	0.403 (0.316 -0.484)	0.01*

Table A.1 (continued)

III C2	III C5	III D1	III D2	III E1	III E11
No reps.	0.00755	0.000136	9.33E -05	8.28E -05	0.00267
4.333	0.495 (0.363 -0.606)	27.56 (11.2 -77)	2.292 (1.815 -3.078)	4.41 (3.974 -4.944)	0.287 (0.231 -0.426)
NO DATA	NO DATA	0.0635	0.177	0.719	No reps.
NO DATA	NO DATA	0.69 (0.37 -0.899)	1.525 (0.858 -4.014)	0.905 (0.377 -1.57)	3.212
0.116	0.00111	0.0327	0.0228	0.0167	0.000506
1.129 (0.969 -1.335)	0.537 (0.516 -0.552)	1.809 (0.865 -3.388)	1.44 (1.066 -2.013)	1.344 (1.036 -1.631)	0.549 (0.414 -0.655)
No reps.	No reps.	0.0946	0.754	0.0649	0.183
12	0.187	1.334 (0.867 -2.149)	1.162 (0.252 -4.582)	1.115 (0.976 -1.301)	0.595 (0.377 -0.921)
NO DATA	NO DATA	0.336	0.00319	0.297	0.65
NO DATA	NO DATA	0.866 (0.575 -1.246)	0.446 (0.261 -0.653)	0.888 (0.632 -1.103)	0.934 (0.586 -1.244)
NO DATA	No reps.	0.0897	0.000457	0.123	0.422
NO DATA	1.498	0.793 (0.55 -1.033)	0.451 (0.337 -0.617)	1.1 (0.973 -1.332)	2.326 (1.203 -4.5)

Table A.1 (continued)

III E3	III E6	III F12	JJ B10	JJ B11	JJ C2
0.000415	9.09E -05	0.754	0.000237	0.000589	0.0266
4.824 (3.328 -9.849)	2.367 (1.752 -2.711)	0.949 (0.603 -1.397)	22.01 (12.72 -71.9)	5.968 (2.613 -14.84)	11.22 (5.303 -21)
0.348	0.248	No reps.	0.246	0.0878	NO DATA
0.597 (0.437 -0.817)	1.811 (0.918 -9)	4.064	0.729 (0.408 -1.325)	0.769 (0.523 -1.129)	NO DATA
0.0316	0.201	0.217	0.239	0.0178	0.00488
1.768 (1.318 -2.351)	1.407 (0.74 -2.424)	0.686 (0.552 -1.047)	1.162 (0.93 -1.694)	2.015 (1.194 -4.588)	2.346 (1.338 -4.155)
0.0309	0.962	No reps.	0.961	0.523	0.00831
1.499 (1.277 -2.042)	1.027 (0.236 -7.023)	0.01*	0.995 (0.743 -1.323)	1.057 (0.815 -1.355)	2.477 (1.766 -3.54)
0.841	0.00391	No reps.	0.146	0.236	0.928
1.034 (0.615 -1.835)	0.437 (0.264 -0.745)	1.473	0.793 (0.583 -0.961)	0.875 (0.65 -1.066)	0.982 (0.573 -1.275)
0.525	0.00182	0.5	0.762	0.451	0.272
1.093 (0.799 -1.671)	0.449 (0.289 -0.681)	1.533 (1 -2.349)	0.971 (0.737 -1.223)	0.936 (0.732 -1.221)	0.843 (0.463 -1.206)

Table A.1 (continued)

JJ C6	JJ D11	JJ D12	JJ D3	JJ D5	JJ D7
0.0752	2.47E -05	0.000614	2.61E -05	0.0603	0.00338
7.204 (1.362 -218.6)	4.174 (3.064 -5.9)	19.76 (9.739 -72.79)	2.2 (1.881 -2.623)	2.024 (1.114 -5.933)	6.389 (2.798 -14.5)
0.129	0.0241	0.0903	0.587	0.013	0.941
0.885 (0.721 -1.07)	0.738 (0.551 -0.89)	0.852 (0.705 -1.089)	0.892 (0.496 -1.861)	0.82 (0.695 -0.93)	1.033 (0.499 -1.814)
0.117	0.265	0.197	0.133	0.475	0.0662
1.27 (0.87 -1.915)	1.287 (0.994 -1.746)	1.468 (0.809 -2.822)	1.291 (0.82 -1.947)	1.077 (0.822 -1.529)	1.324 (0.921 -1.689)
0.369	0.102	0.208	0.979	0.628	0.833
1.075 (0.859 -1.315)	1.155 (0.975 -1.603)	1.25 (0.844 -2.22)	1.011 (0.333 -3.143)	1.016 (0.943 -1.134)	1.078 (0.594 -1.714)
0.000989	0.0788	0.3	0.00168	0.00154	0.101
0.741 (0.608 -0.82)	0.845 (0.725 -1.155)	0.901 (0.765 -1.265)	0.477 (0.323 -0.705)	0.749 (0.673 -0.89)	0.737 (0.38 -1.055)
0.0395	0.523	0.0823	0.00614	0.0162	0.966
0.85 (0.665 -1.014)	0.884 (0.554 -1.24)	0.789 (0.582 -1.094)	0.525 (0.402 -0.827)	0.916 (0.851 -0.999)	0.994 (0.612 -1.458)

Table A.1 (continued)

JJ E10	JJ E11	JJ E2	JJ E9	JJ G10	JJ G12
0.000704	6.63E -05	2.11E -05	0.000116	0.000344	0.00127
30.31 (10.4 -129)	12.05 (9.025 -20.78)	9.948 (6.682 -14.82)	22.58 (11.71 -64)	5.116 (3.644 -12.82)	3.245 (1.505 -4.925)
0.561	0.607	0.582	0.0709	0.201	0.506
0.917 (0.561 -1.245)	0.942 (0.695 -1.096)	0.893 (0.398 -1.402)	0.747 (0.484 -1.1)	0.768 (0.331 -1.103)	1.444 (0.993 -2.101)
0.0459	0.00132	0.226	0.00469	0.00716	0.0146
1.565 (1.015 -3.159)	1.919 (1.306 -2.452)	1.164 (0.786 -1.799)	1.593 (1.242 -2.071)	1.588 (1.29 -2.66)	2.015 (0.917 -2.73)
0.401	0.0136	0.815	0.202	0.0263	0.0244
1.158 (0.813 -1.857)	1.376 (1.141 -2.075)	0.975 (0.737 -1.317)	1.173 (0.832 -1.654)	1.377 (1.106 -1.798)	2.769 (1.3 -5.441)
0.00531	0.000299	0.0066	0.616	0.00224	0.641
0.804 (0.7 -0.9)	2.191 (1.739 -3.02)	0.611 (0.39 -0.845)	0.947 (0.75 -1.309)	0.805 (0.687 -0.902)	0.935 (0.634 -1.627)
0.0757	0.0067	0.00714	0.174	0.0932	0.743
0.801 (0.608 -1.065)	1.25 (1.103 -1.388)	0.697 (0.604 -0.832)	0.906 (0.726 -1.141)	1.059 (0.963 -1.186)	1.06 (0.716 -1.666)

Table A.1 (continued)

JJ G2	JJ H2	JJ H4	JJ H5	K A12	K A3
0.000141	0.0129	0.00304	0.182	0.0211	No reps.
9.664 (6.081 -16.24)	20.9 (4.781 -308.5)	3.596 (1.943 -6.363)	0.792 (0.461 -1.17)	0.363 (0.276 -0.462)	3.671
0.756	0.12	0.165	No reps.	NO DATA	NO DATA
1.108 (0.757 -1.95)	0.774 (0.558 -1.013)	2.214 (1.29 -4.5)	0.01*	NO DATA	NO DATA
0.0017	0.131	0.325	0.614	0.00279	0.01
2.134 (1.445 -3.64)	1.43 (0.79 -2.475)	1.152 (0.925 -1.288)	1.065 (0.712 -1.342)	0.298 (0.16 -0.729)	1.96 (1.293 -2.989)
0.00233	0.37	0.0124	0.947	No reps.	No reps.
1.746 (1.286 -2.035)	1.158 (0.865 -2.006)	4.75 (4.608 -4.896)	0.961 (0.273 -9.666)	0.01*	7.947
0.0218	0.00106	0.214	0.00952	No reps.	0.103
0.793 (0.568 -0.909)	0.776 (0.68 -0.863)	1.183 (0.864 -1.838)	0.636 (0.455 -0.941)	0.793	0.175 (0.131 -0.232)
0.584	0.00539	0.0283	0.0534	No reps.	0.199
0.871 (0.444 -1.528)	0.744 (0.601 -0.935)	1.524 (1.244 -1.944)	0.64 (0.283 -1.023)	1.5	0.627 (0.389 -0.883)

Table A.1 (continued)

K A7	K B1	K B7	K C2	K C6	K C7
0.000182	0.857	0.00253	0.00198	0.0455	0.000242
15.01 (10.97 -18.72)	1.038 (0.82 -1.811)	2.639 (1.5 -5.003)	1.798 (1.251 -2.529)	1.763 (0.982 -3.209)	7.039 (3.396 -15.55)
0.294	0.576	No reps.	0.372	0.661	0.51
1.152 (0.947 -1.518)	1.144 (0.964 -1.357)	1.529	1.977 (0.997 -6.5)	1.162 (0.9 -1.5)	1.113 (0.796 -1.649)
0.00394	0.0134	0.000594	0.533	0.0114	0.00199
2.379 (1.394 -4.388)	0.337 (0.137 -0.877)	1.793 (1.49 -2.344)	1.027 (0.931 -1.158)	1.407 (1.039 -1.957)	1.517 (1.074 -1.696)
0.0049	0.00038	0.0636	0.914	0.854	0.151
1.484 (1.224 -2.097)	0.167 (0.119 -0.281)	2.239 (1.178 -4.178)	0.982 (0.524 -1.335)	1.072 (0.59 -1.878)	1.318 (0.736 -2.195)
0.065	0.0112	0.478	0.0109	0.0092	0.00774
1.524 (0.724 -2.397)	0.183 (0.104 -0.432)	1.082 (0.749 -1.469)	0.611 (0.475 -0.957)	0.572 (0.364 -0.726)	1.243 (1.02 -1.419)
0.00194	NO DATA	0.387	0.000904	0.0855	0.0436
1.532 (1.263 -1.95)	NO DATA	0.855 (0.484 -1.47)	0.498 (0.384 -0.599)	0.757 (0.441 -1.074)	1.38 (0.807 -1.961)

Table A.1 (continued)

K D3	K D5	K E12	K E9	K F12	K F6
0.000526	5.14E -05	0.0179	0.0449	0.0456	0.00144
2.806 (1.809 -3.638)	3.088 (2.398 -3.826)	2.316 (1.44 -5.309)	1.498 (1.042 -2.306)	0.594 (0.289 -1.091)	7.484 (3.006 -18.98)
No reps.	0.689	NO DATA	0.226	0.274	0.334
3.364	0.94 (0.581 -1.489)	NO DATA	0.644 (0.321 -1.715)	2.14 (1.51 -3.035)	0.746 (0.256 -1.849)
0.0518	0.0698	0.0258	0.118	0.00115	0.143
1.432 (1.068 -2.421)	1.308 (0.81 -1.796)	1.268 (0.967 -1.668)	1.393 (0.598 -2.095)	0.307 (0.129 -0.421)	1.245 (0.984 -1.975)
0.00527	0.84	0.172	0.17	0.104	0.276
1.674 (1.227 -2.036)	0.954 (0.476 -1.534)	0.784 (0.512 -1.224)	0.78 (0.457 -1.254)	0.853 (0.831 -0.876)	0.866 (0.599 -1.13)
0.139	0.1	0.565	0.0164	0.613	0.0674
0.411 (0.107 -2.028)	0.813 (0.635 -1.251)	0.899 (0.548 -1.699)	1.907 (1.281 -2.817)	1.146 (0.767 -3.054)	0.768 (0.564 -1.06)
0.0197	0.275	0.232	0.0225	0.312	0.629
0.593 (0.305 -0.954)	0.871 (0.603 -1.372)	0.893 (0.744 -1.16)	1.513 (1.17 -2.217)	1.219 (0.959 -1.596)	0.953 (0.702 -1.214)

Table A.1 (continued)

K F8	K G10	K G2	K H1	K H10	K H8
5.51E -05	0.00103	0.00167	0.00043	0.00115	2.33E -06
0.487 (0.385 -0.56)	2.837 (1.902 -4.76)	5.202 (1.732 -13.39)	0.072 (0.0363 -0.117)	0.0995 (0.0656 -0.163)	0.312 (0.258 -0.364)
0.158	0.117	0.261	0.542	0.303	NO DATA
0.0421 (0.01 -0.747)	0.604 (0.444 -0.852)	0.881 (0.703 -1.248)	0.822 (0.372 -1.876)	1.857 (1.349 -2.555)	NO DATA
0.00129	0.00297	0.0346	0.000285	0.000101	1.13E -06
0.516 (0.421 -0.821)	1.353 (1.118 -1.655)	1.407 (1.033 -2.158)	0.302 (0.177 -0.47)	0.21 (0.149 -0.326)	0.391 (0.347 -0.442)
No reps.	0.816	0.408	0.0473	0.0205	0.36
0.578	1.034 (0.677 -1.557)	1.111 (0.751 -1.671)	0.444 (0.325 -0.928)	0.264 (0.0783 -0.707)	0.512 (0.335 -0.783)
0.478	0.0421	0.596	0.837	0.775	0.511
0.889 (0.512 -1.242)	1.262 (0.859 -1.527)	1.041 (0.759 -1.263)	0.964 (0.589 -1.377)	1.073 (0.613 -2.356)	1.229 (0.554 -2.432)
0.186	0.907	0.328	0.623	0.133	0.511
0.749 (0.481 -1.173)	1.005 (0.896 -1.137)	0.899 (0.571 -1.071)	0.955 (0.693 -1.14)	1.38 (0.787 -1.917)	1.336 (0.99 -1.805)

Table A.1 (continued)

KK A6	KK A7	KK B10	KK B5	KK B7	KK C10
0.00135	0.0945	0.00259	6.22E -05	6.32E -05	0.00107
3.975 (1.714 -6.628)	3.401 (1.382 -22.2)	3.24 (1.633 -7.258)	0.209 (0.15 -0.321)	4.875 (3.478 -7.535)	5.075 (2.281 -12.84)
0.21	0.112	0.227	0.141	0.301	0.169
1.257 (0.67 -1.94)	0.876 (0.761 -1.091)	0.706 (0.393 -1.086)	0.59 (0.381 -0.795)	0.789 (0.356 -1.657)	0.742 (0.367 -0.968)
7.25E -05	0.145	0.000957	0.00569	0.0124	0.000925
1.919 (1.551 -2.219)	1.172 (0.933 -1.561)	1.783 (1.353 -2.405)	0.439 (0.245 -0.913)	1.192 (1.021 -1.363)	1.938 (1.395 -2.851)
0.00749	0.739	0.0514	0.0463	0.605	0.0203
1.434 (1.02 -1.723)	0.989 (0.921 -1.115)	1.898 (1.285 -3.1)	0.622 (0.375 -1.252)	1.041 (0.767 -1.253)	1.434 (0.928 -1.991)
0.00447	0.0173	0.565	0.0628	0.942	0.505
1.506 (1.263 -1.797)	0.838 (0.692 -0.962)	1.09 (0.842 -1.801)	0.539 (0.256 -0.906)	1.008 (0.732 -1.284)	0.919 (0.726 -1.543)
0.000155	0.771	0.243	0.0152	0.675	0.114
1.818 (1.506 -2.294)	0.968 (0.777 -1.5)	1.153 (0.814 -1.653)	0.739 (0.578 -0.836)	0.97 (0.792 -1.157)	0.726 (0.402 -1.11)

Table A.1 (continued)

KK C4	KK D2	KK D8	KK D9	KK E4	KK E5
0.000277	0.038	0.0209	0.000307	0.000355	0.0127
0.411 (0.325 -0.647)	1.907 (0.994 -4.438)	2.243 (1.272 -3.951)	2.67 (1.722 -3.623)	5.271 (4.18 -6.298)	2.059 (1.023 -3.34)
NO DATA	0.364	NO DATA	NO DATA	0.719	0.00151
NO DATA	1.269 (0.774 -2)	NO DATA	NO DATA	0.896 (0.641 -1.516)	2.075 (1.438 -2.942)
0.0577	0.632	0.26	0.00335	0.00551	0.0134
0.494 (0.17 -0.665)	1.079 (0.874 -1.393)	1.245 (0.683 -1.92)	2.419 (1.535 -4.236)	2.443 (1.667 -5.974)	1.587 (1.165 -2.11)
0.301	0.554	0.175	No reps.	0.101	0.361
0.611 (0.271 -0.81)	0.891 (0.452 -1.303)	17.18 (7.694 -38.38)	1.885	1.552 (1.159 -2.686)	1.159 (0.829 -1.749)
0.41	0.0306	0.502	0.631	0.923	0.0902
1.219 (0.689 -2.522)	0.688 (0.475 -1.087)	0.69 (0.247 -2.291)	1.061 (0.749 -1.428)	0.987 (0.622 -1.399)	1.219 (0.922 -1.622)
0.118	0.00857	No reps.	0.978	0.896	0.748
1.531 (0.97 -2.292)	0.494 (0.232 -0.797)	0.373	0.995 (0.655 -1.6)	0.97 (0.687 -1.408)	1.022 (0.842 -1.23)

Table A.1 (continued)

KK F8	KK G6	KK G8	KK H10	KK H3	L A10
0.152	0.000672	1.19E -06	0.00514	0.225	0.000496
7.619 (4.643 -12.5)	0.433 (0.291 -0.646)	0.404 (0.358 -0.444)	0.425 (0.305 -0.682)	1.354 (0.774 -1.827)	5.811 (2.061 -9.278)
0.375	No reps.	NO DATA	NO DATA	NO DATA	0.18
0.563 (0.383 -0.827)	0.786	NO DATA	NO DATA	NO DATA	1.276 (0.869 -2.618)
0.00845	0.000552	0.934	0.0259	0.222	0.0111
2.282 (1.182 -5.229)	0.396 (0.296 -0.617)	0.983 (0.731 -2.42)	0.817 (0.704 -0.98)	0.76 (0.309 -1.09)	1.706 (1.282 -2.363)
0.00482	0.0169	0.631	0.412	No reps.	0.529
3.165 (2.429 -4.627)	0.373 (0.206 -0.864)	0.946 (0.869 -1.03)	0.864 (0.705 -1.135)	0.164	1.11 (0.681 -1.567)
0.706	0.993	0.68	0.205	0.165	0.942
0.891 (0.357 -1.692)	0.998 (0.488 -1.578)	0.945 (0.738 -1.523)	0.816 (0.657 -1.3)	1.17 (0.799 -1.567)	1.007 (0.772 -1.372)
0.856	0.667	0.737	0.217	0.0371	0.0188
0.955 (0.316 -1.603)	1.139 (0.464 -2.57)	1.048 (0.872 -1.526)	1.244 (1.151 -1.344)	1.515 (1.114 -2.829)	1.328 (0.948 -1.63)

Table A.1 (continued)

L A5	L B10	L B11	L B5	L C1	L C12
0.00309	0.015	0.000116	0.000574	0.00296	0.0155
2.059 (1.467 -3.285)	8.63 (2.376 -88.51)	2.811 (2.001 -3.79)	2.731 (2.111 -3.615)	7.168 (1.851 -27.04)	4.063 (1.819 -6.729)
No reps.	0.17	0.183	No reps.	0.235	0.0821
0.859	0.762 (0.488 -1.211)	0.729 (0.345 -1.084)	1.12	0.825 (0.568 -0.99)	2.185 (1.526 -3.436)
0.012	0.252	0.0412	0.00243	0.00124	0.00136
1.297 (1.088 -1.519)	1.205 (0.882 -1.964)	1.237 (0.881 -1.445)	1.325 (1.132 -1.639)	1.958 (1.51 -2.927)	2.234 (1.329 -3.276)
0.106	0.957	0.202	0.7	0.00126	0.0161
0.82 (0.583 -1.019)	1.004 (0.871 -1.379)	1.292 (0.842 -2.379)	0.943 (0.497 -1.472)	1.591 (1.25 -1.961)	1.514 (1.165 -2.141)
0.559	0.0475	0.0591	0.00246	0.24	0.283
1.045 (0.925 -1.45)	0.766 (0.595 -0.993)	0.659 (0.319 -0.916)	1.446 (1.207 -1.916)	0.85 (0.576 -1.176)	1.1 (0.954 -1.43)
0.0385	0.242	0.000182	0.000119	0.511	0.309
1.356 (0.989 -1.778)	0.866 (0.662 -1.309)	0.608 (0.537 -0.717)	1.527 (1.353 -1.722)	0.914 (0.646 -1.395)	1.132 (0.767 -1.435)

Table A.1 (continued)

L C2	L C3	L C4	L C6	L D1	L D10
0.00297	0.000203	0.00245	0.000866	0.00709	0.0354
0.473 (0.384 -0.681)	4.882 (3.642 -10.88)	8.616 (2.465 -30.46)	3.99 (2.489 -8.106)	2.458 (1.372 -4.872)	5.063 (1.742 -25.56)
0.461	0.124	0.104	0.279	0.229	0.232
0.981 (0.965 -0.998)	0.716 (0.39 -1.159)	1.24 (0.898 -1.8)	0.647 (0.528 -0.793)	0.704 (0.265 -0.969)	0.735 (0.369 -1.218)
4.08E -06	0.0329	0.0192	0.049	0.0162	0.413
0.489 (0.418 -0.517)	1.939 (1.326 -2.893)	1.678 (0.874 -2.436)	1.42 (1.048 -2.182)	1.674 (0.84 -2.195)	1.197 (0.746 -2.163)
0.174	0.0591	0.698	0.00641	0.258	0.818
0.76 (0.5 -1.356)	1.324 (0.821 -1.873)	1.074 (0.661 -2.293)	1.155 (1.062 -1.255)	1.124 (0.851 -1.462)	1.034 (0.758 -1.623)
0.883	0.87	0.412	0.207	0.0302	0.0122
0.971 (0.433 -1.503)	1.034 (0.509 -1.506)	1.411 (0.337 -2.934)	0.962 (0.87 -1.008)	0.693 (0.466 -0.981)	0.745 (0.522 -0.899)
0.103	0.295	0.902	0.0365	0.00811	0.00483
1.169 (0.837 -1.424)	0.908 (0.644 -1.154)	1.018 (0.523 -1.317)	0.7 (0.415 -0.951)	0.777 (0.65 -0.978)	0.771 (0.661 -0.928)

Table A.1 (continued)

L D11	L D3	L D7	L E11	L E12	L E6
0.00214	6.54E -05	0.0462	0.000187	0.00209	0.00249
0.552 (0.455 -0.705)	4.833 (3.478 -8.425)	2.848 (1.369 -14.78)	0.036 (0.0116 -0.0869)	0.478 (0.379 -0.66)	0.507 (0.367 -0.617)
0.0341	0.992	0.797	0.715	0.48	0.494
1.404 (1.278 -1.588)	1.002 (0.358 -1.633)	0.942 (0.37 -1.811)	0.909 (0.581 -1.234)	1.473 (1.024 -2.119)	1.074 (0.684 -1.336)
0.0237	0.00141	0.802	0.0235	0.000646	0.676
0.734 (0.505 -0.911)	1.803 (1.296 -2.595)	1.03 (0.752 -1.455)	0.114 (0.0107 -0.296)	0.652 (0.58 -0.724)	0.957 (0.776 -1.487)
0.0397	0.113	0.108	0.024	0.371	0.0107
0.723 (0.557 -1.143)	1.153 (0.977 -1.546)	0.875 (0.693 -1.124)	0.286 (0.272 -0.299)	0.5 (0.316 -0.789)	0.945 (0.896 -0.985)
0.511	0.0054	0.0179	0.931	0.264	0.0183
0.951 (0.807 -1.263)	1.475 (1.114 -2.012)	0.782 (0.56 -0.908)	1.054 (0.389 -3.324)	0.868 (0.651 -1.053)	0.929 (0.847 -0.981)
0.105	0.000252	0.00474	0.713	0.82	0.643
1.223 (0.91 -1.671)	1.394 (1.254 -1.596)	0.799 (0.67 -0.945)	0.94 (0.705 -1.125)	1.044 (0.754 -1.306)	1.072 (0.611 -1.8)

Table A.1 (continued)

L E9	L F2	L G6	L G9	L H10	L H12	L H2
0.00411	0.0115	0.0108	0.000664	0.000254	0.00203	0.00188
0.286 (0.144 -0.435)	3.611 (2.398 -11.41)	2.077 (1.031 -3.754)	0.303 (0.184 -0.451)	6.955 (3.63 -15.08)	3.452 (2.039 -5.828)	0.152 (0.0339 -0.312)
0.587	0.529	0.265	0.413	0.291	No reps.	0.569
0.746 (0.178 -1.502)	0.869 (0.672 -1.246)	1.637 (1.316 -2.036)	0.84 (0.46 -1.381)	1.353 (1.041 -2.062)	1.641	1.181 (0.96 -1.452)
0.000128	0.00671	0.00396	0.0477	0.00127	0.00706	6.16E -05
0.535 (0.459 -0.662)	1.581 (0.962 -1.984)	1.928 (1.305 -2.909)	0.822 (0.659 -1.135)	2.316 (1.81 -3.059)	1.85 (1.22 -2.62)	0.356 (0.255 -0.48)
0.312	0.0351	0.0149	0.28	0.0181	0.699	0.317
0.769 (0.366 -1.503)	1.454 (1.114 -1.797)	1.515 (1.067 -1.844)	0.914 (0.683 -1.188)	2.491 (1.056 -3.682)	0.797 (0.454 -2.201)	0.491 (0.333 -0.723)
0.405	0.0118	0.269	0.0728	0.399	0.269	0.33
1.488 (0.0659 -2.701)	0.624 (0.457 -0.804)	1.105 (0.804 -1.383)	0.805 (0.626 -1.069)	1.102 (0.769 -1.555)	0.791 (0.418 -1.242)	0.715 (0.325 -1.536)
0.344	0.0314	0.129	0.798	0.154	0.31	No reps.
1.207 (0.703 -2.057)	0.697 (0.456 -1.04)	0.834 (0.646 -1.153)	0.956 (0.422 -1.271)	0.886 (0.686 -1.08)	0.814 (0.477 -1.39)	1.451

Table A.1 (continued)

L H9	LL A11	LL A9	LL C10	LL C3	LL D1
0.000332	0.0238	0.162	0.14	0.0736	0.112
0.0345 (0.0168 -0.0633)	0.663 (0.471 -1.17)	1.547 (0.857 -4.839)	1.516 (0.899 -2.485)	0.662 (0.566 -0.835)	1.929 (0.691 -4.409)
0.775	NO DATA	0.0977	NO DATA	No reps.	NO DATA
1.37 (0.583 -3.221)	NO DATA	0.536 (0.486 -0.59)	NO DATA	3	NO DATA
0.000216	0.00612	0.0841	0.827	0.0586	0.126
0.317 (0.21 -0.445)	0.836 (0.808 -0.903)	0.855 (0.737 -1.132)	1.038 (0.662 -1.884)	0.616 (0.333 -0.861)	1.436 (0.812 -2.449)
0.032	No reps.	0.0337	0.0757	No reps.	No reps.
0.418 (0.258 -0.646)	0.528	0.682 (0.499 -0.902)	1.312 (0.994 -1.554)	0.01*	0.01*
0.158	0.0212	0.0813	0.0267	0.79	0.0129
0.732 (0.335 -1.127)	1.354 (1.236 -1.656)	0.545 (0.225 -1.189)	0.314 (0.0911 -0.689)	1.09 (0.785 -1.925)	3.804 (2.255 -11)
0.463	No reps.	0.00526	0.288	NO DATA	0.15
0.89 (0.552 -1.393)	2.244	0.344 (0.198 -0.831)	0.7 (0.357 -2.002)	NO DATA	1.326 (0.98 -2.326)

Table A.1 (continued)

LL D5	LL E1	LL E12	LL E2	LL E4	LL E7
0.000221	0.00239	0.00012	0.0194	2.32E -05	0.0444
0.0562 (0.0263 -0.103)	0.268 (0.177 -0.796)	11.55 (4.709 -26.84)	0.619 (0.424 -0.893)	11.71 (9.275 -17.28)	1.326 (0.925 -1.943)
NO DATA	0.327	0.0436	No reps.	0.224	No reps.
NO DATA	0.91 (0.721 -1.33)	0.801 (0.561 -0.965)	0.01*	1.176 (0.75 -1.684)	1.257
0.0884	0.0434	0.0164	0.348	0.000104	0.722
0.0579 (0.01 -0.209)	0.777 (0.516 -0.937)	1.921 (1.29 -4.382)	1.143 (0.81 -1.421)	1.61 (1.43 -1.937)	1.047 (0.741 -1.552)
0.0757	0.0201	0.28	No reps.	0.0208	0.0442
0.213 (0.0734 -0.687)	0.664 (0.432 -0.858)	1.212 (0.749 -2.337)	0.687	1.187 (0.948 -1.346)	1.514 (0.959 -2.871)
0.911	0.181	0.885	0.927	0.00534	0.0539
1.031 (0.442 -2.25)	0.883 (0.696 -1.096)	0.987 (0.725 -1.26)	0.983 (0.822 -1.359)	1.37 (1.053 -1.762)	0.644 (0.327 -1.18)
No reps.	0.309	0.29	0.449	0.0213	0.0087
1.705	0.839 (0.453 -1.261)	0.958 (0.836 -1.076)	1.285 (0.785 -2.495)	1.196 (1.039 -1.431)	0.674 (0.514 -0.986)

Table A.1 (continued)

LL F12	LL G10	LL G12	LL G5	M A11	M A12
0.0146	0.418	0.00389	0.0194	3.75E -06	0.0707
0.515 (0.508 -0.523)	0.862 (0.617 -1.899)	4.17 (2.711 -5.815)	0.826 (0.79 -0.936)	0.271 (0.213 -0.312)	0.477 (0.196 -0.945)
NO DATA	No reps.	0.122	No reps.	0.957	0.197
NO DATA	9.5	1.876 (1.159 -2.531)	0.01*	1.017 (0.703 -2.355)	1.159 (0.837 -1.734)
0.574	0.129	0.0942	0.0178	0.00196	0.156
1.151 (0.722 -2.688)	1.375 (0.932 -3.195)	1.519 (0.62 -2.379)	0.836 (0.718 -0.984)	0.435 (0.246 -0.613)	0.754 (0.438 -1.425)
No reps.	0.443	0.019	0.386	0.139	0.986
0.01*	1.225 (0.742 -2.035)	3.053 (2.001 -6.1)	0.782 (0.262 -1.378)	0.588 (0.146 -1.065)	0.995 (0.348 -1.758)
No reps.	0.988	0.00827	0.0646	0.0392	0.00267
3.125	0.997 (0.592 -1.899)	1.881 (1.466 -2.95)	0.668 (0.463 -1.156)	1.404 (0.851 -1.936)	2.003 (1.237 -2.982)
0.351	0.51	0.673	0.00396	0.796	0.374
2.197 (1.354 -3.567)	0.891 (0.486 -1.229)	0.931 (0.594 -1.517)	0.562 (0.39 -0.789)	0.948 (0.564 -1.533)	0.914 (0.687 -1.279)

Table A.1 (continued)

M A6	M A7	M B6	M C2	M C5	M D11
0.00147	0.197	0.105	0.00752	0.000141	0.00352
2.282 (1.283 -3.2)	1.294 (0.987 -2.006)	1.205 (0.922 -1.776)	1.664 (0.978 -2.11)	0.0758 (0.0452 -0.118)	0.396 (0.228 -0.535)
0.0309	No reps.	0.501	0.278	0.0593	0.435
1.632 (0.979 -2.637)	0.01*	1.11 (0.709 -1.689)	0.801 (0.387 -1.549)	0.628 (0.453 -0.965)	0.839 (0.684 -1.205)
0.327	0.0577	0.0224	0.197	0.00119	0.49
1.285 (0.48 -2.053)	1.307 (0.899 -1.625)	0.706 (0.439 -0.975)	1.145 (0.865 -1.528)	0.307 (0.229 -0.716)	0.868 (0.536 -1.697)
0.774	0.158	0.412	0.689	0.00895	0.06
0.87 (0.2 -3.351)	1.944 (1.643 -2.3)	0.867 (0.589 -1.558)	0.95 (0.565 -1.173)	0.491 (0.278 -0.943)	0.632 (0.318 -0.864)
0.0465	0.77	0.305	0.226	0.0056	0.0571
0.489 (0.233 -1.325)	0.848 (0.434 -2.219)	1.139 (0.803 -1.449)	0.848 (0.638 -1.369)	0.351 (0.303 -0.396)	1.479 (1.071 -2.172)
0.000245	0.589	0.0113	0.000908	0.000643	0.413
0.561 (0.466 -0.701)	1.121 (0.695 -2.275)	1.632 (1.12 -2.638)	0.732 (0.639 -0.871)	0.569 (0.452 -0.611)	1.159 (0.559 -1.765)

Table A.1 (continued)

M D8	M E11	M E12	M E2	M E3	M E4
0.00385	0.0461	0.00258	0.00386	0.0102	0.0901
6.167 (3.304 -8.963)	0.55 (0.344 -0.831)	0.467 (0.379 -0.704)	2.257 (1.348 -4.453)	0.45 (0.206 -0.718)	1.23 (0.954 -1.583)
0.142	0.681	0.336	0.0363	0.246	0.372
1.579 (1.28 -2.326)	0.926 (0.707 -1.233)	1.149 (0.65 -1.487)	1.307 (1.063 -2)	1.246 (0.579 -1.707)	0.0625 (0.01 -0.39)
0.0534	0.113	0.0489	0.0753	0.0293	0.295
1.581 (1.015 -1.853)	0.85 (0.65 -1.089)	0.701 (0.427 -1.155)	1.205 (1.014 -1.586)	0.826 (0.684 -0.997)	1.188 (0.719 -1.963)
0.013	0.00806	0.135	0.572	0.113	0.585
2.373 (1.488 -3.067)	0.68 (0.464 -0.843)	0.704 (0.388 -1.208)	1.052 (0.806 -1.43)	0.848 (0.645 -1.109)	0.854 (0.462 -1.344)
0.0217	0.071	0.895	0.884	0.196	0.848
1.474 (1.219 -2.145)	1.355 (1.009 -1.622)	0.979 (0.586 -1.585)	0.986 (0.678 -1.218)	1.152 (0.857 -1.409)	0.961 (0.624 -1.541)
0.822	0.208	0.596	0.0281	0.119	0.0818
0.959 (0.416 -1.414)	0.929 (0.748 -1.04)	1.046 (0.843 -1.265)	1.422 (0.874 -2.013)	1.234 (0.76 -1.573)	1.183 (1.068 -1.252)

Table A.1 (continued)

M E5	M E9	M F12	M F2	M F6	M G1
0.00379	0.00239	0.00164	6.65E -05	0.00515	0.000255
0.656 (0.51 -0.755)	6.183 (1.688 -16.29)	0.0659 (0.0265 -0.152)	0.289 (0.202 -0.404)	0.625 (0.423 -0.771)	4.391 (3.172 -6.37)
0.911	0.415	0.93	0.105	0.0555	0.356
0.968 (0.588 -1.364)	0.938 (0.785 -1.168)	1.016 (0.483 -1.524)	0.737 (0.637 -0.909)	2.225 (1.501 -2.71)	0.819 (0.409 -1.554)
0.00378	0.68	0.000506	0.00806	0.232	0.00327
0.603 (0.448 -0.83)	0.967 (0.815 -1.33)	0.594 (0.442 -0.695)	0.33 (0.155 -0.616)	0.85 (0.662 -1.423)	1.814 (1.13 -2.297)
0.0473	0.649	0.116	0.0233	0.0514	0.0481
0.504 (0.287 -0.784)	0.963 (0.751 -1.313)	0.803 (0.454 -0.944)	0.482 (0.204 -0.824)	0.633 (0.478 -1.203)	1.703 (1.226 -3.443)
0.235	0.041	0.101	0.203	0.606	0.673
1.282 (0.73 -2.31)	0.824 (0.657 -1.036)	0.93 (0.854 -1.08)	1.285 (0.74 -2.362)	1.131 (0.641 -2.481)	0.914 (0.457 -1.709)
0.0346	0.0745	0.491	0.127	0.575	0.582
1.24 (1.051 -1.381)	0.839 (0.63 -0.949)	0.903 (0.499 -1.229)	1.337 (0.745 -1.896)	0.924 (0.635 -1.579)	1.096 (0.534 -1.525)

Table A.1 (continued)

M G10	M G11	M G12	M G2	M G3	M H1
0.000358	0.00506	0.000147	0.00127	0.00529	0.00262
0.146 (0.0872 -0.251)	0.175 (0.0747 -0.416)	0.042 (0.0237 -0.0823)	0.337 (0.234 -0.458)	0.258 (0.112 -0.616)	0.295 (0.198 -0.583)
No reps.	0.351	No reps.	0.533	0.0357	No reps.
1.608	0.733 (0.38 -1.355)	1.608	1.398 (0.727 -3.308)	1.481 (0.993 -2.051)	0.897
0.0013	2.00E -05	0.00405	0.0146	0.0116	2.74E -05
0.318 (0.186 -0.404)	0.371 (0.315 -0.47)	0.142 (0.0564 -0.373)	0.468 (0.252 -0.682)	0.418 (0.187 -0.724)	0.418 (0.367 -0.453)
0.158	0.0196	0.0505	0.217	0.103	0.00638
0.507 (0.277 -0.76)	0.507 (0.349 -0.861)	0.255 (0.0769 -0.739)	0.796 (0.487 -1.167)	0.592 (0.36 -0.965)	0.477 (0.382 -0.641)
0.0334	0.0107	0.582	0.085	0.479	0.000162
0.737 (0.564 -0.949)	0.48 (0.263 -0.866)	0.861 (0.514 -1.619)	1.248 (0.933 -1.618)	1.223 (0.375 -2.673)	0.62 (0.557 -0.684)
0.909	0.00995	0.491	0.0285	0.0354	0.722
0.986 (0.631 -1.467)	0.587 (0.351 -0.835)	0.866 (0.627 -1.462)	1.345 (1.085 -2.088)	0.814 (0.664 -1.007)	1.044 (0.912 -1.287)

Table A.1 (continued)

M H10	M H5	M H6	MM A2	MM C2	MM D8
0.000199	0.00964	8.53E -05	0.00173	0.386	0.0131
0.0581 (0.0325 -0.101)	0.336 (0.149 -0.669)	0.0815 (0.034 -0.157)	2.911 (1.853 -4.282)	0.826 (0.562 -1.269)	11.45 (3.714 -25)
No reps.	0.5	0.589	0.0323	NO DATA	0.5
0.691	0.904 (0.571 -1.297)	0.919 (0.602 -1.479)	3.545 (2.152 -9.5)	NO DATA	0.796 (0.342 -1.378)
0.000199	0.238	0.000785	0.0351	0.0846	0.0193
0.159 (0.0893 -0.241)	0.891 (0.587 -1.018)	0.378 (0.269 -0.533)	2.708 (0.672 -7.483)	0.858 (0.708 -1.018)	1.823 (1.278 -2.38)
0.0123	0.026	0.122	0.911	No reps.	0.0766
0.243 (0.0877 -0.801)	0.819 (0.672 -0.976)	0.584 (0.181 -1.245)	1.038 (0.382 -2.386)	0.01*	2.94 (1.176 -16.62)
0.977	0.0602	0.693	0.2	NO DATA	0.813
0.988 (0.462 -1.557)	0.843 (0.653 -1.018)	1.074 (0.576 -1.756)	0.696 (0.244 -1.159)	NO DATA	1.028 (0.778 -1.439)
0.608	0.0582	0.00332	4.34E -05	No reps.	0.148
0.876 (0.483 -1.545)	1.103 (0.998 -1.254)	1.35 (1.176 -1.604)	1.629 (1.483 -1.871)	5	1.171 (1.128 -1.216)

Table A.1 (continued)

MM F10	MM H10	MM H7	N A10	N A11	N A3
0.00423	0.0045	0.018	0.000915	0.00279	0.000195
17.5 (7.5 -33)	13.83 (4.212 -42.5)	5.86 (2.914 -16.18)	8.945 (3.854 -26.9)	6.625 (4.258 -11)	14.52 (8.178 -49.3)
0.232	0.501	0.746	0.439	0.71	0.0582
0.732 (0.434 -1.631)	0.875 (0.569 -1.298)	0.919 (0.699 -1.447)	0.877 (0.563 -1.033)	1.333 (0.742 -2.394)	0.603 (0.374 -0.817)
0.0337	0.0355	0.00874	0.125	0.0252	0.0359
2.543 (1.821 -3.305)	1.434 (0.988 -1.837)	1.507 (0.985 -1.996)	1.185 (0.92 -1.562)	2.7 (2.062 -5.521)	1.671 (0.961 -2.682)
0.023	0.0103	0.0309	0.89	0.0436	0.0545
1.604 (1.028 -2.293)	1.901 (1.116 -3.557)	2.265 (1.743 -2.901)	0.986 (0.767 -1.394)	1.867 (1.088 -3.699)	1.48 (0.994 -3)
0.94	0.806	0.72	0.0211	0.736	0.741
0.989 (0.706 -1.493)	0.966 (0.721 -1.599)	1.042 (0.745 -1.288)	0.819 (0.661 -0.921)	0.953 (0.681 -1.705)	1.038 (0.735 -1.387)
0.945	0.0407	0.251	0.029	0.176	0.479
1.008 (0.756 -1.659)	1.299 (0.965 -1.722)	0.813 (0.514 -1.097)	0.821 (0.677 -1.088)	1.099 (0.944 -1.301)	1.099 (0.897 -1.754)

Table A.1 (continued)

N A5	N A6	N A9	N B1	N B9	N C5
0.000148	0.00176	0.00387	0.000224	6.57E -05	0.237
0.0943 (0.0688 -0.178)	7.978 (5.277 -12.5)	2.137 (1.751 -2.747)	30.18 (12.38 -95)	22.62 (11.37 -57.5)	0.813 (0.669 -1.233)
No reps.	0.709	0.0307	0.387	0.536	No reps.
0.669	0.817 (0.218 -2.25)	0.58 (0.351 -1.072)	0.843 (0.611 -1.336)	0.849 (0.445 -1.319)	0.01*
0.00152	0.000507	0.429	0.0389	0.00759	0.167
0.17 (0.0739 -0.336)	2.016 (1.74 -2.534)	1.167 (0.782 -1.922)	1.549 (0.999 -2.905)	1.695 (1.186 -2.57)	0.503 (0.114 -1.351)
0.0522	0.0987	0.124	0.344	0.155	No reps.
0.245 (0.0709 -1.086)	1.741 (0.64 -3.991)	0.465 (0.212 -1.049)	1.142 (0.857 -1.813)	1.221 (0.73 -1.694)	0.494
0.462	0.587	0.761	0.247	0.909	0.869
0.862 (0.54 -1.608)	0.895 (0.53 -1.663)	1.076 (0.707 -1.343)	0.879 (0.679 -1.278)	1.013 (0.731 -1.387)	1.042 (0.856 -1.268)
0.9	0.259	0.272	0.11	0.164	0.108
1.054 (0.509 -1.697)	1.107 (0.913 -1.342)	1.258 (0.711 -2.081)	0.83 (0.661 -1.236)	0.9 (0.758 -1.214)	2.041 (1.805 -2.307)

Table A.1 (continued)

N D11	N D4	N D6	N E10	N E2	N E3
0.000159	0.00187	0.00713	0.213	0.000343	3.91E -05
3.608 (2.686 -4.465)	0.189 (0.132 -0.454)	16.32 (5.011 -33)	1.43 (0.943 -2.5)	0.102 (0.0524 -0.272)	2.522 (2.056 -3.288)
0.968	0.5	0.74	No reps.	0.148	0.575
1.006 (0.596 -1.287)	1.083 (1 -1.173)	0.924 (0.499 -1.27)	0.01*	0.864 (0.687 -1.004)	0.923 (0.505 -1.243)
0.00806	0.00513	0.00399	0.0288	0.0203	0.0775
1.434 (1.103 -1.811)	0.296 (0.117 -0.571)	2.5 (1.565 -4.12)	2.304 (1.523 -4.149)	0.485 (0.265 -1.08)	1.167 (0.993 -1.402)
0.171	0.0528	0.00596	0.391	0.00392	0.73
1.478 (0.689 -2.903)	0.316 (0.0687 -0.75)	1.61 (1.262 -1.908)	1.572 (1.143 -2.162)	0.462 (0.3 -0.827)	1.027 (0.826 -1.318)
0.0209	0.989	0.0519	0.15	0.0654	0.235
2.072 (0.788 -3.273)	0.997 (0.629 -1.949)	1.58 (1.088 -2.619)	0.547 (0.293 -1.055)	0.65 (0.369 -1.11)	1.201 (0.899 -1.771)
0.00459	0.565	0.0322	0.0892	0.0595	0.407
1.262 (1.14 -1.555)	1.171 (0.749 -1.627)	1.32 (1.198 -1.643)	0.617 (0.378 -0.978)	0.799 (0.573 -1.013)	1.077 (0.899 -1.397)

Table A.1 (continued)

N E6	N F1	N F10	N F2	N F9	N G8
0.00455	0.000309	0.0226	0.00111	0.000256	0.000302
8.879 (5.086 -19.5)	7.299 (4.647 -12.09)	0.356 (0.343 -0.369)	19.82 (8.609 -48.42)	6.336 (2.672 -12.12)	4.462 (2.76 -7.489)
0.46	0.623	NO DATA	0.756	0.558	0.243
1.402 (0.725 -2.627)	0.905 (0.606 -1.702)	NO DATA	1.07 (0.905 -1.265)	0.888 (0.566 -2.039)	2.259 (0.0521 -5.26)
0.00035	0.00558	0.0503	0.0465	0.00721	0.0195
2.269 (1.989 -2.925)	1.69 (1.366 -2.242)	1.338 (1.018 -1.84)	1.608 (1.216 -3.041)	2.095 (1.574 -3.588)	1.61 (1.057 -2.186)
0.0365	0.52	No reps.	0.201	0.337	0.513
1.759 (1.131 -2.888)	1.094 (0.692 -1.786)	0.01*	1.431 (0.768 -3.047)	1.204 (0.636 -1.891)	1.313 (0.177 -3.365)
0.385	0.835	0.765	0.991	0.793	0.663
0.912 (0.718 -1.232)	1.034 (0.704 -1.533)	0.914 (0.725 -1.153)	1.002 (0.489 -1.539)	1.042 (0.838 -1.54)	1.033 (0.773 -1.212)
0.012	0.0693	0.323	0.273	0.196	0.775
1.365 (1.199 -1.972)	0.818 (0.674 -1.192)	1.431 (0.828 -1.997)	1.137 (0.803 -1.61)	0.828 (0.482 -1.216)	0.965 (0.617 -1.498)

Table A.1 (continued)

N H7	NN A2	NN A4	NN B9	NN C5	NN D12
0.000265	0.136	0.00122	0.000352	0.0553	0.0178
0.59 (0.528 -0.653)	0.39 (0.318 -0.478)	0.226 (0.144 -0.384)	0.499 (0.403 -0.673)	4.174 (1.595 -14.26)	9.35 (6.411 -17)
0.244	No reps.	0.59	No reps.	0.219	0.101
0.9 (0.732 -1.261)	5.5	1.482 (0.878 -2.5)	1.863	0.605 (0.368 -0.984)	2.706 (1.783 -5.347)
0.431	0.083	0.014	0.0364	0.0207	0.0962
0.932 (0.728 -1.179)	0.722 (0.466 -1.25)	0.3 (0.109 -0.704)	0.516 (0.171 -0.802)	1.946 (1.223 -3.476)	2.215 (0.395 -6.134)
0.341	0.514	0.0961	No reps.	0.0181	0.0318
0.915 (0.669 -1.151)	0.868 (0.664 -1.222)	0.193 (0.0954 -0.571)	0.244	2.707 (2.135 -3.415)	3.919 (2.408 -5.497)
0.000429	0.000728	0.972	0.197	0.927	0.0365
0.846 (0.774 -0.896)	0.431 (0.385 -0.505)	0.993 (0.573 -1.732)	1.394 (0.886 -2.045)	0.982 (0.692 -2.426)	1.436 (1.069 -2.313)
0.113	0.6	0.706	0.49	0.868	0.00652
1.153 (0.871 -1.457)	1.305 (0.905 -1.881)	1.23 (0.659 -3.125)	1.164 (0.812 -1.978)	1.042 (0.444 -1.764)	1.458 (1.114 -2.042)

Table A.1 (continued)

NN D2	NN D4	NN D5	NN D7	NN E1	NN E10
0.0015	1.77E -05	0.0184	0.0326	0.0162	0.0666
30.49 (10.82 -171.5)	4.058 (2.853 -5.027)	4.964 (1.699 -31.05)	7.801 (4.8 -16.5)	10.2 (3.564 -43.38)	0.488 (0.452 -0.526)
0.199	0.718	0.108	0.98	0.535	NO DATA
0.833 (0.485 -1.119)	1.104 (0.667 -2.174)	0.893 (0.744 -1.032)	1.016 (0.624 -1.655)	0.954 (0.772 -1.29)	NO DATA
0.215	0.466	0.121	0.0166	0.74	0.0117
1.374 (0.899 -2.41)	1.141 (0.957 -1.838)	1.273 (0.928 -1.845)	1.893 (0.976 -3.165)	0.979 (0.914 -1.166)	0.633 (0.45 -0.809)
0.29	0.411	0.951	0.103	0.246	0.892
1.146 (0.832 -1.741)	1.28 (0.561 -2.831)	1.003 (0.844 -1.135)	3.123 (1.604 -6.334)	0.667 (0.483 -1.09)	0.977 (0.832 -1.316)
0.141	0.0163	0.375	0.85	0.000601	0.0472
0.861 (0.698 -1.238)	1.176 (1.063 -1.427)	0.929 (0.749 -1.201)	0.974 (0.765 -1.367)	1.451 (1.265 -1.666)	0.832 (0.766 -0.869)
0.00462	0.412	0.0507	0.00865	0.841	0.076
0.806 (0.694 -0.91)	0.842 (0.739 -0.959)	1.133 (0.947 -1.27)	1.657 (1.162 -2.504)	1.007 (0.87 -1.093)	1.449 (1.057 -1.951)

Table A.1 (continued)

NN E11	NN E8	NN E9	NN F9	NN G1	NN G6
0.0176	0.000143	0.0758	0.00272	0.0257	0.000385
0.487 (0.33 -0.624)	0.487 (0.412 -0.564)	12.98 (9.549 -17.64)	0.712 (0.62 -0.815)	0.479 (0.297 -0.982)	3.554 (2.561 -5.797)
NO DATA	No reps.	NO DATA	No reps.	0.195	0.0704
NO DATA	1.629	NO DATA	2.585	0.775 (0.4 -1.168)	0.806 (0.629 -1.118)
0.49	0.0428	0.0248	0.0225	0.298	0.311
0.868 (0.472 -1.331)	0.444 (0.12 -1.082)	4.219 (2.745 -6.056)	0.553 (0.368 -0.641)	0.919 (0.786 -1.282)	0.851 (0.466 -1.196)
No reps.	0.346	0.344	No reps.	0.0167	0.25
0.01*	0.175 (0.061 -0.501)	1.543 (1.19 -2.002)	0.398	0.882 (0.761 -0.961)	1.242 (1 -2.347)
No reps.	0.216	NO DATA	0.609	0.00636	9.73E -05
1.452	1.2 (0.979 -1.644)	NO DATA	1.148 (0.804 -1.762)	0.804 (0.675 -0.952)	1.474 (1.301 -1.668)
NO DATA	0.0292	0.0529	0.0857	0.0656	0.0126
NO DATA	2.084 (1 -3.877)	1.237 (0.91 -1.69)	1.31 (1.027 -1.708)	1.14 (0.905 -1.315)	0.759 (0.652 -0.996)

Table A.1 (continued)

NN G7	NN H12	NN H5	NN H6	NN H9	O A10
0.152	0.000501	0.0032	0.124	0.00346	0.000202
0.263 (0.0101 -0.824)	1.373 (1.168 -1.539)	0.501 (0.301 -0.728)	0.721 (0.406 -1.112)	2.008 (1.316 -3.094)	33.23 (12.26 -113.5)
0.269	0.333	NO DATA	NO DATA	No reps.	0.352
0.762 (0.311 -1.295)	0.707 (0.579 -0.864)	NO DATA	NO DATA	0.01*	0.842 (0.408 -1.242)
0.0304	0.149	0.0308	0.00599	0.388	0.172
0.858 (0.752 -0.989)	1.28 (0.788 -1.869)	0.73 (0.576 -0.895)	0.566 (0.537 -0.618)	1.114 (0.902 -1.351)	1.266 (0.729 -1.587)
0.00102	0.0354	No reps.	No reps.	0.139	0.161
0.859 (0.826 -0.912)	1.344 (0.986 -2.127)	0.109	0.155	1.206 (0.964 -1.616)	1.221 (0.806 -1.81)
0.00771	0.964	0.767	0.201	0.546	0.00504
0.896 (0.818 -0.957)	1.003 (0.811 -1.196)	0.947 (0.666 -1.468)	1.252 (0.927 -1.804)	0.936 (0.68 -1.153)	2.471 (1.367 -4.146)
0.257	0.128	0.0435	0.0939	0.388	0.00777
0.768 (0.291 -1.119)	0.781 (0.592 -1.053)	2.82 (1.86 -4)	1.923 (1.291 -2.71)	0.699 (0.331 -1.716)	1.292 (1.024 -1.583)

Table A.1 (continued)

O A8	O A9	O B1	O B10	O B3	O B6
No reps.	0.00522	0.00167	0.00236	0.000542	0.000367
3.563	5.672 (3.789 -11.22)	28.84 (5.336 -172)	4.37 (2.18 -11.15)	7.922 (4.318 -24.24)	5.104 (2.46 -9.375)
NO DATA	No reps.	0.0523	0.258	0.845	0.296
NO DATA	1.42	0.712 (0.54 -1.039)	0.631 (0.4 -1.094)	1.037 (0.752 -1.83)	0.807 (0.521 -1.174)
0.0716	0.00404	0.24	0.0257	0.000372	0.00232
2.146 (1.726 -3.307)	2.249 (1.456 -3.49)	0.868 (0.544 -1.058)	1.656 (0.793 -2.322)	2.135 (1.651 -2.797)	1.773 (1.423 -2.512)
No reps.	0.0865	0.892	0.0524	0.327	0.879
0.01*	2.083 (1.273 -4.634)	0.976 (0.613 -1.602)	1.522 (1.019 -2.259)	1.133 (0.828 -1.663)	0.963 (0.477 -2.298)
0.227	0.503	0.00315	0.114	0.643	0.0634
4.241 (2.477 -7.259)	1.116 (0.704 -1.797)	2.172 (1.313 -3.224)	1.261 (0.937 -1.95)	1.08 (0.822 -1.556)	1.398 (0.858 -1.817)
0.86	0.443	0.375	0.811	0.237	0.0928
0.947 (0.589 -1.498)	1.063 (0.829 -1.331)	1.087 (0.949 -1.657)	0.943 (0.324 -1.516)	1.086 (0.896 -1.274)	0.845 (0.704 -1.189)

Table A.1 (continued)

O B7	O B9	O C10	O C11	O C2	O C6
0.0277	0.0237	0.000518	4.27E -06	0.012	0.0563
7.772 (4 -13)	7.474 (4 -10.99)	4.669 (2.462 -9.592)	7.668 (5.205 -11.88)	1.708 (0.962 -2.707)	5.75 (1.285 -70.12)
0.739	0.906	0.0371	0.257	0.194	0.122
1.063 (0.722 -1.459)	1.048 (0.763 -1.44)	0.666 (0.396 -1.005)	1.527 (0.882 -3.604)	0.787 (0.576 -1.373)	0.745 (0.38 -1.113)
0.00171	0.112	0.0223	0.000303	0.081	0.0766
2.163 (1.728 -3.102)	1.439 (0.776 -2.16)	1.455 (0.983 -2.035)	1.916 (1.578 -2.113)	0.871 (0.763 -0.986)	1.265 (0.964 -1.725)
0.15	0.221	0.0328	0.313	0.456	0.429
1.572 (1.003 -2.745)	3.318 (2.151 -5.118)	1.295 (0.978 -1.598)	1.194 (0.734 -1.823)	0.926 (0.608 -1.213)	1.073 (0.874 -1.396)
0.0106	0.00544	0.156	0.644	0.998	0.0108
1.629 (1.15 -2.256)	1.858 (1.194 -2.825)	0.876 (0.707 -1.153)	0.89 (0.5 -1.499)	1 (0.742 -1.476)	0.746 (0.553 -0.934)
0.337	0.425	0.0761	0.219	0.000228	0.338
1.188 (0.619 -2.081)	1.239 (0.447 -2.193)	0.776 (0.475 -1.078)	0.896 (0.706 -1.09)	0.601 (0.472 -0.661)	0.932 (0.718 -1.121)

Table A.1 (continued)

O C7	O C8	O C9	O D10	O D12	O D3
0.000309	4.28E -05	0.00405	3.15E -05	0.00254	0.722
3.406 (2.668 -4.928)	6.963 (4.796 -10.84)	7.654 (3.609 -26.3)	6.897 (4.956 -11.35)	26.24 (9.087 -219.5)	0.868 (0.535 -1.698)
0.0809	0.106	0.094	0.956	0.342	NO DATA
0.48 (0.211 -1.203)	0.755 (0.53 -1.214)	0.667 (0.454 -1.263)	0.99 (0.511 -1.711)	1.152 (0.809 -2.064)	NO DATA
0.0535	0.0185	0.00363	0.00387	0.269	0.0543
1.521 (1.156 -2.004)	1.598 (1.173 -2.134)	1.911 (1.098 -2.803)	1.546 (1.185 -2.06)	1.244 (0.837 -2.281)	0.659 (0.431 -0.933)
0.0267	0.0769	0.256	0.316	0.22	No reps.
1.587 (1.003 -2.615)	1.383 (0.842 -2.463)	1.298 (0.763 -1.808)	1.178 (0.705 -1.686)	1.191 (0.843 -1.869)	0.01*
0.00606	0.0152	0.00733	0.602	0.00902	0.289
1.451 (1.11 -2.042)	1.728 (1.249 -2.99)	2.034 (1.275 -3.479)	1.068 (0.845 -1.617)	0.786 (0.629 -0.952)	0.695 (0.502 -1.147)
0.0687	0.0327	0.498	0.22	0.968	0.116
1.155 (0.903 -1.373)	1.268 (1.048 -1.494)	0.949 (0.715 -1.166)	1.105 (0.886 -1.434)	0.993 (0.64 -1.502)	0.725 (0.623 -0.92)

Table A.1 (continued)

O D6	O D7	O D9	O E10	O E11	O E6
0.000159	0.00401	0.000124	5.70E -05	0.00161	0.000178
5.634 (3.064 -10.97)	12.34 (2.926 -39)	10.82 (6.975 -30.73)	5.628 (4.716 -8.083)	0.644 (0.535 -0.801)	4.889 (3.373 -6.484)
0.59	0.641	0.144	0.93	No reps.	0.851
0.919 (0.673 -1.288)	0.883 (0.594 -1.729)	0.85 (0.602 -1.012)	0.983 (0.7 -1.925)	7	1.036 (0.667 -2.419)
0.00374	0.0151	0.00579	0.00534	0.315	0.018
2.354 (1.762 -3.674)	2.025 (1.005 -4.018)	1.893 (1.364 -3.128)	2.107 (1.444 -3.467)	0.791 (0.354 -1.093)	1.532 (0.973 -2.345)
0.102	0.00338	0.022	0.0795	0.118	0.131
1.573 (0.871 -2.787)	2.246 (1.562 -3.437)	1.515 (0.934 -2.077)	1.298 (0.928 -1.945)	0.856 (0.832 -0.882)	1.357 (0.689 -2.338)
0.0583	0.00801	0.111	0.288	0.466	0.0889
1.281 (0.943 -1.695)	2.096 (1.311 -2.904)	1.419 (0.942 -2.155)	1.174 (0.78 -1.75)	1.134 (0.774 -1.975)	1.342 (0.927 -1.941)
0.0154	0.0168	0.0682	0.0205	0.711	0.000672
1.381 (1.027 -1.636)	1.198 (1.002 -1.4)	1.147 (0.946 -1.256)	1.185 (0.955 -1.345)	1.078 (0.835 -1.511)	1.289 (1.166 -1.419)

Table A.1 (continued)

O E7	O E8	O E9	O F11	O F6	O F7
0.000387	0.0238	0.00523	0.000484	0.000204	0.00187
9.511 (4.384 -14.63)	6.026 (1.221 -39.12)	7.487 (1.913 -32.02)	6.659 (4.335 -12.53)	3.171 (2.156 -4.459)	5.227 (2.959 -10.57)
0.287	0.459	0.958	0.184	0.379	0.24
1.262 (0.662 -2.322)	0.927 (0.606 -1.177)	1.007 (0.746 -1.684)	1.226 (0.753 -1.553)	0.84 (0.531 -1.184)	0.835 (0.463 -1.233)
0.2	0.082	0.0368	0.0133	0.0227	0.0184
1.516 (0.576 -2.897)	1.205 (1.016 -1.801)	1.335 (1.083 -2.007)	1.962 (1.499 -3.403)	1.356 (1.022 -1.856)	1.162 (1.083 -1.431)
0.0369	0.33	0.543	0.0239	0.0121	0.432
1.738 (1.013 -2.964)	1.052 (0.933 -1.233)	1.061 (0.819 -1.31)	1.372 (0.968 -2.025)	1.364 (1.235 -1.605)	1.048 (0.912 -1.251)
0.0248	0.0115	0.0522	0.0365	0.182	0.715
1.477 (0.907 -2.271)	1.16 (1.094 -1.322)	1.169 (0.89 -1.371)	1.333 (1.025 -2.028)	1.177 (0.93 -1.628)	1.031 (0.78 -1.355)
0.00089	0.0844	0.065	0.817	0.152	0.0255
1.675 (1.395 -1.934)	1.206 (0.791 -1.403)	1.268 (1.176 -1.44)	1.038 (0.57 -1.619)	1.249 (0.755 -1.767)	1.154 (0.948 -1.286)

Table A.1 (continued)

O F8	O G12	O G6	O G7	O G8	O G9
0.000378	0.00985	1.96E -05	0.000606	1.84E -05	0.000943
13.01 (6.912 -42)	3.627 (1.633 -6.432)	5.128 (3.683 -6.233)	3.91 (2.3 -8.048)	3.335 (2.842 -4.68)	3.29 (2.547 -3.819)
0.784	NO DATA	0.573	0.843	0.138	0.0599
1.054 (0.615 -2.079)	NO DATA	1.223 (0.503 -2.907)	1.046 (0.645 -1.796)	0.732 (0.327 -1.214)	0.631 (0.355 -1.089)
0.00585	0.0619	0.00443	0.00127	0.000257	0.00903
2.306 (1.572 -3.83)	5.003 (1.518 -26.71)	1.29 (1.061 -1.502)	1.983 (1.706 -2.256)	1.227 (1.116 -1.314)	1.695 (1.345 -2.526)
0.000322	0.139	0.123	0.0162	0.17	0.0318
2.143 (1.489 -2.695)	7.532 (4.816 -11.78)	1.214 (0.949 -1.555)	2.207 (1.462 -3.081)	1.131 (0.901 -1.363)	1.527 (0.997 -2.302)
0.0688	0.183	0.0463	0.848	0.141	0.766
1.45 (0.914 -2.32)	0.619 (0.29 -1.55)	1.319 (0.971 -1.723)	0.983 (0.79 -1.204)	0.838 (0.565 -1.096)	1.045 (0.745 -1.448)
0.647	0.35	0.13	0.425	0.426	0.422
1.065 (0.573 -1.341)	0.791 (0.386 -1.276)	1.241 (0.812 -1.641)	1.134 (0.798 -1.799)	1.074 (0.81 -1.219)	1.123 (0.828 -1.63)

Table A.1 (continued)

O H12	O H5	OO A10	OO B6	OO C1	OO C7
0.00583	0.00217	0.0695	0.00619	0.00324	0.116
21.79 (12.34 -78.5)	3.652 (2.16 -5.856)	0.434 (0.293 -0.655)	0.526 (0.371 -0.708)	0.452 (0.33 -0.671)	1.321 (0.974 -1.902)
0.391	0.843	No reps.	NO DATA	NO DATA	No reps.
1.187 (0.687 -1.944)	0.952 (0.673 -1.843)	0.01*	NO DATA	NO DATA	7.5
0.00535	0.189	0.0198	5.78E -05	0.000897	0.431
2.15 (1.349 -3.953)	1.422 (0.638 -2.641)	0.704 (0.529 -0.927)	0.317 (0.271 -0.379)	0.518 (0.395 -0.638)	0.884 (0.587 -1.239)
0.00885	0.0695	0.146	No reps.	0.99	0.873
1.663 (1.096 -1.98)	1.296 (0.894 -1.998)	0.751 (0.51 -1.022)	0.01*	1.003 (0.617 -1.75)	0.965 (0.654 -1.253)
0.119	0.806	0.182	No reps.	0.73	0.0184
1.284 (0.955 -1.763)	1.024 (0.807 -1.486)	1.278 (0.786 -1.984)	1.819	1.062 (0.695 -2.009)	1.574 (1.206 -2.181)
5.06E -05	0.241	0.459	NO DATA	0.965	0.0308
1.514 (1.441 -1.61)	1.111 (0.864 -1.456)	1.284 (0.532 -1.866)	NO DATA	1.011 (0.443 -1.748)	1.455 (0.918 -2.076)

Table A.1 (continued)

OO D11	OO E4	OO F3	OO G10	OO G12	OO G2
0.0477	0.0245	No reps.	0.000108	0.000443	0.00293
1.935 (1.103 -4.973)	0.417 (0.247 -0.662)	11.5	7.135 (4.888 -16.13)	0.298 (0.17 -0.404)	0.37 (0.28 -0.59)
0.864	NO DATA	No reps.	0.419	NO DATA	No reps.
1.024 (0.616 -1.484)	NO DATA	1.939	0.888 (0.599 -1.341)	NO DATA	1
0.703	0.142	0.0448	0.00347	0.000201	3.37E -05
1.05 (0.771 -1.615)	0.589 (0.271 -0.916)	1.726 (1.113 -3.204)	2.021 (1.269 -2.98)	0.406 (0.282 -0.547)	0.469 (0.376 -0.544)
0.782	1	0.0325	0.0336	0.109	0.325
1.016 (0.881 -1.171)	0.01*	3.156 (2.976 -3.347)	1.593 (1.119 -2.385)	0.448 (0.33 -0.798)	1.2 (0.905 -1.389)
0.000555	NO DATA	0.542	0.778	NO DATA	0.354
0.725 (0.621 -0.811)	NO DATA	1.112 (0.616 -1.649)	1.05 (0.673 -1.708)	NO DATA	0.741 (0.241 -1.094)
0.0335	0.067	0.238	0.426	0.105	0.188
0.811 (0.641 -0.96)	2.603 (2.353 -2.879)	1.132 (0.889 -1.318)	0.88 (0.555 -1.308)	1.754 (0.917 -2.868)	1.327 (0.815 -2.027)

Table A.1 (continued)

OO G3	OO G8	OO H11	OO H3	OO H4	OO H9
0.0426	0.0458	0.00636	0.00354	0.794	0.514
6.448 (0.622 -12.4)	4.616 (1.475 -40.71)	0.466 (0.359 -0.815)	2.824 (1.647 -6.114)	1.194 (0.704 -2.026)	1.1 (0.996 -1.214)
0.291	0.0067	No reps.	0.555	No reps.	NO DATA
1.688 (0.724 -8)	0.784 (0.63 -0.944)	3	1.147 (0.746 -1.929)	0.016	NO DATA
0.0135	0.129	0.00355	0.00483	0.696	0.0216
2.251 (1.158 -4.762)	1.279 (0.879 -1.985)	0.632 (0.491 -0.844)	1.706 (1.114 -2.174)	0.924 (0.575 -1.808)	1.62 (0.995 -2.08)
0.415	0.226	0.238	0.0381	0.589	NO DATA
1.432 (0.945 -2.886)	1.138 (0.88 -1.512)	0.739 (0.656 -0.832)	1.737 (1.133 -4.152)	0.932 (0.85 -1.023)	NO DATA
0.38	0.00115	0.931	0.696	No reps.	No reps.
1.122 (0.754 -1.625)	0.664 (0.583 -0.763)	1.022 (0.567 -1.773)	0.958 (0.796 -1.252)	0.726	0.01*
0.396	0.0201	0.162	0.188	0.916	No reps.
0.911 (0.69 -1.23)	0.927 (0.833 -0.966)	1.435 (0.897 -2.963)	1.104 (0.95 -1.287)	1.013 (0.84 -1.39)	2.074

Table A.1 (continued)

P A10	P B3	P B6	P C10	P C2	P D1
0.00137	0.348	0.00949	0.00209	0.00433	0.851
4.231 (0.647 -8.88)	0.649 (0.4 -0.933)	5.092 (4.241 -7)	4.69 (0.0915 -9.777)	2.434 (0.484 -5.29)	0.955 (0.01 -4)
0.0702	No reps.	No reps.	0.0334	0.471	No reps.
0.667 (0.554 -0.82)	1.518	2.747	0.584 (0.381 -1.051)	0.883 (0.67 -1.491)	0.01*
0.359	0.157	0.342	0.0117	0.19	0.574
1.361 (0.291 -4.25)	1.472 (0.01 -3.277)	1.726 (0.01 -8.5)	2.091 (0.508 -5.058)	1.436 (0.01 -4.151)	1.085 (0.355 -1.46)
0.021	No reps.	0.102	0.375	0.633	0.672
1.499 (1.12 -2.007)	2.175 (0.916 -3.435)	6.219 (4.626 -8.361)	1.392 (0.809 -3.596)	1.232 (0.312 -2.808)	1.064 (0.919 -1.259)
0.161	No reps.	0.425	0.972	0.38	0.198
1.624 (0.01 -5.291)	5	0.83 (0.384 -1.206)	1.009 (0.174 -2.4)	0.795 (0.101 -1.849)	1.263 (0.627 -2.715)
0.712	0.948	0.758	0.722	0.613	0.442
1.101 (0.119 -2.679)	1.035 (0.01 -1.95)	1.115 (0.01 -3.7)	1.1 (0.01 -2.91)	0.858 (0.01 -2.067)	1.145 (0.33 -1.979)

Table A.1 (continued)

P D10	P D12	P D3	P D6	P E11	P E2
0.00198	0.409	0.00198	0.00152	0.0278	0.00114
12.42 (0.159 -151.7)	0.6 (0.01 -1.197)	6.931 (1.587 -29.98)	3.204 (2.35 -3.66)	2.616 (1.435 -8.347)	2.054 (0.792 -4.307)
0.449	No reps.	0.277	No reps.	0.797	0.438
0.754 (0.112 -1.271)	0.396	0.848 (0.411 -1.589)	1.615	0.98 (0.829 -1.367)	1.339 (0.01 -2.248)
0.35	0.637	0.0603	0.186	0.988	0.238
1.363 (0.01 -3.039)	1.063 (0.548 -1.777)	1.451 (0.707 -3.249)	1.529 (1.129 -1.794)	1.005 (0.01 -1.965)	1.256 (0.307 -2.61)
0.787	0.386	0.178	NO DATA	0.0489	0.331
1.093 (0.0307 -3.307)	1.246 (0.919 -2.079)	1.192 (0.686 -2.074)	NO DATA	0.896 (0.793 -1.058)	1.228 (0.453 -2.32)
0.369	0.732	0.565	0.604	0.948	0.609
0.712 (0.0227 -1.312)	1.176 (0.01 -1.635)	0.955 (0.631 -1.382)	1.123 (0.261 -1.866)	0.973 (0.0215 -2.152)	0.9 (0.472 -1.637)
0.443	0.247	0.995	0.277	0.753	0.186
0.788 (0.01 -1.675)	1.569 (0.802 -2.405)	1 (0.742 -1.495)	1.268 (0.925 -1.562)	1.11 (0.01 -2.579)	0.692 (0.01 -1.877)

Table A.1 (continued)

P E3	P E5	P E6	P E8	P E9	P F11
0.561	0.103	0.00101	0.0015	0.853	0.0456
0.848 (0.116 -1.934)	4.875 (2.617 -23.14)	6.458 (0.01 -16.63)	5.872 (0.01 -55.97)	0.972 (0.652 -1.704)	2.962 (0.01 -29.48)
0.362	0.45	0.271	0.321	0.0704	0.107
1.311 (0.12 -3.678)	0.826 (0.491 -1.246)	1.163 (0.89 -1.774)	0.821 (0.507 -1.641)	3.522 (3.063 -4.051)	1.116 (0.973 -1.308)
0.709	0.0975	0.102	0.646	0.532	0.794
1.109 (0.121 -2.306)	1.726 (0.331 -3.424)	1.78 (0.01 -4.542)	1.159 (0.0109 -4.102)	1.099 (0.0762 -1.819)	1.09 (0.01 -2.51)
0.785	0.924	0.914	0.632	0.276	0.226
1.08 (0.0986 -2.427)	1.03 (0.34 -2.208)	1.036 (0.0678 -2.73)	0.832 (0.104 -1.759)	0.759 (0.418 -1.123)	0.908 (0.661 -1.054)
0.705	0.735	0.192	0.766	0.721	0.233
1.11 (0.108 -3.007)	1.102 (0.0129 -3.076)	1.189 (0.821 -1.711)	0.907 (0.01 -2.208)	1.064 (0.0152 -2.899)	0.846 (0.539 -1.146)
0.255	0.733	0.952	0.766	0.894	0.746
1.408 (0.156 -3.561)	0.896 (0.0773 -1.931)	1.02 (0.01 -2.141)	0.89 (0.01 -2.115)	0.984 (0.65 -1.542)	1.119 (0.01 -2.309)

Table A.1 (continued)

P F12	P F3	P F5	P F8	P G1	P G3
0.266	0.00398	0.00986	0.00788	0.000819	0.00147
1.856 (0.531 -3.715)	4.225 (0.0985 -30.9)	4.284 (0.648 -12.74)	4.106 (0.01 -16.99)	9.549 (0.979 -33.38)	6.152 (0.01 -16.66)
0.309	0.951	0.756	0.702	0.717	0.317
0.711 (0.573 -0.988)	1.019 (0.0126 -3.939)	1.186 (0.01 -3.665)	0.868 (0.0462 -1.544)	1.056 (0.22 -1.54)	0.754 (0.2 -1.799)
0.286	0.596	0.0887	0.868	0.972	0.0816
1.295 (0.643 -2.548)	1.174 (0.0716 -2.669)	1.748 (0.0537 -4.504)	1.053 (0.01 -3.057)	1.01 (0.171 -2.201)	1.771 (0.125 -4.24)
0.398	0.817	0.726	0.796	0.539	0.309
1.451 (0.225 -2.409)	1.074 (0.0499 -2.676)	1.137 (0.145 -2.292)	0.907 (0.0522 -1.904)	0.884 (0.223 -1.718)	1.565 (0.632 -3.437)
0.84	0.518	0.995	0.997	0.0898	0.123
0.945 (0.0857 -1.804)	0.82 (0.0513 -1.809)	1.002 (0.092 -2.944)	1.001 (0.01 -2.044)	1.952 (0.102 -4.789)	1.85 (0.0897 -5.151)
0.92	0.777	0.821	0.477	0.931	0.957
0.978 (0.01 -2.223)	0.92 (0.0387 -2.975)	1.049 (0.209 -2.131)	0.787 (0.01 -1.6)	1.02 (0.289 -2.27)	1.017 (0.01 -2.614)

Table A.1 (continued)

P G6	P G8	P G9	P H5	PINK A12	PINK A3
0.00788	0.0481	0.0704	8.07E -06	0.0426	0.00466
3.112 (0.832 -14.43)	4.222 (0.0154 -12.49)	14.28 (0.707 -35.59)	34.77 (0.872 -296)	0.73 (0.489 -1.159)	2.512 (1.299 -3.903)
0.886	0.639	0.0148	0.0286	NO DATA	0.269
1.045 (0.818 -1.336)	0.916 (0.579 -1.507)	0.544 (0.259 -0.872)	0.737 (0.502 -0.982)	NO DATA	0.833 (0.457 -1.182)
0.0382	0.48	0.0331	0.678	0.108	0.406
2.041 (0.639 -4.612)	1.281 (0.0507 -2.211)	1.393 (1.103 -2.049)	1.124 (0.0536 -2.587)	1.196 (0.834 -1.49)	1.09 (0.926 -1.707)
0.109	0.931	0.953	0.699	0.0296	0.466
1.533 (0.912 -3.15)	1.026 (0.0277 -3.595)	0.987 (0.581 -2.034)	1.135 (0.239 -1.658)	0.58 (0.566 -0.595)	1.067 (0.851 -1.395)
0.978	0.511	0.00504	0.0347	0.0259	0.0948
0.993 (0.302 -2.505)	0.802 (0.0143 -1.74)	2.01 (1.445 -2.98)	2.249 (0.0325 -5.923)	2.015 (1.254 -3.45)	1.115 (0.916 -1.326)
0.802	0.372	0.29	0.888	0.67	0.321
0.942 (0.01 -1.845)	0.739 (0.01 -1.968)	1.108 (0.817 -1.317)	1.042 (0.0345 -2.653)	1.092 (0.936 -1.275)	1.062 (0.887 -1.188)

Table A.1 (continued)

PINK A6	PINK B1	PINK B10	PINK B11	PINK B8	PINK C1
0.0193	2.45E -05	0.000217	3.66E -05	0.00299	0.000582
20.89 (0.831 -266)	0.233 (0.151 -0.29)	0.339 (0.293 -0.366)	0.318 (0.282 -0.383)	33.98 (11.17 -142.5)	0.277 (0.179 -0.38)
0.268	0.662	No reps.	NO DATA	0.092	0.384
0.868 (0.66 -1.259)	1.099 (0.936 -1.29)	1.518	NO DATA	1.277 (0.815 -1.697)	0.789 (0.438 -1.356)
0.0379	0.000179	0.00154	0.00399	0.0408	0.000104
1.404 (0.921 -1.739)	0.449 (0.392 -0.55)	0.406 (0.278 -0.529)	0.313 (0.204 -0.384)	1.789 (1.183 -2.57)	0.42 (0.331 -0.564)
0.702	0.0552	0.0328	0.104	0.117	0.378
1.048 (0.791 -1.378)	0.461 (0.337 -0.65)	0.555 (0.269 -1.026)	0.257 (0.102 -1.23)	1.272 (0.928 -2.214)	0.716 (0.283 -1.207)
0.1	0.069	0.725	0.979	0.671	0.318
1.59 (1.162 -2.702)	0.646 (0.566 -0.823)	0.887 (0.216 -1.901)	1.01 (0.434 -2.518)	0.967 (0.786 -1.206)	0.84 (0.613 -1.487)
0.00445	0.267	0.0296	0.0747	0.753	0.107
1.187 (1.029 -1.302)	1.363 (0.758 -2.295)	1.251 (1.012 -1.54)	1.289 (0.86 -1.549)	0.965 (0.591 -1.285)	0.877 (0.67 -0.995)

Table A.1 (continued)

PINK C11	PINK C2	PINK C5	PINK C7	PINK C8	PINK D10
0.00175	1.67E -05	0.000168	0.0145	0.000168	0.000505
0.486 (0.343 -0.683)	0.294 (0.208 -0.359)	0.375 (0.312 -0.46)	0.56 (0.347 -0.794)	6.01 (3.217 -10.47)	0.313 (0.219 -0.446)
0.79	NO DATA	No reps.	NO DATA	0.00348	0.191
1.19 (0.715 -1.98)	NO DATA	3	NO DATA	2.416 (1.54 -3.343)	1.311 (1.206 -1.426)
0.01	3.66E -05	0.000595	0.0152	0.0423	3.24E -05
0.606 (0.417 -0.793)	0.327 (0.25 -0.397)	0.474 (0.374 -0.563)	0.545 (0.285 -0.792)	1.532 (0.928 -2.353)	0.545 (0.478 -0.625)
0.177	0.122	0.45	No reps.	0.828	0.697
0.744 (0.513 -1.04)	0.491 (0.262 -1.054)	0.701 (0.204 -1.683)	0.29	1.087 (0.01 -2.231)	0.913 (0.559 -1.45)
0.137	0.143	0.545	0.0714	0.0341	0.29
1.408 (0.655 -2.292)	1.35 (0.741 -2.006)	1.13 (0.624 -1.968)	1.374 (0.88 -1.817)	1.668 (1.1 -3.213)	1.174 (0.687 -1.709)
0.262	0.202	0.00922	0.27	0.652	0.565
1.2 (0.784 -1.809)	1.225 (0.8 -1.811)	1.788 (1.185 -3.128)	1.484 (0.48 -2.694)	1.079 (0.647 -1.893)	1.124 (0.73 -1.692)

Table A.1 (continued)

PINK D3	PINK D4	PINK D5	PINK D6	PINK E10	PINK E11
8.50E -05	0.0561	0.00463	0.00313	0.000231	0.00025
0.261 (0.185 -0.425)	0.356 (0.139 -0.615)	0.195 (0.0867 -0.322)	0.467 (0.292 -0.853)	0.0504 (0.0288 -0.0859)	0.0704 (0.0374 -0.126)
0.13	NO DATA	0.00275	NO DATA	0.123	0.473
1.936 (1.249 -5)	NO DATA	2.514 (1.654 -4.525)	NO DATA	0.752 (0.553 -1.231)	0.0907 (0.01 -0.822)
0.0963	0.0509	0.335	0.00642	0.00126	0.00383
0.198 (0.01 -0.46)	0.484 (0.232 -0.794)	0.951 (0.794 -1.061)	0.426 (0.244 -0.702)	0.5 (0.304 -0.61)	0.245 (0.111 -0.565)
0.508	0.061	0.669	0.415	0.0179	0.0482
0.665 (0.242 -1.26)	0.384 (0.297 -0.63)	0.933 (0.575 -1.398)	0.628 (0.288 -1.396)	0.587 (0.325 -0.841)	0.376 (0.0906 -0.932)
0.346	0.0766	0.00106	0.0571	0.0189	0.042
1.33 (0.453 -2.602)	1.648 (1.045 -2.832)	1.753 (1.318 -2.181)	1.401 (0.914 -1.793)	0.829 (0.655 -0.954)	1.593 (1.005 -2.491)
0.0561	0.0719	0.295	0.277	0.106	0.11
1.83 (0.987 -3.738)	1.875 (1 -3.015)	1.113 (0.821 -1.478)	1.427 (0.744 -2.314)	0.932 (0.813 -1.012)	1.243 (1.01 -1.613)

Table A.1 (continued)

PINK E12	PINK E2	PINK E3	PINK E4	PINK E5	PINK E6
0.000134	0.000426	6.51E -05	0.000571	7.62E -06	0.000125
0.272 (0.179 -0.418)	0.0268 (0.0118 -0.0605)	0.151 (0.0935 -0.249)	0.0395 (0.0181 -0.0776)	0.0284 (0.0173 -0.0604)	0.0993 (0.0438 -0.202)
No reps.	0.277	0.139	NO DATA	No reps.	0.594
1.593	0.788 (0.308 -1.041)	0.785 (0.744 -0.829)	NO DATA	0.395	1.205 (0.696 -1.926)
0.0294	0.00348	0.00236	0.00297	0.0117	0.00104
0.242 (0.0274 -0.529)	0.216 (0.11 -0.369)	0.546 (0.408 -0.729)	0.145 (0.0341 -0.303)	0.246 (0.119 -0.393)	0.362 (0.237 -0.513)
0.0824	0.0763	0.00377	0.109	0.0797	0.28
0.3 (0.18 -0.614)	0.379 (0.0886 -0.969)	0.573 (0.423 -0.734)	0.169 (0.0487 -1.334)	0.191 (0.0709 -0.325)	0.488 (0.187 -0.943)
0.636	0.0678	0.619	0.991	0.397	0.324
1.079 (0.795 -1.57)	1.578 (0.771 -2.787)	0.916 (0.598 -1.301)	1.003 (0.527 -1.786)	1.259 (0.771 -2.633)	1.212 (0.813 -2.225)
0.00155	0.000601	0.665	0.0898	0.503	0.0184
1.561 (1.225 -1.969)	1.642 (1.297 -1.982)	0.964 (0.748 -1.199)	1.307 (0.979 -1.986)	1.367 (0.997 -1.875)	1.23 (1.032 -1.369)

Table A.1 (continued)

PINK E7	PINK E8	PINK E9	PINK F1	PINK F10	PINK F11
0.02	0.000293	5.99E -05	0.00112	1.37E -05	0.0183
0.635 (0.455 -0.919)	0.473 (0.408 -0.587)	0.0408 (0.0175 -0.0766)	0.318 (0.212 -0.42)	4.873 (3.667 -6.663)	2.602 (0.929 -5.236)
0.42	No reps.	0.0581	0.688	0.658	0.187
1.357 (0.551 -2.39)	10.5	0.804 (0.654 -1.213)	0.942 (0.564 -1.366)	0.904 (0.619 -1.192)	0.618 (0.454 -1)
0.0498	0.0104	0.00198	0.0979	0.0324	0.151
0.543 (0.192 -0.808)	0.416 (0.213 -1.018)	0.366 (0.205 -0.668)	0.799 (0.526 -1.23)	1.394 (0.977 -1.663)	1.242 (0.86 -1.75)
0.221	0.181	0.0314	0.0599	0.00708	0.15
0.747 (0.359 -1.226)	0.759 (0.579 -1.488)	0.546 (0.257 -0.896)	0.858 (0.665 -0.985)	1.387 (1.09 -1.582)	1.253 (0.732 -1.825)
0.0204	0.396	0.0165	0.0527	0.00154	0.0546
1.648 (0.857 -2.506)	0.724 (0.283 -1.502)	0.807 (0.69 -0.923)	0.679 (0.469 -0.797)	0.787 (0.694 -0.875)	0.775 (0.52 -1.031)
0.0184	6.66E -05	0.641	0.0137	0.595	0.0551
1.556 (1.196 -2.246)	0.499 (0.421 -0.579)	0.955 (0.795 -1.463)	1.154 (1.023 -1.312)	1.057 (0.894 -1.469)	0.762 (0.469 -1.026)

Table A.1 (continued)

PINK F2	PINK F3	PINK F7	PINK F9	PINK G1	PINK G12
8.55E -05	0.00246	0.000211	0.035	5.34E -05	0.00156
0.15 (0.103 -0.199)	0.174 (0.0861 -0.421)	0.0857 (0.05 -0.162)	0.651 (0.434 -1.099)	1.934 (1.576 -2.217)	3.042 (2.031 -6.465)
0.326	0.885	No reps.	0.483	0.941	0.909
0.822 (0.447 -1.252)	0.958 (0.504 -1.807)	0.592	1.104 (0.642 -1.552)	0.983 (0.461 -1.641)	0.99 (0.806 -1.331)
0.000537	0.0017	0.00144	0.65	0.623	0.0425
0.548 (0.462 -0.628)	0.308 (0.17 -0.502)	0.155 (0.0721 -0.302)	1.046 (0.903 -1.619)	0.96 (0.749 -1.176)	1.446 (1.048 -2.413)
0.139	0.434	0.0619	0.101	0.211	0.519
0.61 (0.248 -1.159)	0.51 (0.136 -1.413)	0.103 (0.0189 -0.417)	0.811 (0.602 -1.126)	1.204 (0.813 -1.644)	1.112 (0.579 -1.844)
0.862	0.148	0.422	0.0613	0.681	0.00436
1.022 (0.745 -1.493)	1.563 (0.936 -2.447)	1.406 (0.516 -3.09)	1.348 (1.05 -1.798)	0.955 (0.715 -1.319)	1.161 (1.062 -1.227)
0.798	0.702	0.236	2.96E -05	0.297	0.213
0.961 (0.482 -1.259)	1.099 (0.393 -1.73)	1.914 (1.488 -2.462)	1.728 (1.563 -1.991)	1.098 (0.86 -1.46)	1.183 (0.707 -1.54)

Table A.1 (continued)

PINK G2	PINK G3	PINK G5	PINK H1	PINK H3	PINK H4
0.0962	0.0017	0.00821	0.000145	8.59E -05	0.024
0.835 (0.583 -1.014)	3.006 (2.111 -6.761)	0.284 (0.184 -0.476)	0.345 (0.285 -0.448)	2.687 (1.947 -3.649)	0.275 (0.112 -0.736)
0.561	0.984	No reps.	NO DATA	0.119	0.309
1.237 (0.747 -2.163)	0.993 (0.517 -2.498)	1.518	NO DATA	0.796 (0.55 -1.053)	1.258 (0.789 -1.804)
0.0749	0.0294	0.00422	0.00235	0.00535	0.207
0.74 (0.435 -0.959)	1.313 (1.012 -1.699)	0.339 (0.153 -0.525)	0.469 (0.293 -0.599)	1.327 (1.118 -1.539)	0.868 (0.556 -1.058)
0.452	0.0153	0.381	0.0425	0.225	0.377
0.807 (0.588 -1.691)	1.705 (1.169 -2.267)	0.577 (0.212 -1.642)	0.415 (0.391 -0.44)	1.209 (0.764 -1.726)	1.233 (0.61 -2.294)
0.00107	0.746	0.0219	0.0235	0.172	1.16E -06
1.767 (1.328 -2.327)	1.026 (0.811 -1.303)	1.531 (0.884 -2.102)	1.363 (1.099 -1.816)	1.198 (0.7 -1.479)	2.644 (2.333 -3.006)
0.00287	0.904	0.434	0.418	0.00702	0.0633
1.572 (1.291 -1.973)	1.014 (0.764 -1.476)	1.123 (0.823 -2.119)	1.293 (0.623 -3.135)	1.377 (1.149 -1.783)	1.164 (0.956 -1.483)

Table A.1 (continued)

PINK H7	PINK H9	PP A7	PP B12	PP B7	PP B8
0.00269	0.000357	0.0123	0.485	0.00376	0.138
0.148 (0.0592 -0.262)	10.54 (5.043 -34.98)	0.38 (0.263 -0.619)	0.751 (0.467 -1.443)	0.563 (0.407 -0.919)	0.641 (0.336 -0.873)
0.227	0.578	NO DATA	NO DATA	No reps.	NO DATA
2.022 (1.556 -2.629)	0.876 (0.477 -1.828)	NO DATA	NO DATA	3.285	NO DATA
0.00409	0.027	0.0345	0.252	0.013	0.34
0.315 (0.156 -0.45)	1.31 (1.018 -1.726)	0.52 (0.322 -1.126)	0.796 (0.546 -1.871)	0.541 (0.329 -0.78)	0.838 (0.575 -1.533)
0.123	0.0933	0.356	No reps.	0.135	No reps.
0.375 (0.0829 -1.042)	1.23 (0.796 -1.494)	1.155 (0.925 -1.405)	0.01*	0.679 (0.442 -1.118)	0.01*
0.441	0.221	0.000936	0.71	0.532	0.548
1.166 (0.82 -1.738)	1.069 (0.886 -1.222)	2.484 (2.384 -2.62)	1.152 (0.863 -1.537)	1.067 (0.78 -1.552)	1.785 (0.91 -3.5)
0.0204	0.0423	0.585	No reps.	0.334	No reps.
1.341 (1.178 -1.586)	1.256 (0.843 -1.464)	1.303 (0.662 -2.726)	2.5	0.822 (0.418 -1.373)	2.837

Table A.1 (continued)

PP B9	PP C2	PP C3	PP C8	PP D1	PP D10
0.0409	0.000517	0.00151	3.40E -05	0.000626	0.000672
0.425 (0.213 -0.694)	0.0905 (0.0435 -0.162)	0.276 (0.221 -0.37)	0.275 (0.251 -0.291)	0.213 (0.146 -0.38)	2.71 (1.473 -3.608)
NO DATA	No reps.	0.615	0.201	No reps.	0.0535
NO DATA	0.961	1.275 (0.897 -1.812)	2.57 (1.887 -3.5)	3.509	0.766 (0.532 -0.939)
0.0114	0.000134	0.0129	0.00862	0.00111	0.331
0.413 (0.197 -0.608)	0.193 (0.105 -0.285)	0.358 (0.207 -0.472)	0.403 (0.182 -0.638)	0.209 (0.102 -0.339)	1.097 (0.838 -1.443)
No reps.	0.0385	0.217	0.179	0.126	0.22
0.0938	0.186 (0.0733 -0.466)	0.518 (0.183 -1.288)	0.442 (0.0925 -1.024)	0.357 (0.124 -0.981)	1.22 (0.766 -1.846)
No reps.	0.907	0.967	0.434	0.883	0.000195
0.461	0.972 (0.46 -1.578)	0.99 (0.458 -1.711)	1.204 (0.881 -2.112)	1.036 (0.6 -1.928)	2.044 (1.658 -2.683)
No reps.	0.752	0.221	1	0.595	0.0827
0.01*	0.964 (0.701 -1.482)	1.253 (0.868 -1.752)	1 (0.497 -1.647)	1.036 (0.907 -1.169)	0.909 (0.823 -1.119)

Table A.1 (continued)

PP D11	PP D3	PP D4	PP D6	PP D7	PP D8
1.10E -05	5.47E -05	0.000506	0.00391	0.011	0.000857
4.554 (3.832 -6.447)	0.364 (0.299 -0.499)	0.391 (0.298 -0.608)	0.208 (0.103 -0.34)	0.238 (0.0745 -0.495)	0.14 (0.0685 -0.248)
0.000895	No reps.	No reps.	0.0119	0.00942	0.000543
0.624 (0.54 -0.711)	3	0.01*	1.478 (1.166 -1.823)	2.076 (1.027 -3.527)	3.442 (3.251 -3.567)
0.183	0.000665	0.0687	1.24E -05	0.77	7.89E -05
1.428 (0.71 -3.244)	0.432 (0.378 -0.477)	0.735 (0.594 -1.075)	0.695 (0.634 -0.73)	0.981 (0.767 -1.186)	0.519 (0.42 -0.59)
0.18	0.0848	0.138	0.233	0.983	0.183
1.165 (0.919 -1.582)	0.44 (0.394 -0.491)	0.81 (0.571 -1.207)	0.884 (0.635 -1.141)	1.004 (0.557 -1.77)	0.752 (0.418 -1.258)
0.21	0.0771	0.194	0.438	0.00223	0.23
0.79 (0.362 -1.016)	1.272 (1.235 -1.31)	0.81 (0.515 -1.035)	1.11 (0.756 -1.575)	1.502 (1.193 -1.919)	1.136 (0.912 -1.607)
0.17	0.04	0.55	0.14	0.134	0.371
0.884 (0.755 -1.077)	1.249 (1.056 -1.498)	0.876 (0.575 -1.334)	1.188 (0.91 -1.713)	1.18 (0.872 -1.509)	0.854 (0.614 -1.482)

Table A.1 (continued)

PP D9	PP E10	PP E2	PP E4	PP E5	PP E6
0.00531	3.02E -05	0.000326	0.043	0.000593	0.00395
0.249 (0.111 -0.45)	0.137 (0.104 -0.188)	0.135 (0.0767 -0.212)	0.503 (0.268 -1.113)	0.183 (0.0793 -0.302)	0.134 (0.0451 -0.272)
0.694	0.434	0.379	0.472	0.121	0.105
1.099 (0.721 -1.732)	1.619 (1.095 -2.394)	1.187 (0.799 -1.991)	0.908 (0.602 -1.274)	1.566 (0.979 -3.395)	1.271 (0.852 -1.667)
1.62E -05	0.0147	0.000281	0.0374	0.0267	0.00506
0.56 (0.483 -0.622)	0.13 (0.01 -0.462)	0.315 (0.213 -0.487)	0.667 (0.461 -1.164)	0.658 (0.425 -1.004)	0.583 (0.421 -0.884)
0.364	0.0676	0.212	0.3	0.215	0.0265
0.784 (0.332 -1.403)	0.262 (0.0532 -0.97)	0.567 (0.226 -1.406)	0.846 (0.502 -1.208)	0.77 (0.555 -1.071)	0.686 (0.475 -1.022)
0.00776	0.661	0.57	0.00536	0.000405	0.000107
1.155 (0.988 -1.246)	1.137 (0.394 -2.681)	1.205 (0.567 -2.957)	1.493 (1.125 -1.952)	1.422 (1.207 -1.558)	1.774 (1.538 -2.205)
0.0133	0.266	0.0984	0.459	0.416	0.0194
0.738 (0.599 -0.88)	0.766 (0.598 -1.073)	1.268 (0.941 -1.779)	1.044 (0.883 -1.191)	1.091 (0.799 -1.388)	1.225 (1.001 -1.572)

Table A.1 (continued)

PP E7	PP E8	PP E9	PP F4	PP F7	PP F9
0.0109	0.00756	0.00222	5.07E -06	0.00116	0.00361
0.346 (0.257 -0.585)	0.241 (0.193 -0.296)	0.268 (0.145 -0.416)	0.211 (0.168 -0.259)	0.308 (0.16 -0.522)	0.234 (0.104 -0.497)
NO DATA	No reps.	0.673	No reps.	NO DATA	0.449
NO DATA	0.01*	0.897 (0.485 -1.372)	1.978	NO DATA	1.118 (0.661 -1.751)
0.0011	0.00312	0.00752	0.00445	0.00312	0.0354
0.31 (0.174 -0.573)	0.334 (0.137 -0.561)	0.556 (0.308 -0.835)	0.22 (0.108 -0.345)	0.463 (0.242 -0.699)	0.657 (0.377 -0.883)
0.138	0.035	0.0037	0.181	0.00254	0.287
0.296 (0.0524 -0.887)	0.415 (0.204 -0.592)	0.554 (0.42 -0.725)	0.386 (0.176 -0.897)	0.58 (0.549 -0.598)	0.859 (0.602 -1.149)
0.101	0.227	0.0292	0.811	0.397	0.00532
1.585 (0.846 -4.339)	1.238 (0.954 -1.834)	1.446 (1.196 -2.209)	0.934 (0.49 -1.815)	1.167 (0.85 -2.127)	1.597 (1.26 -1.995)
0.0181	0.691	0.371	0.584	0.166	0.0296
1.516 (1.177 -1.746)	0.935 (0.682 -1.397)	1.056 (0.965 -1.298)	0.831 (0.652 -1.058)	1.414 (1.166 -1.951)	1.25 (1.034 -1.616)

Table A.1 (continued)

PP G1	PP G2	PP G3	PP G6	PP G7	PP H2
6.37E -05	0.000403	0.406	0.000495	0.000568	3.43E -07
0.118 (0.0915 -0.18)	0.081 (0.0574 -0.2)	0.773 (0.274 -1.365)	0.259 (0.194 -0.371)	0.403 (0.307 -0.571)	0.146 (0.121 -0.177)
No reps.	NO DATA	1.77E -05	0.141	NO DATA	NO DATA
1.095	NO DATA	0.121 (0.0635 -0.154)	0.854 (0.748 -0.916)	NO DATA	NO DATA
3.49E -05	0.000457	3.94E -07	0.000163	0.00282	0.000199
0.245 (0.184 -0.332)	0.154 (0.0741 -0.271)	0.123 (0.0927 -0.14)	0.552 (0.48 -0.679)	0.441 (0.284 -0.595)	0.19 (0.0821 -0.24)
0.0343	0.035	0.535	0.381	No reps.	0.178
0.402 (0.183 -0.831)	0.161 (0.0641 -0.552)	1.189 (0.607 -2.952)	0.867 (0.488 -1.48)	0.01*	0.362 (0.0809 -1.397)
9.74E -05	0.186	0.00631	0.146	0.181	0.689
0.409 (0.375 -0.434)	0.741 (0.473 -1.139)	1.871 (1.304 -2.405)	0.758 (0.416 -1.187)	1.23 (0.955 -1.62)	1.214 (0.628 -2.643)
0.0786	0.487	2.03E -07	0.0159	0.872	0.792
0.798 (0.671 -1.301)	0.915 (0.704 -1.302)	0.116 (0.101 -0.142)	0.558 (0.312 -0.968)	1.09 (0.443 -2.207)	1.112 (0.654 -2.174)

Table A.1 (continued)

PP H6	PP H7	PP H8	Q B12	Q C4	Q C7
0.000378	0.00212	0.000442	0.00019	0.03	3.17E -05
0.104 (0.044 -0.199)	0.597 (0.456 -0.815)	0.26 (0.199 -0.372)	9.661 (3.65 -20.45)	0.706 (0.539 -1.18)	3.818 (2.745 -4.944)
0.0461	0.389	NO DATA	0.0679	No reps.	0.3
1.314 (1.009 -1.97)	1.289 (1.079 -1.54)	NO DATA	1.612 (1.008 -3.146)	19.71	1.386 (0.882 -1.948)
0.0421	0.00055	0.000437	0.0231	0.246	0.00447
0.876 (0.741 -1.006)	0.596 (0.486 -0.703)	0.4 (0.295 -0.476)	1.479 (1.074 -2.286)	0.629 (0.243 -1.038)	2.427 (1.923 -3.122)
0.0994	0.315	0.131	0.144	No reps.	0.012
0.911 (0.751 -1.018)	0.843 (0.592 -1.59)	0.615 (0.26 -1.744)	1.17 (0.919 -1.663)	0.727 (0.0938 -1.36)	2.089 (1.191 -3.609)
0.461	0.908	0.114	0.00315	0.676	0.179
1.379 (0.131 -2.549)	0.988 (0.82 -1.586)	0.879 (0.74 -1.034)	1.261 (1.05 -1.405)	1.159 (0.755 -2.091)	1.669 (0.766 -2.255)
0.27	0.48	0.844	0.0158	0.000332	0.995
1.131 (0.858 -1.725)	1.082 (0.82 -1.61)	1.025 (0.828 -1.196)	1.272 (0.959 -1.49)	1.539 (1.463 -1.604)	1.001 (0.544 -1.62)

Table A.1 (continued)

Q D2	Q D3	Q F6	Q F9	Q G9	Q H3
0.000239	0.000108	0.267	0.0219	0.113	0.000351
0.402 (0.27 -0.52)	6.223 (3.851 -12.21)	0.693 (0.378 -1.659)	2.62 (1.965 -5.016)	0.508 (0.339 -0.802)	0.0255 (0.0106 -0.049)
0.626	0.149	No reps.	NO DATA	NO DATA	0.174
0.842 (0.482 -1.365)	2.264 (1.862 -2.753)	4.961	NO DATA	NO DATA	1.21 (1.036 -1.424)
0.00105	0.000562	0.552	0.0369	0.154	1.51E -05
0.608 (0.431 -0.699)	2.067 (1.806 -2.713)	1.17 (0.751 -1.485)	1.453 (1.214 -1.803)	0.697 (0.587 -0.961)	0.199 (0.138 -0.26)
0.145	0.00239	No reps.	No reps.	No reps.	0.00871
0.671 (0.377 -1.37)	1.767 (1.158 -2.381)	0.01*	2.816	0.01*	0.286 (0.131 -0.553)
0.412	0.198	NO DATA	0.289	0.279	0.965
0.849 (0.46 -1.394)	1.37 (0.507 -2.252)	NO DATA	0.899 (0.694 -1.251)	1.234 (1.118 -1.362)	1.008 (0.629 -1.772)
0.0995	0.0196	No reps.	0.839	0.311	0.314
1.35 (1.015 -1.741)	1.414 (0.962 -1.972)	1.889	1.034 (0.71 -1.437)	1.328 (0.892 -1.831)	1.198 (0.831 -1.767)

Table A.1 (continued)

Q H5	QQ A10	QQ A6	QQ B1	QQ B5	QQ B6
9.30E -05	7.70E -05	0.00709	0.000478	0.225	0.972
0.0673 (0.0397 -0.102)	5.278 (2.752 -7.484)	29.11 (8.151 -171)	0.182 (0.113 -0.245)	0.759 (0.636 -1.042)	0.997 (0.82 -1.147)
0.284	0.255	0.701	NO DATA	NO DATA	NO DATA
1.299 (0.998 -1.837)	0.894 (0.718 -1.048)	0.943 (0.493 -1.421)	NO DATA	NO DATA	NO DATA
4.76E -05	0.00134	0.0145	0.000261	0.00245	0.0654
0.298 (0.245 -0.403)	1.975 (1.501 -3.206)	1.632 (1.143 -2.756)	0.217 (0.115 -0.334)	3.054 (2.734 -3.24)	0.73 (0.597 -1)
0.00311	0.0402	0.283	0.0454	NO DATA	No reps.
0.425 (0.275 -0.689)	1.467 (0.97 -2.665)	1.133 (0.897 -1.608)	0.251 (0.16 -0.449)	NO DATA	0.237
0.817	0.832	0.0275	0.612	NO DATA	No reps.
0.961 (0.548 -1.308)	1.044 (0.702 -1.467)	0.77 (0.607 -0.898)	0.786 (0.556 -1.11)	NO DATA	0.53
0.657	0.0814	0.271	0.844	NO DATA	No reps.
1.092 (0.65 -1.691)	0.889 (0.749 -1.004)	0.926 (0.776 -1.154)	1.068 (0.82 -1.392)	NO DATA	2

Table A.1 (continued)

QQ C2	QQ C9	QQ D1	QQ D12	QQ D4	QQ D6
0.000474	0.0522	0.189	0.842	0.000158	0.0171
0.132 (0.0766 -0.224)	2.331 (0.946 -6.424)	0.658 (0.396 -1.067)	1.035 (0.729 -2.02)	0.244 (0.166 -0.295)	0.503 (0.391 -0.74)
0.803	0.311	NO DATA	NO DATA	NO DATA	NO DATA
0.852 (0.517 -1.406)	0.864 (0.565 -1.219)	NO DATA	NO DATA	NO DATA	NO DATA
0.000532	0.103	0.883	0.0185	0.000264	0.00753
0.415 (0.286 -0.557)	1.219 (0.902 -1.848)	1.03 (0.781 -1.71)	0.575 (0.437 -0.737)	0.269 (0.154 -0.462)	0.813 (0.67 -0.93)
0.0434	0.447	No reps.	NO DATA	0.0234	0.0294
0.615 (0.407 -1.309)	1.077 (0.842 -1.411)	0.01*	NO DATA	0.504 (0.29 -0.952)	2.204 (1.748 -2.823)
0.0114	0.000546	NO DATA	0.00273	0.0707	0.372
0.512 (0.296 -0.859)	0.707 (0.631 -0.812)	NO DATA	1.736 (1.608 -2.07)	0.632 (0.433 -0.884)	0.652 (0.3 -1.78)
0.0668	0.0395	0.375	0.0995	0.537	0.00818
0.657 (0.629 -0.687)	0.693 (0.382 -0.925)	3.404 (1.5 -7.723)	1.427 (1.193 -1.799)	0.729 (0.31 -1.183)	0.499 (0.368 -0.732)

Table A.1 (continued)

QQ E1	QQ E2	QQ F1	QQ F3	QQ G10	QQ G4
0.909	0.00136	0.00373	3.66E -05	0.000647	0.000744
1.02 (0.738 -1.4)	0.446 (0.291 -0.656)	3.124 (1.65 -4.751)	2.018 (1.743 -2.417)	0.47 (0.393 -0.617)	6.701 (4.102 -21.45)
No reps.	NO DATA	0.437	0.455	0.0868	0.44
0.881	NO DATA	1.264 (0.702 -2.414)	0.878 (0.624 -1.149)	1.32 (1.271 -1.371)	1.116 (0.761 -1.927)
0.146	0.00148	0.00232	0.16	0.0246	0.333
1.355 (1.187 -1.761)	0.501 (0.3 -0.591)	1.406 (1.178 -1.688)	1.096 (0.918 -1.228)	0.641 (0.453 -0.947)	1.126 (0.706 -1.492)
0.0283	No reps.	0.0413	0.0267	0.0651	0.826
3.851 (3.627 -4.089)	0.204	1.543 (0.867 -2.312)	1.161 (0.95 -1.307)	0.818 (0.632 -1.032)	0.929 (0.306 -2.66)
0.666	0.0319	0.558	0.84	0.041	0.255
1.024 (0.867 -1.146)	0.83 (0.75 -0.908)	0.875 (0.526 -1.755)	0.979 (0.695 -1.458)	0.691 (0.51 -0.957)	1.59 (0.725 -5.431)
0.318	0.291	0.235	0.37	0.000573	0.116
1.275 (0.881 -1.998)	1.2 (0.936 -1.437)	0.87 (0.627 -1.278)	0.828 (0.372 -1.367)	0.548 (0.49 -0.676)	0.762 (0.469 -1.022)

Table A.1 (continued)

QQ H12	RR A2	RR B11	RR B12	RR B9	RR C3
0.00551	0.000454	0.0372	6.76E -05	0.00163	0.000207
0.534 (0.355 -0.725)	9.105 (4.972 -23.86)	0.287 (0.267 -0.309)	5.179 (3.087 -7.777)	0.408 (0.212 -0.574)	9.937 (4.674 -25.26)
No reps.	0.576	NO DATA	0.185	NO DATA	0.0924
7.5	1.032 (0.894 -1.299)	NO DATA	0.69 (0.352 -1.242)	NO DATA	0.654 (0.336 -0.925)
0.000145	0.00501	0.00355	0.0748	0.00153	0.0131
0.582 (0.49 -0.678)	1.46 (1.155 -1.849)	0.447 (0.338 -0.508)	1.773 (1.272 -2.114)	0.36 (0.181 -0.536)	1.851 (1.262 -2.885)
0.313	0.374	No reps.	0.0222	0.0459	0.135
0.662 (0.268 -1.963)	1.102 (0.855 -1.422)	0.01*	1.636 (1.189 -2.968)	0.279 (0.255 -0.306)	1.484 (0.776 -3.48)
0.0116	0.108	No reps.	0.921	0.15	0.786
1.349 (1.059 -1.663)	0.831 (0.635 -1.145)	1	0.984 (0.718 -1.656)	1.75 (1.53 -2.001)	1.026 (0.841 -1.418)
0.0322	0.566	NO DATA	0.866	0.111	0.841
1.466 (1.061 -1.95)	0.96 (0.803 -1.262)	NO DATA	0.985 (0.764 -1.291)	1.762 (1.225 -2.5)	0.986 (0.824 -1.234)

Table A.1 (continued)

RR D11	RR D4	RR F5	RR G1	RR G5	RR G7
4.94E -05	3.57E -05	1.61E -05	0.0112	0.019	0.00683
6.828 (5.128 -11.49)	3.782 (2.871 -5.042)	10.18 (7.304 -17.86)	0.588 (0.467 -0.713)	12.78 (6.273 -18.5)	9.313 (0.785 -54.46)
0.0293	0.794	0.966	No reps.	0.555	0.224
0.619 (0.526 -0.695)	0.964 (0.614 -1.673)	1.004 (0.686 -1.238)	6.5	0.902 (0.626 -1.598)	0.78 (0.356 -1.231)
0.0567	0.0426	0.0106	0.025	0.000263	0.498
1.885 (0.876 -4.369)	1.329 (0.997 -1.685)	1.585 (1.145 -2.657)	0.693 (0.616 -0.743)	2.115 (1.949 -2.255)	0.882 (0.491 -1.229)
0.127	0.0892	0.0709	No reps.	0.0138	0.75
2.311 (0.987 -12.22)	1.243 (0.949 -1.881)	1.2 (0.946 -1.683)	0.0724	2.118 (1.38 -2.569)	1.067 (0.508 -1.522)
0.0891	0.492	0.92	0.028	0.933	0.000371
1.258 (0.89 -1.788)	1.099 (0.824 -1.728)	0.989 (0.734 -1.438)	1.208 (1.166 -1.288)	1.014 (0.763 -1.707)	2.12 (1.57 -2.748)
0.452	0.0168	0.0624	No reps.	0.956	0.201
1.052 (0.864 -1.213)	1.376 (1.268 -1.457)	0.928 (0.843 -1.024)	3.648	1.007 (0.666 -1.405)	0.788 (0.381 -1.092)

Table A.1 (continued)

RR H5	S A1	S A2	S B1	S B2	S B7
0.000479	0.125	4.83E -06	0.117	0.000284	0.000347
0.323 (0.245 -0.462)	0.656 (0.463 -0.985)	13.19 (9.07 -20.73)	1.824 (0.954 -5.351)	6.52 (3.256 -11.22)	2.22 (1.471 -2.684)
No reps.	0.00901	8.04E -05	0.506	0.603	0.364
2.164	0.232 (0.157 -0.465)	2.143 (1.808 -2.802)	0.956 (0.801 -1.171)	0.948 (0.696 -1.258)	1.176 (0.662 -2.101)
0.00822	0.000375	0.00368	0.455	0.000136	0.654
0.451 (0.237 -0.553)	0.394 (0.247 -0.498)	2.397 (1.696 -3.583)	1.077 (0.877 -1.434)	1.866 (1.64 -2.069)	1.049 (0.788 -1.296)
0.0541	0.0115	0.00698	0.386	0.0242	0.345
0.304 (0.212 -0.538)	0.163 (0.0601 -0.548)	1.624 (1.103 -2.218)	1.052 (0.903 -1.252)	2.126 (1.451 -4.387)	0.941 (0.806 -1.151)
0.026	0.000348	0.00619	0.00149	0.00478	0.627
0.733 (0.723 -0.742)	0.107 (0.0439 -0.221)	1.511 (1.194 -2.018)	0.757 (0.659 -0.901)	0.769 (0.605 -0.87)	0.911 (0.525 -1.62)
No reps.	0.69	0.0359	0.0278	0.0269	0.645
2.271	1.142 (0.788 -2.011)	1.338 (0.973 -1.72)	0.87 (0.803 -0.948)	0.794 (0.591 -0.926)	1.064 (0.814 -1.927)

Table A.1 (continued)

S D4	S E12	S E2	S E9	S F11	S G2
0.284	0.000391	0.00582	0.00399	0.000484	0.00802
1.061 (0.942 -1.223)	0.52 (0.472 -0.653)	2.297 (1.39 -3.998)	0.468 (0.314 -0.661)	14.88 (5.933 -51.5)	5.364 (2.099 -26.64)
No reps.	0.12	0.608	NO DATA	0.422	0.673
0.01*	1.939 (1.709 -2.2)	0.924 (0.635 -1.364)	NO DATA	0.772 (0.385 -1.219)	0.933 (0.511 -1.525)
0.671	0.037	0.479	0.0193	0.000318	0.123
0.949 (0.609 -1.169)	0.676 (0.411 -0.843)	1.1 (0.62 -1.442)	0.761 (0.588 -1.027)	2.111 (1.717 -2.539)	1.24 (0.957 -1.721)
0.579	0.055	0.857	0.15	0.0854	0.0981
0.913 (0.812 -1.026)	0.597 (0.317 -0.997)	1.014 (0.841 -1.276)	0.756 (0.384 -1.178)	1.189 (0.942 -1.526)	1.182 (0.98 -1.553)
0.978	0.114	0.937	0.682	0.0698	5.00E -05
0.994 (0.572 -1.621)	1.472 (0.75 -2.449)	0.987 (0.734 -1.444)	1.096 (0.593 -2.055)	1.299 (1.022 -2.123)	1.465 (1.283 -1.58)
0.338	0.567	0.523	0.0184	0.465	0.673
0.945 (0.854 -1.043)	1.124 (0.682 -1.721)	0.964 (0.755 -1.088)	1.35 (1.04 -1.669)	1.098 (0.681 -1.451)	0.843 (0.01 -1.977)

Table A.1 (continued)

S G3	T A1	T A11	T A2	T B10	T B3
0.0801	0.5	0.000572	0.000387	0.000927	0.00912
7.968 (1.067 -12.89)	1.064 (1 -1.132)	5.153 (2.299 -8.743)	4.419 (2.094 -7.215)	4.543 (2.937 -8.258)	0.47 (0.37 -0.668)
0.412	NO DATA	No reps.	0.0878	0.372	No reps.
1.198 (0.642 -2.027)	NO DATA	1.293	1.521 (0.937 -2.025)	1.12 (0.971 -1.357)	0.01*
0.00228	0.000341	0.0045	0.0123	0.00181	0.0062
1.462 (1.187 -1.7)	1.811 (1.646 -1.884)	1.661 (1.488 -1.955)	1.861 (1.447 -2.359)	2.331 (1.58 -3.22)	0.433 (0.235 -0.554)
0.0245	No reps.	0.188	0.0136	0.0726	0.0369
1.276 (1.023 -1.53)	3.309	1.698 (0.991 -2.248)	1.523 (1.059 -2.373)	1.987 (1.178 -3.422)	0.623 (0.354 -0.832)
0.0119	NO DATA	0.486	0.00961	0.0188	0.304
1.712 (1.016 -2.537)	NO DATA	1.092 (0.715 -1.35)	1.52 (1.114 -2.116)	0.784 (0.592 -0.941)	0.819 (0.526 -1.394)
0.411	No reps.	0.0516	0.000582	0.124	0.834
0.845 (0.43 -1.26)	1.604	2.394 (1.47 -5.323)	1.363 (1.285 -1.509)	0.784 (0.502 -1.303)	0.959 (0.709 -1.308)

Table A.1 (continued)

T B7	T C1	T C12	T C3	T C7	T C8
0.161	5.53E -05	0.000515	0.0527	0.115	1.94E -05
0.592 (0.272 -1.327)	2.749 (2.128 -3.324)	0.45 (0.32 -0.582)	1.56 (1.121 -2.782)	0.406 (0.306 -0.853)	8.804 (5.257 -14.63)
0.0334	0.784	NO DATA	0.0423	0.105	0.219
1.552 (0.968 -2.651)	0.928 (0.291 -1.585)	NO DATA	1.708 (1.369 -1.999)	1.406 (1.2 -1.78)	1.426 (0.993 -2.688)
0.134	0.208	0.00236	0.135	0.104	0.000133
1.128 (0.954 -1.485)	1.287 (0.753 -1.995)	0.548 (0.419 -0.729)	1.174 (0.818 -1.444)	0.792 (0.638 -1.41)	1.778 (1.495 -2.002)
0.149	0.869	No reps.	0.04	0.128	0.0844
0.936 (0.86 -1.065)	1.081 (0.254 -3.501)	0.126	1.647 (0.942 -3.22)	0.616 (0.452 -0.874)	1.306 (0.902 -1.656)
0.154	0.0105	0.988	0.676	0.727	0.06
1.169 (0.751 -1.413)	0.439 (0.284 -0.772)	1.007 (0.696 -1.459)	0.921 (0.416 -1.481)	1.025 (0.759 -1.342)	1.109 (1.015 -1.285)
0.0578	0.000362	0.0304	0.00823	0.35	0.359
1.306 (0.788 -1.603)	0.58 (0.484 -0.767)	1.619 (1.19 -2.572)	0.652 (0.424 -0.885)	0.716 (0.295 -1.138)	0.929 (0.756 -1.127)

Table A.1 (continued)

T D11	T E10	T E2	T E3	T E4	T E6
0.188	0.138	0.000924	0.000416	3.37E -05	5.37E -05
0.734 (0.373 -1.005)	0.82 (0.614 -1.052)	3.47 (2.271 -5.096)	17.67 (7.522 -58.93)	5.287 (3 -6.527)	8.983 (6.039 -17.53)
0.00453	0.00199	0.982	0.639	0.583	0.505
2.267 (1.796 -3)	2.419 (2.239 -2.547)	0.994 (0.369 -1.926)	1.051 (0.764 -1.404)	1.323 (0.566 -2.293)	1.198 (0.654 -1.883)
0.0774	0.212	0.000552	0.017	0.011	2.17E -05
1.563 (1.008 -3.126)	1.208 (0.846 -1.745)	1.955 (1.499 -2.707)	1.464 (1.146 -2.015)	3.068 (2.016 -8.197)	2.211 (1.738 -2.488)
0.0231	0.745	0.00557	0.233	0.0442	0.015
1.364 (1.176 -1.579)	1.091 (0.685 -1.407)	1.57 (0.999 -1.993)	1.185 (0.831 -1.801)	2.103 (1.551 -2.692)	1.415 (0.982 -1.99)
0.446	0.847	0.0656	0.109	0.179	0.0146
1.081 (0.793 -1.319)	1.034 (0.736 -1.89)	1.308 (1.03 -1.881)	0.895 (0.744 -1.094)	1.185 (0.819 -1.596)	1.428 (1.126 -1.994)
0.662	0.824	0.085	0.693	0.235	0.000973
1.062 (0.724 -1.532)	0.983 (0.815 -1.262)	1.357 (0.969 -2.46)	1.049 (0.804 -1.588)	1.244 (0.828 -2.194)	1.474 (1.22 -1.689)

Table A.1 (continued)

T F10	T F2	T F5	T F6	T F7	T G2
0.00914	0.0372	0.0102	0.00226	0.809	0.0332
5.473 (2.825 -10.74)	8.755 (1.891 -73.1)	3.236 (1.828 -4.728)	0.571 (0.461 -0.732)	1.03 (0.681 -1.297)	1.467 (1.038 -2.523)
No reps.	0.392	0.272	0.805	0.133	0.735
1.333	0.871 (0.586 -1.377)	0.82 (0.467 -1.048)	1.064 (0.875 -1.292)	0.534 (0.407 -0.89)	1.053 (0.687 -1.582)
0.00383	0.982	0.0823	0.00695	0.448	0.391
1.765 (1.152 -2.457)	1.002 (0.846 -1.324)	1.525 (0.957 -2.718)	0.747 (0.572 -0.906)	0.905 (0.718 -1.243)	1.081 (0.788 -1.249)
0.11	0.221	0.585	0.124	0.517	0.0149
1.605 (1.177 -2.953)	0.909 (0.774 -1.096)	1.136 (0.649 -2.007)	0.673 (0.291 -1.18)	0.886 (0.477 -1.352)	0.57 (0.418 -0.913)
0.251	0.339	0.00079	0.283	0.0128	0.00277
0.875 (0.705 -1.026)	0.692 (0.198 -1.468)	1.376 (1.273 -1.542)	1.085 (0.861 -1.327)	1.448 (1.047 -2.096)	1.957 (1.195 -2.876)
0.482	0.497	0.00437	0.156	0.000783	0.0011
0.926 (0.646 -1.226)	1.093 (0.728 -1.648)	1.492 (1.116 -1.992)	1.448 (0.646 -2.271)	1.82 (1.447 -2.445)	1.908 (1.45 -2.697)

Table A.1 (continued)

T G6	T G9	T H3	T H9	TT A2	TT A9
0.44	0.000878	0.0155	0.00344	0.0798	0.00285
0.875 (0.505 -1.295)	5.014 (2.455 -11.69)	0.415 (0.316 -0.697)	0.522 (0.379 -0.77)	8.55 (1.12 -285)	0.514 (0.425 -0.665)
0.0399	0.589	0.108	0.271	0.469	0.53
0.716 (0.492 -0.978)	0.905 (0.516 -1.418)	1.38 (0.993 -1.976)	0.627 (0.254 -1.433)	0.902 (0.527 -1.365)	0.522 (0.255 -1.068)
0.238	0.00624	0.0785	0.00396	0.192	0.0692
0.831 (0.476 -1.122)	1.912 (1.389 -3.709)	0.724 (0.535 -0.907)	0.663 (0.592 -0.841)	1.166 (0.919 -1.572)	0.703 (0.386 -1.135)
0.922	0.127	0.0866	0.0377	0.35	0.017
1.019 (0.571 -1.588)	1.239 (0.914 -1.714)	0.706 (0.352 -0.988)	0.467 (0.204 -0.969)	1.043 (0.944 -1.258)	0.593 (0.479 -0.801)
5.37E -05	0.949	0.362	0.0566	0.0112	0.148
2.291 (1.773 -2.797)	0.992 (0.733 -1.356)	0.835 (0.541 -1.632)	1.329 (0.952 -2.092)	0.801 (0.628 -0.905)	1.121 (0.924 -1.292)
0.697	0.432	0.348	0.00138	0.134	0.0439
1.039 (0.739 -1.46)	0.804 (0.43 -1.455)	1.329 (0.835 -1.754)	1.777 (1.346 -2.231)	0.865 (0.678 -1.232)	1.294 (0.973 -1.77)

Table A.1 (continued)

TT B12	TT B6	TT B9	TT C1	TT C12	TT D10
0.00489	0.00939	0.00429	0.039	0.0813	0.00306
2.372 (1.405 -3.405)	0.619 (0.378 -0.779)	0.275 (0.189 -0.387)	0.563 (0.35 -0.729)	1.534 (1.074 -2.759)	0.494 (0.408 -0.748)
No reps.	0.336	NO DATA	No reps.	0.945	No reps.
0.01*	1.148 (0.876 -1.775)	NO DATA	0.745	1.009 (0.703 -1.649)	4.022
0.00102	0.765	0.00837	0.393	0.243	0.013
1.835 (1.311 -2.286)	0.976 (0.762 -1.297)	0.537 (0.398 -0.6)	0.897 (0.645 -1.483)	1.144 (0.855 -1.619)	0.55 (0.428 -0.926)
0.092	0.0763	1	0.495	0.993	No reps.
1.997 (1.208 -4.31)	0.887 (0.806 -1.043)	0.01*	1.379 (0.926 -3)	1.003 (0.0781 -2.026)	0.465
0.348	0.896	0.207	0.853	0.000721	0.0933
0.818 (0.506 -1.459)	0.987 (0.74 -1.302)	1.116 (1.075 -1.158)	1.026 (0.684 -1.387)	0.699 (0.557 -0.754)	0.713 (0.587 -1.068)
0.267	0.00372	NO DATA	0.000209	0.0444	0.207
0.64 (0.396 -2.541)	1.451 (1.13 -1.723)	NO DATA	2.106 (1.589 -2.82)	0.712 (0.469 -0.901)	2.542 (1.856 -3.482)

Table A.1 (continued)

TT D11	TT D6	TT E12	TT E5	TT E9	TT F3
0.0633	0.000517	0.00554	0.00423	9.28E -05	0.0304
0.646 (0.369 -0.993)	0.501 (0.424 -0.635)	0.466 (0.268 -0.567)	0.474 (0.315 -0.787)	0.55 (0.472 -0.669)	2.351 (1.094 -5.131)
0.00214	0.361	NO DATA	0.118	0.0398	0.206
2.196 (1.754 -3.339)	0.658 (0.504 -0.859)	NO DATA	1.196 (1.157 -1.237)	0.513 (0.39 -0.596)	1.208 (0.742 -1.717)
0.676	0.215	0.0907	0.174	0.00604	0.223
0.948 (0.656 -1.249)	0.936 (0.813 -1.035)	1.481 (0.819 -3.11)	0.728 (0.366 -1.587)	0.62 (0.532 -0.882)	0.846 (0.553 -1.322)
0.231	0.251	0.26	0.956	0.0179	0.817
1.156 (0.873 -1.686)	0.725 (0.51 -1.336)	1.071 (0.983 -1.138)	1.02 (0.385 -1.659)	0.605 (0.429 -1.086)	1.05 (0.524 -1.727)
0.958	0.198	0.668	0.00899	0.0901	0.000491
1.008 (0.695 -1.402)	1.395 (0.673 -2.693)	1.098 (0.628 -1.605)	1.337 (1.092 -1.569)	1.228 (0.877 -1.648)	3.333 (2.595 -5.051)
0.000901	0.334	0.0264	0.00636	0.163	0.0299
1.999 (1.629 -2.499)	1.11 (0.919 -1.594)	1.476 (1.452 -1.5)	1.739 (1.195 -2.292)	1.422 (0.802 -2.27)	1.481 (0.851 -1.952)

Table A.1 (continued)

TT F9	TT G10	TT G12	TT G3	TT G4	TT G6
0.000533	0.00385	0.355	0.00139	0.0002	9.73E -06
0.247 (0.159 -0.348)	3.032 (1.54 -5.879)	1.306 (0.499 -2.933)	0.647 (0.528 -0.95)	0.316 (0.222 -0.427)	1.989 (1.714 -2.278)
0.99	0.0464	5.83E -05	NO DATA	No reps.	0.916
1.007 (0.386 -1.882)	1.367 (0.799 -1.705)	2.57 (1.999 -3.209)	NO DATA	1.671	1.018 (0.483 -1.422)
0.000996	0.0796	0.000679	0.0144	0.0055	0.00185
0.268 (0.141 -0.407)	1.207 (0.931 -1.599)	1.606 (1.21 -1.861)	0.52 (0.4 -0.738)	0.413 (0.177 -0.642)	1.198 (1.109 -1.336)
0.125	0.111	0.0266	No reps.	0.138	0.651
0.334 (0.0979 -1.006)	1.22 (0.836 -1.513)	1.539 (0.981 -2.707)	0.536	0.216 (0.106 -0.772)	1.07 (0.691 -1.643)
0.578	1.47E -06	0.000429	0.153	0.0421	0.00457
0.892 (0.639 -1.815)	2.012 (1.83 -2.202)	2.989 (1.968 -4.72)	0.891 (0.724 -1.114)	1.544 (1.24 -2.153)	1.435 (1.149 -1.855)
0.993	0.517	0.0264	0.202	0.486	0.00042
0.997 (0.468 -2.122)	1.053 (0.826 -1.326)	1.368 (0.95 -1.842)	1.249 (0.817 -2.063)	1.614 (1.021 -2.552)	1.523 (1.314 -1.805)

Table A.1 (continued)

TT G9	TT H1	TT H5	TT H6	U A1	U A4
0.0885	0.00686	0.000439	0.000249	0.0136	0.000859
0.706 (0.591 -0.866)	0.342 (0.168 -0.524)	0.384 (0.294 -0.496)	1.701 (1.372 -2.042)	4.359 (1.629 -14)	3.472 (2.853 -4.343)
No reps.	NO DATA	NO DATA	0.197	0.449	NO DATA
0.412	NO DATA	NO DATA	0.706 (0.463 -1.258)	1.028 (1.004 -1.052)	NO DATA
0.00233	0.00408	0.00494	0.62	0.00276	0.00504
0.498 (0.448 -0.616)	0.636 (0.442 -0.874)	0.332 (0.22 -0.414)	0.934 (0.728 -1.63)	2.397 (1.577 -3.289)	2.385 (1.3 -4.779)
No reps.	No reps.	No reps.	0.306	0.0231	0.0669
0.365	0.392	0.239	0.509 (0.19 -1.624)	2.23 (1.437 -4.467)	2.687 (2.421 -2.982)
0.199	0.215	0.783	0.0888	0.013	0.958
2.294 (1.754 -3)	1.913 (1.158 -3.869)	0.963 (0.698 -1.232)	1.391 (0.81 -2.145)	1.429 (1.079 -2.096)	0.989 (0.576 -1.695)
No reps.	0.113	0.615	0.00115	0.19	No reps.
1	1.449 (0.799 -2.393)	1.175 (0.681 -1.571)	1.93 (1.548 -2.917)	1.191 (0.847 -1.726)	1.363

Table A.1 (continued)

U B8	U C5	U C7	U D5	U D9	U E4
1.35E -05	0.0681	0.0381	5.04E -05	0.0312	0.11
15.24 (10.64 -19.74)	2.645 (0.722 -6.988)	0.74 (0.539 -0.99)	5.915 (4.422 -11.24)	1.63 (1.073 -2.922)	2.135 (0.81 -3.511)
0.025	0.192	No reps.	0.375	0.183	No reps.
1.789 (1.295 -3.736)	0.714 (0.29 -1.385)	2.687	0.746 (0.254 -1.521)	0.783 (0.507 -1.231)	0.01*
0.000705	0.0122	0.00608	0.00496	0.398	0.0756
2.149 (1.513 -3.229)	0.656 (0.464 -0.844)	0.53 (0.334 -0.675)	1.505 (1.114 -1.888)	1.123 (0.788 -1.656)	1.835 (1.05 -3.198)
0.0117	0.556	No reps.	0.0417	0.556	0.548
1.629 (1.259 -2.929)	0.896 (0.496 -1.457)	0.822	1.167 (0.991 -1.396)	0.977 (0.831 -1.069)	1.562 (0.93 -2.625)
0.228	0.0715	0.0608	0.582	0.0166	0.337
1.213 (0.744 -1.69)	1.625 (0.739 -2.179)	0.856 (0.704 -0.984)	0.965 (0.798 -1.158)	0.779 (0.588 -0.985)	1.464 (0.818 -2.287)
0.466	0.245	0.391	0.983	0.0432	0.471
1.08 (0.688 -1.316)	1.167 (0.665 -1.475)	0.89 (0.61 -1.397)	0.999 (0.804 -1.224)	0.766 (0.562 -1.017)	1.162 (0.772 -1.609)

Table A.1 (continued)

U E6	U F5	U G1	U G11	U G7	U H1
0.00969	0.00163	0.000646	0.00022	0.00497	0.000375
0.416 (0.267 -0.699)	0.283 (0.125 -0.446)	6.671 (3.862 -10.81)	2.218 (1.595 -2.79)	3.312 (2.437 -4.707)	13.78 (6.668 -41.5)
0.495	0.711	0.0951	0.209	No reps.	0.329
0.875 (0.385 -1.324)	0.96 (0.599 -1.226)	1.86 (0.908 -4.449)	1.185 (0.861 -1.687)	1.553	1.419 (0.772 -3.541)
0.669	0.00846	0.0214	0.782	0.00116	0.0306
0.958 (0.76 -1.424)	0.733 (0.532 -0.866)	2.243 (1.125 -6.79)	1.037 (0.687 -1.346)	1.896 (1.626 -2.03)	1.766 (0.987 -3.002)
0.0219	0.224	0.0149	0.204	No reps.	0.758
0.882 (0.794 -0.987)	0.755 (0.385 -1.309)	2.095 (1.263 -4.072)	1.403 (0.774 -2.531)	1.913	0.864 (0.154 -2.503)
0.772	0.00376	0.0776	0.00474	0.0555	0.0936
1.015 (0.866 -1.14)	1.451 (1.254 -1.687)	1.832 (0.662 -3.161)	2.267 (1.553 -2.969)	1.311 (1.04 -1.854)	0.756 (0.579 -1.162)
0.814	0.0154	0.0117	0.934	0.858	0.0683
1.022 (0.756 -1.469)	1.227 (1.02 -1.509)	1.487 (1.072 -2.276)	1.02 (0.38 -1.873)	0.98 (0.608 -1.25)	1.213 (0.871 -1.504)

Table A.1 (continued)

U H2	U H3	U H5	UU A11	UU A4	UU A9
0.0164	0.009	5.80E -06	0.00157	0.0111	0.000153
0.338 (0.164 -0.575)	4.033 (1.075 -11.41)	0.425 (0.369 -0.494)	9.46 (4.65 -21.5)	9.563 (2.102 -39.5)	3.465 (2.378 -5.484)
0.57	0.549	NO DATA	0.589	0.445	0.0014
0.943 (0.696 -1.194)	0.867 (0.617 -1.611)	NO DATA	0.949 (0.782 -1.309)	1.071 (0.904 -1.539)	0.394 (0.341 -0.459)
0.15	0.000101	8.35E -06	0.00285	0.0292	0.266
0.845 (0.645 -1.113)	1.63 (1.532 -1.76)	0.334 (0.266 -0.408)	1.522 (1.289 -2.12)	1.361 (1.008 -1.78)	1.399 (1.024 -2.916)
0.814	0.603	0.24	0.195	0.447	0.0483
0.983 (0.756 -1.16)	1.087 (0.629 -1.463)	0.348 (0.126 -1.129)	1.145 (0.92 -1.505)	1.073 (0.857 -1.551)	2.74 (1.396 -6.27)
0.0983	0.586	0.57	0.324	0.00762	0.776
0.908 (0.728 -1.01)	0.934 (0.789 -1.456)	0.892 (0.5 -1.335)	1.094 (0.834 -1.376)	0.848 (0.705 -0.905)	1.096 (0.516 -2.834)
0.338	0.804	0.929	0.308	0.295	0.00696
0.943 (0.813 -1.172)	0.959 (0.569 -1.809)	1.016 (0.642 -1.356)	1.17 (0.726 -1.784)	1.072 (0.938 -1.382)	1.459 (1.179 -1.962)

Table A.1 (continued)

UU B5	UU B6	UU B7	UU B9	UU C10	UU C12
0.000165	0.000275	2.81E -06	0.000803	0.065	0.0462
2.593 (1.928 -3.912)	3.679 (2.829 -5.472)	0.414 (0.391 -0.441)	2.516 (1.758 -3.187)	1.458 (1.005 -1.814)	15.94 (2.786 -188)
0.422	0.176	NO DATA	0.187	No reps.	0.0308
1.15 (0.655 -1.715)	0.78 (0.534 -1.001)	NO DATA	0.734 (0.338 -1.158)	0.01*	0.814 (0.635 -0.936)
0.0409	0.0309	0.895	0.106	0.654	0.084
1.434 (0.848 -2.151)	1.498 (1.043 -2.214)	0.983 (0.616 -1.387)	1.242 (0.853 -1.588)	1.066 (0.687 -1.913)	1.274 (0.946 -1.88)
0.311	0.0179	0.494	0.0285	0.0562	0.117
1.184 (0.834 -2.314)	1.287 (1.033 -1.657)	0.779 (0.388 -1.454)	1.696 (1.195 -3.059)	2.993 (1.892 -4.84)	1.194 (0.957 -1.589)
0.0763	0.653	0.188	0.0257	0.373	0.000427
1.144 (0.977 -1.412)	0.933 (0.655 -1.467)	0.917 (0.815 -1.188)	0.714 (0.565 -0.951)	1.526 (0.0863 -4.27)	0.691 (0.64 -0.765)
0.275	0.275	0.236	0.529	0.159	0.15
1.116 (0.777 -1.455)	1.08 (0.89 -1.32)	1.222 (1.028 -1.538)	0.958 (0.784 -1.242)	0.697 (0.4 -1.272)	0.918 (0.745 -1.03)

Table A.1 (continued)

UU C9	UU D3	UU D5	UU D6	UU E1	UU E12
0.00599	0.0513	0.00156	0.025	0.000188	0.00113
0.534 (0.351 -0.784)	0.657 (0.507 -0.937)	0.425 (0.229 -0.568)	0.516 (0.269 -0.926)	1.853 (1.497 -2.232)	4.527 (2.006 -9.763)
0.277	No reps.	NO DATA	NO DATA	0.734	0.0129
1.136 (1.071 -1.206)	0.01*	NO DATA	NO DATA	0.945 (0.606 -1.156)	1.411 (0.956 -1.727)
0.0292	0.493	0.00138	0.399	0.00354	0.00364
0.65 (0.503 -1.032)	1.146 (0.574 -1.847)	0.571 (0.425 -0.79)	1.107 (0.638 -1.295)	1.925 (1.454 -2.354)	1.5 (1.161 -1.661)
0.128	0.625	0.0898	0.431	0.438	0.222
0.896 (0.824 -0.957)	1.265 (0.889 -1.798)	0.52 (0.394 -0.786)	1.439 (1.074 -1.927)	1.275 (0.849 -2.027)	1.439 (0.523 -3.287)
0.26	0.0348	0.159	0.0765	0.457	8.79E -08
0.64 (0.283 -1.124)	0.719 (0.631 -0.935)	0.786 (0.5 -1.095)	0.365 (0.244 -0.65)	1.087 (0.863 -1.517)	2.097 (2.031 -2.23)
0.00973	No reps.	0.0134	0.0152	0.903	0.781
2.009 (1.5 -3.221)	0.492	2.103 (1.736 -3.174)	0.529 (0.349 -0.769)	0.981 (0.597 -1.669)	0.95 (0.401 -1.277)

Table A.1 (continued)

UU E2	UU E6	UU F2	UU F7	UU F9	UU G1
0.00217	0.00101	0.000138	0.0106	0.0355	0.000561
0.485 (0.407 -0.573)	0.478 (0.312 -0.647)	4.789 (2.587 -7.083)	1.57 (1.285 -2.24)	6.146 (1.679 -30.45)	2.993 (1.76 -4.345)
0.197	NO DATA	0.19	NO DATA	0.765	0.573
1.39 (0.749 -2.513)	NO DATA	1.388 (1.255 -1.535)	NO DATA	0.955 (0.661 -1.459)	1.248 (0.647 -1.867)
0.00204	0.454	0.0104	0.0116	0.217	0.127
0.656 (0.502 -0.85)	0.854 (0.462 -1.871)	1.409 (1.03 -1.795)	1.58 (1.063 -2.177)	1.155 (0.927 -1.599)	1.421 (0.763 -2.281)
0.271	No reps.	0.0588	0.199	0.972	0.998
0.759 (0.473 -1.286)	0.788	1.493 (0.815 -2.592)	1.322 (1.208 -1.447)	1.003 (0.844 -1.309)	1.001 (0.193 -5.325)
0.193	0.33	0.139	0.155	0.334	0.00869
1.602 (1.042 -3.668)	1.286 (0.719 -2.688)	0.657 (0.335 -1.116)	0.728 (0.41 -1.399)	0.928 (0.746 -1.088)	0.366 (0.144 -0.67)
0.00517	0.00738	0.1	0.354	0.219	0.00614
1.47 (1.303 -1.615)	1.649 (1.257 -2.271)	0.777 (0.565 -1.121)	0.92 (0.795 -1.127)	1.141 (0.904 -1.542)	0.501 (0.339 -0.722)

Table A.1 (continued)

UU G8	UU H10	UU H5	UU H9	V B1	V D3
0.0294	0.0399	0.0343	3.82E -05	NO DATA	0.000616
1.515 (0.851 -2.083)	4.333 (2.99 -7.884)	2.313 (1.244 -5.98)	6.792 (5.213 -9.494)	NO DATA	9.704 (4.881 -16.87)
No reps.	NO DATA	0.00916	0.0678	NO DATA	0.641
3.541	NO DATA	0.628 (0.423 -0.891)	1.725 (1.045 -2.547)	NO DATA	0.886 (0.463 -1.394)
0.032	0.00491	0.0527	0.000325	0.000299	0.0264
1.231 (1.002 -1.496)	2.005 (1.263 -3.273)	0.692 (0.363 -0.938)	2.795 (1.922 -4.23)	4.643 (4.64 -4.646)	2.207 (1.318 -5.165)
0.323	0.102	0.52	0.00515	No reps.	0.00646
1.459 (0.539 -1.881)	1.643 (1.167 -1.99)	0.87 (0.397 -1.27)	2.683 (1.385 -5.627)	10	2.242 (1.368 -3.994)
0.169	0.169	0.0179	0.0125	NO DATA	0.00197
0.914 (0.775 -1.167)	1.15 (0.857 -1.542)	1.544 (0.893 -2.086)	1.291 (0.936 -1.459)	NO DATA	2.777 (1.426 -4.968)
0.169	0.0722	0.134	0.137	NO DATA	0.0057
0.82 (0.54 -1.083)	1.288 (1.154 -1.475)	1.072 (0.996 -1.27)	1.142 (0.876 -1.505)	NO DATA	1.723 (1.318 -2.367)

Table A.1 (continued)

V E8	W A2	W B10	W B7	W B9	W C10
0.0156	No reps.	0.0011	0.000196	0.0041	0.00749
9.569 (1.667 -63.42)	5	19.58 (6.831 -80.66)	19.58 (8.927 -62.5)	0.521 (0.348 -0.798)	0.313 (0.207 -0.739)
0.299	NO DATA	0.174	0.327	0.734	0.0406
1.256 (0.699 -2.498)	NO DATA	0.852 (0.613 -1.108)	0.883 (0.617 -1.429)	1.026 (0.869 -1.235)	0.744 (0.477 -0.965)
0.039	No reps.	0.02	0.00476	0.00692	0.00258
1.451 (1.049 -2.33)	2.499	1.307 (1.065 -1.616)	2.045 (1.347 -3.593)	0.527 (0.423 -0.842)	0.788 (0.666 -0.878)
0.478	0.0633	0.265	0.0226	0.0311	0.0259
0.962 (0.809 -1.178)	5.485 (4.629 -6.5)	1.157 (0.845 -1.857)	1.575 (1.102 -2.383)	0.39 (0.222 -0.68)	0.887 (0.817 -1.004)
0.92	0.0449	0.0057	0.436	0.401	0.0191
1.009 (0.712 -1.215)	0.325 (0.3 -0.352)	0.816 (0.681 -0.9)	0.91 (0.631 -1.362)	1.149 (0.845 -1.516)	0.81 (0.699 -0.926)
0.411	0.258	0.0402	0.951	0.356	0.03
1.081 (0.818 -1.374)	0.805 (0.431 -1.142)	0.871 (0.733 -1.027)	1.007 (0.679 -1.433)	0.809 (0.533 -1.264)	0.78 (0.616 -0.948)

Table A.1 (continued)

W C3	W C6	W D1	W D12	W E11	W E3
0.00214	0.057	0.000239	0.316	0.0551	7.23E -05
0.124 (0.0565 -0.286)	6.598 (2.148 -20)	0.123 (0.0865 -0.223)	0.568 (0.243 -0.94)	0.554 (0.252 -0.922)	0.147 (0.103 -0.195)
No reps.	0.00748	NO DATA	0.0437	0.0093	No reps.
1.26	3.395 (2.833 -4.094)	NO DATA	0.0869 (0.01 -0.211)	9.477 (1.965 -38.5)	2.809
0.0428	0.0078	0.00551	0.00154	0.299	0.00014
0.304 (0.0957 -0.67)	8.664 (3.796 -25)	0.35 (0.227 -0.414)	0.175 (0.0887 -0.336)	1.335 (0.441 -2.673)	0.322 (0.222 -0.483)
0.133	0.0504	0.142	0.00326	0.217	0.0295
0.583 (0.273 -0.846)	1.877 (1.786 -1.973)	0.276 (0.0672 -1.2)	0.0722 (0.0149 -0.134)	1.518 (0.748 -4.114)	0.444 (0.251 -0.891)
0.259	0.372	0.74	0.00258	0.0106	0.0676
1.234 (0.666 -2.026)	1.293 (0.739 -3.088)	0.857 (0.437 -1.782)	0.069 (0.0119 -0.191)	4.195 (2.035 -11.62)	1.47 (1.133 -2.039)
0.882	0.585	0.172	0.284	0.00374	0.454
1.032 (0.425 -1.57)	1.046 (0.751 -1.23)	1.291 (0.902 -1.647)	1.381 (0.551 -2.76)	1.601 (1.272 -2.154)	1.134 (0.538 -1.523)

Table A.1 (continued)

W E5	W F1	W F3	W G10	W G11	W G7
0.000164	0.000258	0.000142	0.00138	0.0802	0.00087
0.239 (0.193 -0.347)	0.0383 (0.0114 -0.101)	11.66 (6.84 -33.65)	5.462 (2.004 -14.53)	0.285 (0.211 -0.625)	0.145 (0.0774 -0.279)
0.472	0.000267	0.578	0.176	0.369	NO DATA
0.679 (0.476 -0.968)	1.99 (1.575 -2.471)	1.114 (0.688 -1.717)	0.469 (0.378 -0.581)	1.334 (1.105 -1.61)	NO DATA
0.00516	1.01E -05	0.0162	0.00193	0.0027	0.00389
0.593 (0.367 -0.771)	0.34 (0.254 -0.377)	1.585 (1.216 -2.496)	2.302 (1.315 -3.738)	0.361 (0.205 -0.548)	0.22 (0.0831 -0.315)
0.0672	0.253	0.999	0.0117	0.108	0.0437
0.469 (0.207 -1.244)	0.76 (0.479 -1.542)	1 (0.669 -1.393)	1.674 (0.988 -2.443)	0.475 (0.286 -0.705)	0.238 (0.1 -0.589)
0.0179	0.36	0.488	0.205	0.168	0.529
0.645 (0.351 -0.801)	1.162 (0.804 -1.94)	1.099 (0.788 -1.565)	0.742 (0.451 -1.004)	1.26 (1.028 -1.826)	1.181 (0.916 -2.381)
0.125	0.106	0.159	0.0447	0.277	0.13
0.743 (0.467 -1.392)	1.147 (1.003 -1.407)	0.811 (0.5 -0.927)	0.804 (0.625 -0.987)	0.888 (0.646 -1.077)	1.744 (0.98 -2.881)

Table A.1 (continued)

W H5	W H8	WW A5	WW A6	WW F7	WW G9
0.95	0.00109	0.21	0.000674	3.42E -05	0.00426
0.986 (0.475 -1.756)	0.361 (0.27 -0.467)	1.556 (1.066 -2.445)	14.84 (7.643 -58.12)	3.343 (2.403 -4.119)	9.797 (4.025 -38.57)
NO DATA	NO DATA	0.0681	0.966	0.379	0.601
NO DATA	NO DATA	0.777 (0.756 -0.798)	0.984 (0.01 -1.546)	0.798 (0.377 -1.386)	0.919 (0.547 -1.393)
0.537	0.000757	0.00198	0.00307	0.0989	0.0037
0.791 (0.378 -4.23)	0.407 (0.364 -0.474)	1.959 (1.409 -2.477)	1.649 (1.232 -2.031)	1.788 (1.208 -2.269)	2.209 (1.11 -3.399)
No reps.	0.483	0.304	0.169	0.00326	0.0179
0.01*	0.659 (0.382 -1.746)	1.275 (0.728 -1.734)	1.233 (0.964 -1.815)	2.271 (2.097 -2.468)	1.697 (1.234 -3.462)
0.0161	0.5	0.0249	0.378	0.0792	0.861
2.758 (1.689 -4.607)	1.22 (1 -1.487)	0.595 (0.435 -1.295)	0.873 (0.516 -1.306)	1.203 (0.933 -1.565)	0.969 (0.622 -1.456)
0.0144	0.101	0.192	0.0345	0.000143	0.69
2.588 (1.705 -4)	3.523 (2.879 -4.312)	0.713 (0.406 -1.239)	0.879 (0.735 -1.019)	1.649 (1.428 -1.924)	0.958 (0.694 -1.378)

Table A.1 (continued)

WW H11	WW H2	X A4	X A6	X B12	X B3
0.359	0.0278	0.000254	0.016	0.022	0.00795
1.127 (0.892 -1.709)	4.964 (3.31 -8.34)	0.21 (0.153 -0.331)	3.095 (1.732 -5.275)	10.55 (5.869 -20)	25.07 (3.497 -240.5)
NO DATA	0.921	No reps.	No reps.	0.425	0.132
NO DATA	0.986 (0.843 -1.277)	0.944	0.916	0.787 (0.561 -1.713)	0.897 (0.682 -1.026)
0.311	0.0147	0.00962	0.00504	0.0015	0.17
1.141 (0.834 -1.378)	1.856 (1.324 -2.225)	0.381 (0.166 -0.873)	2.796 (1.95 -3.828)	2.288 (1.597 -3.948)	1.219 (0.927 -1.819)
No reps.	NO DATA	0.0595	5.40E -05	0.0337	0.7
0.01*	NO DATA	0.318 (0.138 -0.784)	2.003 (1.756 -2.574)	2.447 (1.9 -3.369)	1.049 (0.743 -1.643)
0.26	0.659	0.348	0.0592	0.979	0.0543
1.728 (0.706 -4.741)	1.062 (0.794 -1.672)	0.8 (0.37 -1.161)	1.459 (0.891 -2.313)	1.003 (0.785 -1.478)	0.908 (0.762 -0.986)
0.00643	0.546	0.0719	0.856	0.237	0.205
2.072 (1.246 -3.211)	1.244 (0.703 -4.443)	1.521 (0.805 -2.264)	1.044 (0.598 -2.442)	0.884 (0.702 -1.235)	1.155 (0.767 -1.559)

Table A.1 (continued)

X C1	X C2	X D4	X E11	X E2	X E3
0.00973	0.000688	0.000331	0.00205	0.0212	NO DATA
5.109 (2.486 -12.49)	17.24 (8.075 -60.5)	7.981 (4.015 -16.53)	0.411 (0.256 -0.517)	36.04 (20.46 -104)	NO DATA
0.129	0.122	0.903	NO DATA	0.871	NO DATA
1.947 (1.199 -3)	0.58 (0.299 -0.938)	1.035 (0.507 -1.64)	NO DATA	0.978 (0.615 -1.306)	NO DATA
0.0147	0.168	0.00565	0.0406	0.0064	0.00199
1.328 (1.126 -1.428)	1.517 (0.953 -2.809)	1.719 (1.257 -2.38)	0.547 (0.313 -0.781)	1.783 (1.201 -2.454)	1.626 (1.624 -1.629)
0.0228	0.194	0.437	No reps.	0.219	No reps.
1.787 (1.223 -3.032)	1.184 (0.706 -1.596)	1.249 (0.517 -2.043)	0.096	1.215 (0.774 -1.947)	5.691
0.0772	0.0525	0.000499	0.38	0.168	0.0479
1.232 (0.855 -1.693)	0.893 (0.736 -0.994)	1.413 (1.234 -1.574)	1.281 (0.635 -1.888)	1.219 (0.93 -1.621)	1.319 (1.292 -1.347)
0.254	0.148	0.00888	No reps.	0.0177	0.000161
1.141 (0.818 -1.424)	1.086 (0.927 -1.204)	1.415 (1.085 -1.732)	1.881	1.308 (1.025 -1.752)	1.497 (1.456 -1.571)

Table A.1 (continued)

X F12	X F4	X G1	X G11	X G2	X G4
0.000407	0.0314	0.0148	0.00375	0.000229	1.55E -05
8.198 (6.103 -17.55)	1.857 (0.924 -3.324)	11.05 (2.311 -129.2)	1.655 (1.203 -2.133)	3.596 (2.411 -6.294)	3.863 (2.825 -5.044)
0.092	0.0406	0.0684	NO DATA	0.715	0.0822
0.723 (0.396 -1.235)	0.66 (0.455 -1.072)	0.776 (0.581 -1.121)	NO DATA	0.938 (0.515 -1.352)	0.602 (0.428 -1.027)
0.00171	0.738	0.362	0.016	0.0046	0.00041
1.554 (1.217 -2.107)	1.021 (0.794 -1.143)	1.166 (0.839 -1.68)	1.813 (1.34 -3.048)	2.129 (1.666 -3.42)	1.783 (1.462 -2.198)
0.151	0.516	0.744	0.226	0.414	0.0769
1.431 (0.865 -3.04)	1.036 (0.86 -1.223)	0.973 (0.827 -1.375)	1.273 (0.878 -1.693)	1.24 (0.393 -2.058)	1.434 (0.962 -2.726)
0.298	0.897	0.0166	0.0156	0.173	0.000705
0.882 (0.663 -1.364)	1.016 (0.703 -1.405)	0.83 (0.708 -0.976)	0.436 (0.311 -0.888)	1.203 (0.845 -1.751)	2.472 (1.704 -3.373)
0.144	0.737	0.825	0.0114	0.205	0.0845
0.847 (0.559 -1.027)	0.868 (0.01 -1.808)	0.979 (0.683 -1.364)	0.763 (0.604 -0.959)	1.251 (0.82 -2.154)	1.307 (0.79 -1.819)

Table A.1 (continued)

X G6	X G7	X G8	X G9	X H4	X H7
0.000167	0.0732	0.00301	0.00229	0.00119	0.00524
18.82 (7.749 -50)	3.578 (1.201 -25.58)	4.558 (2.777 -10.99)	5.713 (2.994 -12.48)	6.101 (3.112 -21.5)	6.474 (2.047 -24.39)
0.496	0.642	0.747	0.173	0.435	0.945
0.835 (0.596 -1.663)	0.925 (0.537 -1.553)	1.082 (0.694 -1.796)	1.347 (0.706 -1.983)	1.223 (1.038 -1.441)	1.015 (0.573 -2.172)
0.0186	0.292	0.0282	0.227	0.00254	0.672
1.93 (1.611 -2.153)	1.084 (0.885 -1.293)	1.892 (0.905 -2.636)	3.014 (0.466 -11.62)	3.003 (1.761 -5.703)	1.055 (0.833 -1.712)
0.00402	0.332	0.355	0.767	0.412	0.177
1.806 (1.372 -3.072)	0.944 (0.874 -1.158)	1.556 (0.175 -2.659)	1.077 (0.48 -2.032)	1.318 (0.7 -3.881)	0.889 (0.698 -1.049)
0.0704	0.25	0.912	0.0195	0.3	0.97
1.202 (0.869 -1.5)	0.942 (0.789 -1.078)	1.014 (0.64 -1.298)	0.604 (0.407 -0.957)	0.759 (0.462 -1.64)	1.002 (0.896 -1.124)
0.362	0.23	0.0579	0.00285	0.24	0.146
0.84 (0.631 -1.386)	0.791 (0.43 -1.088)	1.37 (1.021 -1.609)	1.562 (1.092 -1.951)	0.835 (0.543 -1.104)	1.108 (0.966 -1.407)

Table A.1 (continued)

X H8	X H9	XX A10	XX B11	XX C12	XX D1
7.45E -05	0.00123	0.00901	0.0089	0.000578	0.0263
10.01 (6.719 -20.09)	3.027 (1.987 -4.193)	4.708 (2.416 -8.35)	0.502 (0.45 -0.564)	4.385 (2.072 -7.7)	7.23 (2.266 -83.87)
0.431	0.873	0.033	NO DATA	0.0154	0.26
0.886 (0.656 -1.212)	1.053 (0.679 -2.547)	1.327 (1.116 -1.612)	NO DATA	0.685 (0.461 -0.947)	0.786 (0.364 -1.288)
0.244	0.0271	0.0712	0.0826	0.751	0.296
1.156 (0.886 -1.75)	1.459 (0.895 -2.146)	1.875 (0.671 -2.592)	0.557 (0.516 -0.601)	0.983 (0.854 -1.194)	1.178 (0.789 -1.658)
0.525	0.0859	0.0596	0.0677	0.153	0.883
1.079 (0.756 -1.433)	1.38 (0.924 -2.504)	2.12 (1.04 -3.107)	2.288 (2.095 -2.5)	0.937 (0.839 -1.069)	1.009 (0.857 -1.308)
0.246	0.856	0.119	NO DATA	0.000138	0.00552
0.948 (0.848 -1.08)	1.021 (0.615 -1.333)	0.804 (0.567 -1.062)	NO DATA	0.701 (0.632 -0.757)	0.805 (0.687 -0.922)
0.394	0.494	0.503	0.272	0.0564	0.32
1.174 (0.843 -1.963)	0.852 (0.389 -1.716)	0.813 (0.403 -1.801)	1.79 (1.373 -2.334)	0.841 (0.681 -0.964)	0.919 (0.677 -1.145)

Table A.1 (continued)

XX D10	XX E12	XX E3	XX E5	XX G1	XX G4
0.000345	0.204	0.00105	0.43	0.000101	0.0428
27.84 (13.03 -112)	0.836 (0.718 -1)	4.587 (1.866 -8.068)	1.176 (0.929 -2)	2.509 (1.953 -3.059)	5.535 (2.665 -8.229)
0.22	NO DATA	0.139	No reps.	No reps.	0.585
0.875 (0.764 -1.23)	NO DATA	1.849 (0.958 -3.635)	0.681	1.656	1.068 (0.98 -1.165)
0.158	0.0689	0.000152	0.578	0.00576	0.00235
1.657 (0.938 -4.073)	4.349 (3.707 -5.102)	2.514 (2.03 -2.953)	1.123 (0.968 -1.303)	1.642 (1.051 -2.278)	2.93 (1.833 -4.864)
0.159	No reps.	0.00243	No reps.	0.018	0.0035
1.331 (0.777 -2.349)	4.382	2.095 (1.228 -3.091)	0.01*	1.803 (1.284 -2.243)	2.817 (2.575 -3.168)
0.954	0.173	0.0212	NO DATA	0.811	0.0666
1.007 (0.713 -1.499)	0.458 (0.368 -0.569)	1.431 (0.982 -1.882)	NO DATA	0.965 (0.514 -1.24)	1.369 (0.998 -1.918)
0.288	No reps.	0.00637	0.304	0.796	0.703
0.922 (0.704 -1.067)	2.994	1.46 (1.024 -1.742)	1.688 (1 -3.551)	0.955 (0.596 -1.655)	0.942 (0.558 -1.292)

Table A.1 (continued)

XX H10	XX H12	YY A12	YY A4	YY B6	YY C4
0.00124	0.000446	0.000162	0.137	0.000834	0.00511
15.4 (7.114 -46.43)	30.2 (14.54 -118.5)	10.6 (6.597 -24.8)	0.646 (0.322 -1.184)	5.861 (3.535 -18.66)	3.724 (1.744 -11.08)
0.814	0.232	0.669	NO DATA	0.146	0.83
0.961 (0.669 -1.288)	0.775 (0.391 -1.12)	0.917 (0.532 -1.74)	NO DATA	0.814 (0.733 -0.971)	1.044 (0.51 -1.963)
0.0297	0.0814	0.0129	0.0137	0.0549	0.386
2.382 (1.076 -5.65)	1.642 (0.847 -3.082)	1.611 (1.244 -2.726)	0.41 (0.262 -0.848)	1.846 (1.112 -4.23)	1.114 (0.841 -1.778)
0.071	0.571	0.608	No reps.	0.00171	0.684
1.45 (0.906 -2.296)	1.089 (0.79 -1.647)	1.062 (0.679 -1.422)	0.01*	2.139 (1.634 -2.967)	0.872 (0.234 -1.697)
0.816	0.178	0.961	NO DATA	0.963	0.00254
1.042 (0.641 -1.643)	1.166 (0.873 -1.568)	1.006 (0.678 -1.424)	NO DATA	1.008 (0.611 -1.565)	0.787 (0.688 -0.911)
0.884	0.0667	0.596	No reps.	0.783	0.0472
0.979 (0.573 -1.356)	1.102 (0.977 -1.241)	0.965 (0.717 -1.066)	3.195	0.964 (0.555 -1.253)	0.798 (0.599 -1.118)

Table A.1 (continued)

YY C6	YY F4	YY G8	YY H11	Z B11	Z D3
0.00999	0.0609	0.226	0.278	0.534	0.000343
8.653 (4.557 -20.5)	1.635 (0.971 -2.887)	0.855 (0.628 -1.196)	0.649 (0.421 -1.136)	1.575 (0.951 -2.61)	22.12 (9.764 -72.5)
0.772	NO DATA	NO DATA	NO DATA	NO DATA	0.348
1.072 (0.757 -1.565)	NO DATA	NO DATA	NO DATA	NO DATA	0.812 (0.456 -1.554)
0.0329	0.0205	0.377	0.252	0.834	0.0151
3.162 (1.564 -19)	1.272 (1.105 -1.572)	1.09 (1 -1.27)	0.798 (0.643 -1.042)	1.006 (0.983 -1.03)	1.485 (1.168 -2.034)
0.0294	0.000261	NO DATA	No reps.	NO DATA	0.0184
2.288 (1.028 -4.677)	1.807 (1.671 -1.893)	NO DATA	0.01*	NO DATA	1.226 (1 -1.533)
0.988	0.101	NO DATA	NO DATA	No reps.	0.893
1.006 (0.163 -1.743)	0.461 (0.407 -0.522)	NO DATA	NO DATA	0.01*	1.016 (0.658 -1.329)
0.0234	0.424	0.0132	No reps.	0.193	0.294
1.379 (0.92 -1.924)	0.822 (0.358 -1.278)	2.454 (2.409 -2.5)	2.526	1.355 (1.055 -1.809)	0.877 (0.625 -1.184)

Table A.1 (continued)

Z E5	Z G1	Z G8	ZZ A9	ZZ B8	ZZ C3
0.095	0.594	0.0652	0.00622	0.00111	0.000268
43.15 (24.5 -76)	1.151 (0.757 -2.253)	8.993 (3.522 -26.75)	2.33 (1.931 -4.413)	0.469 (0.316 -0.658)	25.81 (12.44 -91)
NO DATA	No reps.	0.727	NO DATA	0.783	0.00998
NO DATA	0.01*	0.939 (0.476 -1.405)	NO DATA	0.947 (0.431 -1.423)	0.724 (0.544 -0.947)
0.841	0.665	0.0209	0.0458	0.0175	0.0104
1.092 (0.285 -2.228)	0.923 (0.613 -1.75)	2.634 (1.441 -6.056)	1.533 (1.294 -2.257)	0.87 (0.755 -0.975)	1.799 (1.261 -2.684)
0.0219	0.0328	0.0523	No reps.	0.646	0.209
2.549 (2.185 -3.376)	1.312 (1.074 -1.513)	2.062 (1.15 -3.47)	0.661	0.978 (0.862 -1.179)	1.138 (0.849 -1.588)
0.0848	0.111	0.012	0.0185	8.59E -05	0.579
3.492 (2.953 -4.129)	0.639 (0.343 -1.362)	0.884 (0.799 -0.963)	1.214 (1.208 -1.221)	0.811 (0.765 -0.87)	0.957 (0.727 -1.19)
0.0768	0.356	0.386	0.119	0.104	0.422
1.533 (0.816 -2.284)	0.873 (0.623 -1.203)	1.032 (0.932 -1.133)	0.883 (0.704 -1.037)	0.778 (0.426 -0.97)	0.939 (0.737 -1.188)

Table A.1 (continued)

ZZ C8	ZZ D9	ZZ E12	ZZ E6	ZZ E8	ZZ F1
7.04E -05	0.000979	0.00668	0.0143	0.000269	0.00923
4.391 (3.059 -6.268)	0.471 (0.334 -0.735)	0.194 (0.133 -0.32)	0.373 (0.167 -0.61)	0.197 (0.128 -0.288)	0.356 (0.215 -0.634)
0.847	No reps.	NO DATA	No reps.	NO DATA	NO DATA
1.055 (0.43 -1.711)	5.377	NO DATA	0.01*	NO DATA	NO DATA
0.000899	0.0254	0.0216	0.0453	0.0119	0.00249
1.798 (1.267 -2.299)	0.55 (0.3 -0.868)	0.248 (0.0949 -0.65)	0.632 (0.478 -0.927)	0.516 (0.297 -0.683)	0.516 (0.352 -0.741)
0.568	0.0891	0.295	0.139	0.155	0.238
1.277 (0.121 -1.7)	0.602 (0.56 -0.646)	0.298 (0.0574 -1.059)	0.355 (0.177 -0.786)	0.196 (0.0489 -0.582)	0.0366 (0.01 -0.134)
0.789	0.392	0.452	0.124	0.473	No reps.
0.96 (0.627 -1.711)	1.233 (0.851 -1.635)	0.847 (0.518 -1.275)	1.541 (0.97 -3.104)	0.832 (0.509 -1.435)	0.799
0.0568	No reps.	0.185	0.0584	0.0151	0.187
1.192 (0.979 -1.518)	0.823	1.396 (0.654 -3.228)	1.709 (1 -2.148)	3.608 (3.5 -3.72)	1.663 (1 -2.298)

Table A.1 (continued)

ZZ F10	ZZ F7	ZZ G12	ZZ G3	ZZ H1	ZZZ A10
0.000269	0.0248	0.0565	0.115	0.00395	0.00277
17.77 (8.606 -48)	0.577 (0.361 -0.947)	0.656 (0.548 -0.788)	0.472 (0.281 -0.731)	0.557 (0.504 -0.69)	7.342 (3.19 -21.12)
0.0746	No reps.	NO DATA	NO DATA	NO DATA	0.169
0.778 (0.588 -1.089)	0.673	NO DATA	NO DATA	NO DATA	0.81 (0.576 -1.477)
0.00531	0.074	0.00739	0.00601	0.000804	0.0159
2.073 (1.262 -3.239)	0.721 (0.48 -1.19)	0.574 (0.347 -0.847)	0.477 (0.295 -0.976)	0.431 (0.318 -0.558)	1.828 (1.053 -2.68)
0.0146	0.441	0.408	0.0223	0.0961	0.00895
1.567 (1.201 -2.786)	0.822 (0.534 -1.268)	0.46 (0.258 -0.821)	3.374 (3.233 -3.521)	0.364 (0.312 -0.424)	1.241 (1.127 -1.334)
0.563	0.451	0.276	0.286	0.321	0.91
1.061 (0.833 -1.56)	1.41 (0.482 -2.642)	2.228 (1.538 -3.229)	0.618 (0.427 -1.202)	1.156 (0.863 -1.8)	0.983 (0.517 -1.366)
0.557	0.00819	0.0706	0.0329	0.0537	0.307
1.043 (0.79 -1.303)	3.177 (2.533 -5.499)	1.415 (0.949 -2.179)	2.175 (1.449 -3.72)	1.918 (1.449 -2.495)	0.889 (0.559 -1.131)

Table A.1 (continued)

ZZZ A6	ZZZ B8	ZZZ C11	ZZZ C5	ZZZ C8	ZZZ D10
0.00072	0.0542	0.0659	0.000521	0.00117	0.000526
0.306 (0.215 -0.431)	0.585 (0.452 -0.686)	0.211 (0.135 -0.345)	8.143 (3.776 -12.59)	7.978 (3.952 -32.49)	0.396 (0.284 -0.627)
0.526	NO DATA	NO DATA	0.49	0.414	No reps.
1.748 (0.954 -3.203)	NO DATA	NO DATA	0.911 (0.64 -1.38)	1.111 (0.786 -1.503)	2.5
0.00323	0.198	0.00106	0.00447	0.0472	0.00341
0.336 (0.172 -0.595)	0.832 (0.53 -1.074)	0.257 (0.131 -0.447)	1.481 (1.192 -1.878)	1.581 (0.891 -2.152)	0.532 (0.346 -0.861)
0.109	0.987	0.419	0.00591	0.00491	0.107
0.167 (0.122 -0.228)	0.997 (0.618 -1.537)	0.254 (0.0878 -0.734)	1.395 (1.152 -1.933)	1.492 (1.196 -2.132)	0.661 (0.555 -0.886)
0.643	0.608	0.784	0.41	0.0141	0.309
0.915 (0.533 -1.569)	1.051 (0.892 -1.297)	1.084 (0.521 -1.962)	1.062 (0.873 -1.421)	1.095 (1.038 -1.156)	0.882 (0.735 -0.995)
0.301	0.0119	0.503	0.0124	0.9	0.0913
1.536 (0.953 -4.268)	1.871 (1.477 -2.5)	1.061 (0.861 -1.246)	1.29 (0.998 -1.61)	0.997 (0.907 -1.059)	1.533 (1.235 -2.575)

Table A.1 (continued)

ZZZ D11	ZZZ D12	ZZZ D4	ZZZ D8	ZZZ E6	ZZZ F11
0.000725	0.00112	0.00161	0.00551	0.303	0.0348
0.0732 (0.0358 -0.135)	0.204 (0.0862 -0.428)	0.12 (0.0522 -0.21)	0.523 (0.365 -0.933)	0.728 (0.405 -2.64)	0.27 (0.176 -0.419)
NO DATA	0.298	NO DATA	NO DATA	0.0225	0.0141
NO DATA	0.389 (0.241 -0.627)	NO DATA	NO DATA	1.768 (1.574 -2.097)	1.387 (1.377 -1.397)
3.93E -05	0.000609	0.000118	0.143	0.0287	0.0143
0.232 (0.163 -0.312)	0.352 (0.233 -0.605)	0.298 (0.24 -0.381)	0.715 (0.465 -1.397)	0.521 (0.195 -0.748)	0.312 (0.104 -0.505)
0.0101	0.0224	0.00817	0.115	0.697	0.194
0.416 (0.354 -0.48)	0.408 (0.156 -0.61)	0.277 (0.168 -0.404)	0.405 (0.343 -0.478)	1.169 (0.589 -1.829)	0.314 (0.133 -1.002)
NO DATA	0.871	0.768	0.26	0.0447	0.0892
NO DATA	0.979 (0.727 -1.359)	0.941 (0.643 -1.694)	1.411 (0.915 -1.897)	2.002 (1.12 -4.805)	1.539 (1.141 -2.486)
0.0748	0.389	0.892	No reps.	0.0233	0.264
1.388 (1.193 -1.655)	1.189 (0.75 -1.99)	0.976 (0.578 -1.311)	4.312	1.769 (1.029 -2.665)	1.685 (0.923 -2.982)

Table A.1 (continued)

ZZZ F2	ZZZ F6	ZZZ F9	ZZZ G11	ZZZ H10	ZZZ H3
0.000653	0.00161	0.000105	0.00965	0.00158	0.547
0.363 (0.237 -0.596)	0.403 (0.25 -0.469)	17.48 (10.47 -54.06)	0.463 (0.306 -1.024)	0.614 (0.433 -0.725)	1.125 (0.649 -1.967)
0.936	NO DATA	0.157	No reps.	0.18	No reps.
1.086 (0.475 -2.485)	NO DATA	0.911 (0.705 -1.011)	0.922	2.039 (1.658 -2.508)	0.01*
8.68E -05	0.00041	0.00272	0.0094	0.0723	0.0796
0.404 (0.289 -0.479)	0.391 (0.256 -0.494)	1.96 (1.475 -2.761)	0.363 (0.152 -0.662)	0.746 (0.561 -1.334)	0.893 (0.756 -1.073)
0.175	0.0488	0.039	No reps.	0.707	0.529
0.67 (0.324 -1.18)	0.426 (0.399 -0.455)	1.446 (0.987 -2.312)	0.306	1.105 (0.783 -2.26)	0.928 (0.639 -1.436)
0.5	0.532	0.334	0.0574	0.989	0.0438
1.178 (0.81 -2.79)	1.04 (0.969 -1.153)	0.858 (0.624 -1.226)	1.527 (0.756 -2.533)	0.998 (0.609 -1.415)	0.524 (0.288 -1.081)
0.0523	0.0938	0.226	0.955	0.0268	0.00846
1.62 (0.712 -2.886)	1.737 (1.432 -2.499)	0.822 (0.441 -1.066)	0.99 (0.594 -1.721)	1.428 (1.136 -1.842)	0.658 (0.486 -1.004)

Table A.1 (continued)

ZZZ H6	ZZZ H7	ZZZ H8
0.000336	1.54E -06	0.0225
0.225 (0.135 -0.28)	0.167 (0.142 -0.224)	9.75 (3.086 -141.4)
NO DATA	0.0246	0.408
NO DATA	1.404 (1.266 -1.52)	1.073 (0.862 -1.419)
0.000456	0.000562	0.167
0.328 (0.255 -0.511)	0.47 (0.386 -0.738)	1.208 (0.964 -1.711)
0.139	0.0771	0.403
0.078 (0.01 -0.354)	0.624 (0.358 -1.032)	1.102 (0.834 -1.575)
0.531	0.123	0.11
1.181 (0.713 -2.824)	0.704 (0.362 -1.318)	0.95 (0.846 -1.026)
0.427	0.0376	0.462
1.333 (0.672 -2.334)	0.619 (0.369 -1.071)	0.957 (0.858 -1.186)

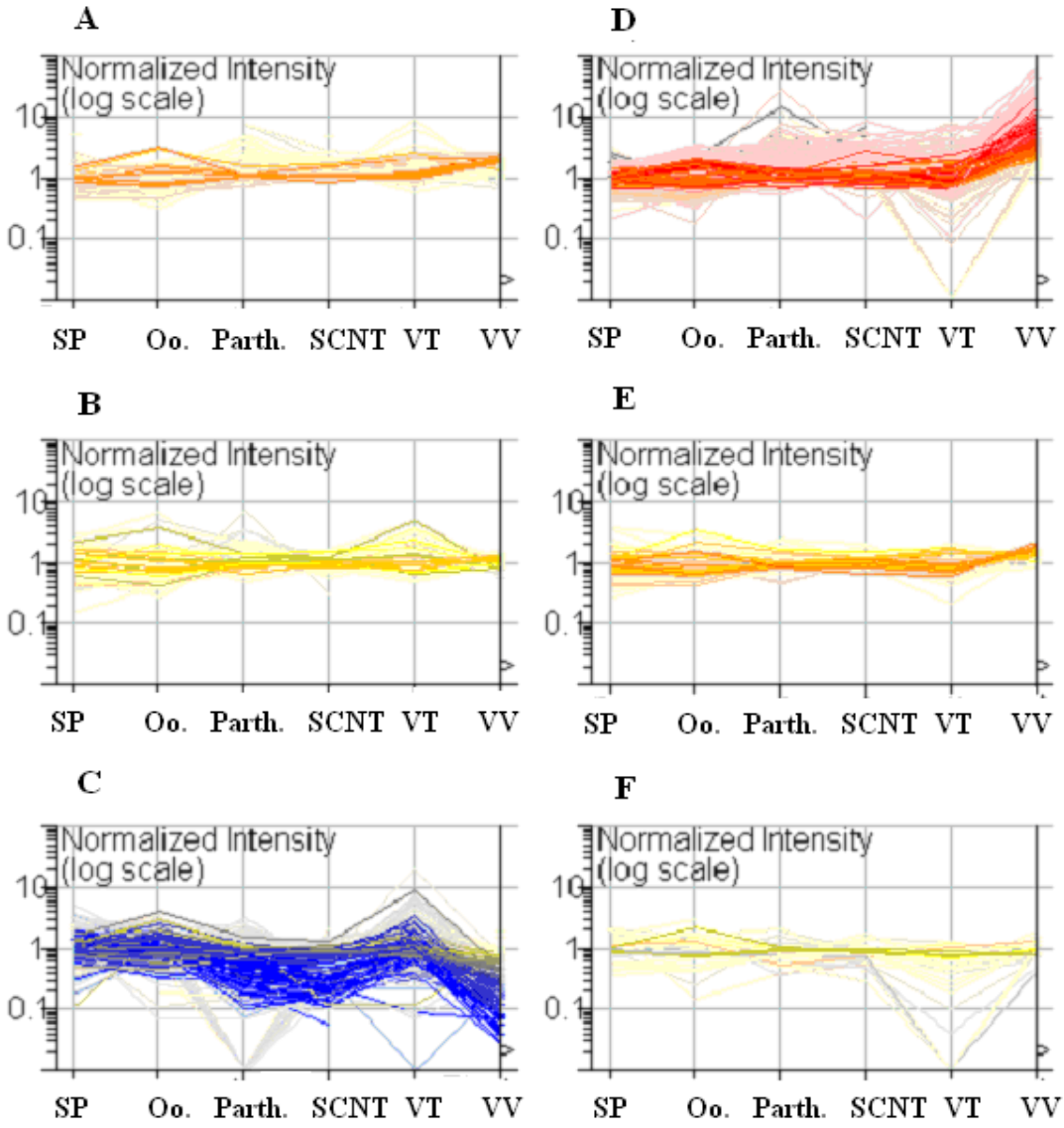

Figure A.1. Self Organizing Map analysis of the significantly different ($P<0.05$) spots (n=1532) in the methylation profiles of sperm, GV oocyte and blastocysts. Spots that do that have an average of one in the sperm (SP), oocytes (Oo.), parthenogenetic-produced blastocysts (Parth.), somatic cell nuclear transfer-produced blastocysts (SCNT), *in vitro*- produced blastocysts (VT), and *in vivo*-produced blastocysts (VV).

Table A.2. Sequenced clones exhibiting similar methylation profiles in the gametes and blastocysts as determined by Self Organizing Map analysis. Blast analysis identified 21% (22/105) of the clones as having similarity to multiple regions, 39% (41/105) of the clones as having no similarity to existing sequenced, and 40% (42/105) of the clones as having similarity to sequenced clones as similar to identified or predicted genes.

Clone	Score	Annotation	Gene	Access #
A				
G A10	396	Human DNA sequence from clone RP11-697G4 on chromosome 6, 5' end of the FOXO3A gene	FOXO3A	AL391646
NN H8		NS		
RR C8	206	PREDICTED: Bos taurus similar to myeloid leukemia factor 1,mRNA.	MLF1	XM_874504
S A11	163	Human DNA sequence from clone RP11-50D16 on chromosome 13		AL445590
W D5	58	PREDICTED: Pan troglodytes similar to frizzled 2 (LOC459881), mRNA	Not found in HUGO	XM_516034
W H6	274	Homo sapiens T-box, brain, 1 (TBR1), mRNA	TBR1	NM_006593
B				
A C6		Multiple		
AA A11		Sus scrofa CC chemokine receptor genes (CCR9)	CCBP2	
E A10		Multiple		
G F5		Multiple immune etc, (STRONG)		
II B3	293	Homo sapiens chromosome 5 clone CTD-2012M11, complete sequence		AC016595.
K D3	262	Homo sapiens BAC clone RP11-73G16 from 4, complete sequence		AC097375.

Table A.2 (continued)

N G6		NS		
QQ A1		NS		
QQ E4	149	PREDICTED: Bos taurus similar to peptidyl prolyl isomerase H	PPIH	XM_873469
T F3	188	Human GLA gene for alpha-D-galactosidase A (EC 3.2.1.22).	GLA	X14448.
U B12		NS		
X G10		NS		
C				
AA A1		NS		
B G2		NS		
BBB A12	301	Sus scrofa glutamate decarboxylase 2 (GAD2), mRNA	GAD2	NM_213895
BBB H7		Multiple		
CCC H12	113	Multiple		
D C10		NS		
D D10		NS		
D D6		Multiple		
EEE B7		Multiple		
EEE B9		Multiple		
EEE E3		only Bac matches		
F E10		Multiple		
F F10		NS		
FF G1		only Bac matches		
G G10	188	PREDICTED: Canis familiaris similar to DEAD (Asp-Glu-Ala-Asp) box	DDX10	XM_536583
GGG D4	226	WNT8B gene	WNT8B	Y11108.
II H10		Multiple		
III D1		Multiple		

Table A.2 (continued)

JJ B10	910	Bos taurus similar to Homeobox protein SIX6 (Sine oculis homeobox homolog 6)	SIX6	XM_589185
JJ D12	129	H.sapiens CpG island DNA gen		
JJ E10		NS		
K G10	157	Human cyclic AMP transcriptional regulator binding protein (CRE-BP1)	ATF2	J05623
LL E4		NS		
NN G9		NS		
D12		Multiple		
P F6	180	Bos taurus similar to protopor-phyrinogen oxidase, Last enzyme of heme synth.	PPOX	XM_593850
P H5	200	PREDICTED: Bos taurus similar to zinc finger, CSL domain	ZCSL2	XM_874300
QQ A6		Multiple		
RR G5	597	H. sapiens genes for histones H2B.1 and H2A	HIST2H2BE	BC069193.
UU C10		NS		
UU H3		NS		
X F12		Homo sapiens prostate antigen PARIS-1 mRNA, complete cds	TBC1D2	
X G2		NS		
XX H10		NS		
XX H12	133	Homo sapiens splicing factor 3a, subunit 3, 60kDa (SF3A3), mRNA	SF3A3	NM_006802
Z D3		NS		
D				
BLUE E3		NS		
CC C1		NS		
CCC B6		Multiple		
EE A11		Membrane bound O-acyltransferase domain	MBOAT5	
EE A12		NS		

Table A.2 (continued)

EE H2		Multiple		
EE H8	159	Homo sapiens aryl hydrocarbon receptor nuclear translocator	ARNT	AY430083.
FF E4	260	Multiple immune components		
G B8	123	Canis familiaris similar to coatomer zeta-1 subunit	COPZ1	XM_843171
HH A7		NS		
L E8		CpG Island plus others (multiple)		
LL D3	NS			
N E2	553	Homo sapiens serine/threonine protein kinase Kp78 (ribosomal)	MARK3	AF159295.
NN F4	151	Mus musculus RIKEN cDNA 2810429O05 gene		NM_134046
PINK E2		NS		
PINK E9		NS		
PINK E10		Multiple		
PP C2		NS		
PP D6		NS		
PP E2		Multiple		
PP E4	293	PREDICTED: Bos taurus similar to malignant T cell amplified sequence 1	MCTS1	XM_593366
PP E5	145	PREDICTED: Canis familiaris similar to Methyltransferase-like	Not found in Hugo	XM_537604
PP E6	180	PREDICTED: Bos taurus similar to Paired box protein Pax-3	PAX3	XM_872034
PP G1		NS		
PP H6	109	Homo sapiens FRG1 (FRG1) gene, complete cds (multiple)	FRG1	AF146191.

Table A.2 (continued)

Q A2	569	Homo sapiens serine/threonine protein kinase Kp78 (ribosomal)	MARK3	AF159295.
Q H5	103	PREDICTED: Bos taurus similar to Forkhead box protein J2	FOXJ2	XM_612715
QQ D3		NS		
T A6		NS		
TT G8	121	790G17 on chromosome 1q21.1-21.3		AL138795.
W E3		NS		
W F1		NS		
E				
B F12	291	Nicotinamide mononucleotide adenylyltransferase 2 isoform 1	NMNAT2	NM_015039
EEE D4	299	Homo sapiens cell division cycle 27 (CDC27) gene, complete cds	CDC27	AY518321
F D1	67.9	PREDICTED: Bos taurus similar to Microtubule-associated protein RP/EB family member 2 (APC-binding protein EB2	APC-binding protein EB2	XM_587271
III C8	196	Homo sapiens UMPS gene for UMP synthase	UMPS	AY691629.
M C9	69.1	Homo sapiens clone RP11-4181C1 on chromosome 10	MLLT10	AL358780.
M D1		NS		
P D2		NS		
P H3	73.8	Homo sapiens similar to ankyrin-repeat protein Nrarp	Nrarp Not found in Hugo	BC053618.
PP F12		NS		
S E3	168	Homo sapiens protopor-phyrinogen oxidase (PPOX) gene, exons 2, 3,	PPOX	AY032686.

Table A.2 (continued)

T G4	167	Homo sapiens RPL18 gene for ribosomal protein L18, complete cds	RPL18	AB061825.
U G4		Multiple ribosomal proteins		
F				
JJ E12		NS		
P G12		NS		
PP D2		NS		
T F1		NS		

Bootstrap analysis of Sperm, GV Oocyte, and Blastocysts PDMH Results

The TIGR Multiple Array Viewer (TMEV) was used to perform additional validation by using the bootstrap analysis to create a hierarchical support tree. Please note TMEV does not use the same algorithm to perform hierarchical clustering and the resulting trees are different than those generated by using GeneSpring software. Specifically, the Standard Correlation used in the GeneSpring software is commonly referred to as Pearson correlation around zero. The TIGR Multiple Array Viewer does not contain this correlation procedure so the Pearson Correlation analysis was substituted. The Pearson Correlation metric was chosen because the hierarchical clustering tree was the most similar, when compared to the other metrics, to the analogous tree produced by using the GeneSpring software.

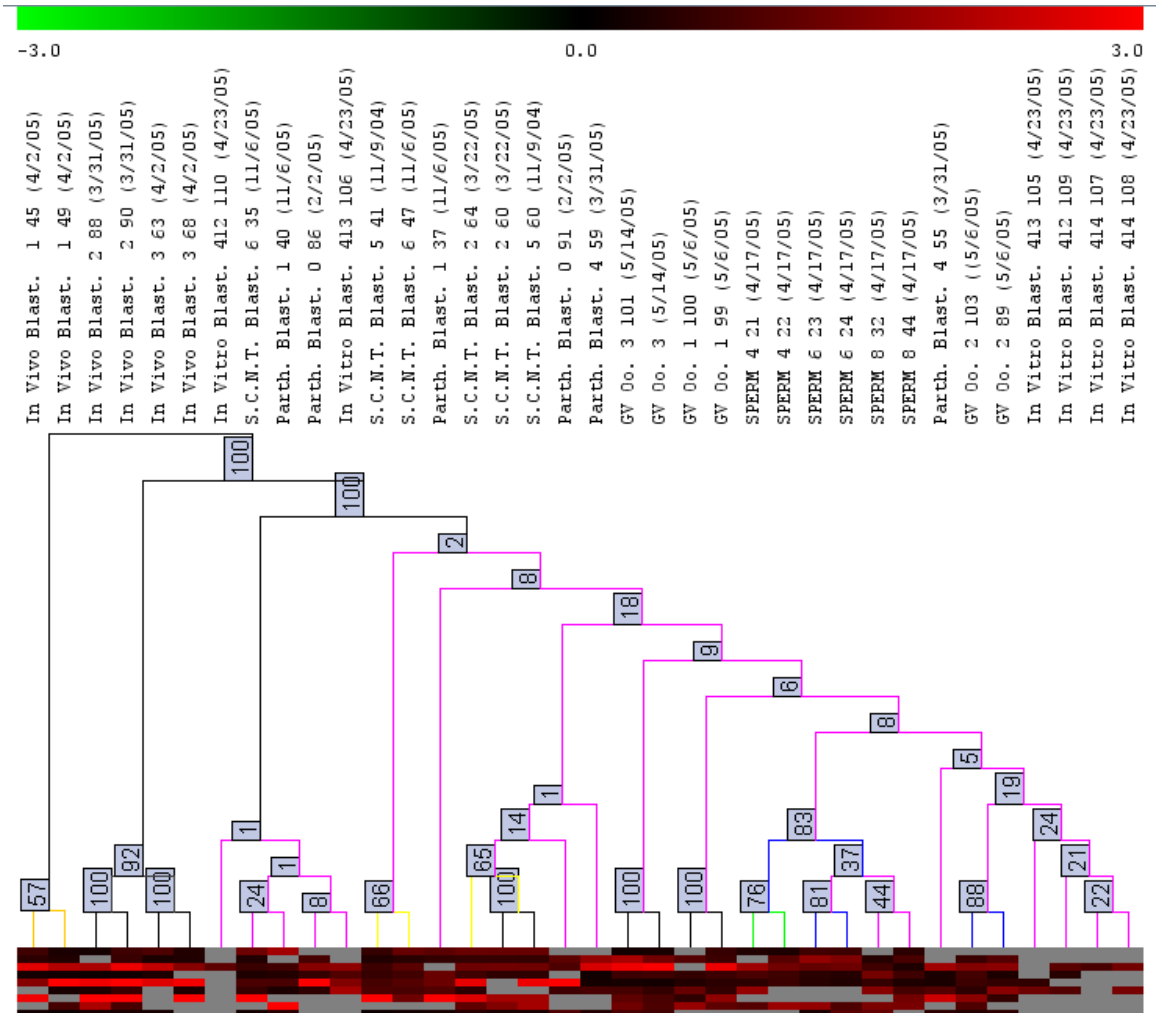

Figure A.2. Bootstrap analysis with replacement after 1000 iterations by using spots that were significantly different (P<0.01) using the Pearson Correlation metric in the TIGR Multiple Array Viewer. High bootstrapping values are identified with the clusters of in vivo developed and matured groups including *in vivo*-produced blastocysts and sperm. The remaining groups were either immature (GC oocytes) or cultured *in vitro* (Parthenogenetic-, *in vitro*-, and SCNT-produced blastocysts) and had low bootstrapping values. These results suggest that *in vivo* development ond maturation increases the consistency of establishing CpG methylation.

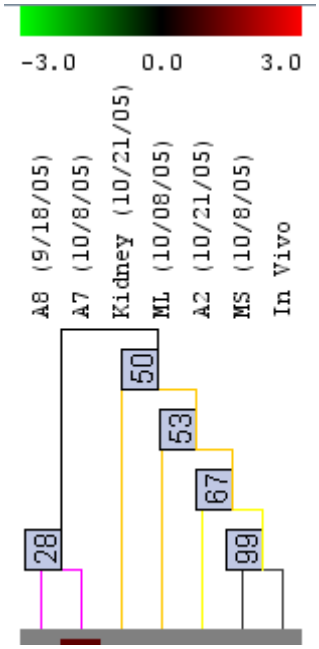

Figure A.3. Hierarchical support tree including bootstrap analysis with replacement after 1000 iterations by using spots that were significantly different ($P<0.01$) between the donor cells and *in vivo*-produced blastocysts. Differential methylation in the gametes and blastocysts was previously identified by using PDMH analysis and bisulfite sequencing (Bonk et al., 2006). Larger numbers at the nodes (range=1 to 100) indicates the support of the clustering. The clustering pattern Generated with by using the Pearson Correlation metric in the TIGR Multiple Array Viewer is not consistent with the clustering pattern produced by using GeneSpring software but these results demonstrate the consistency of the data in each group.

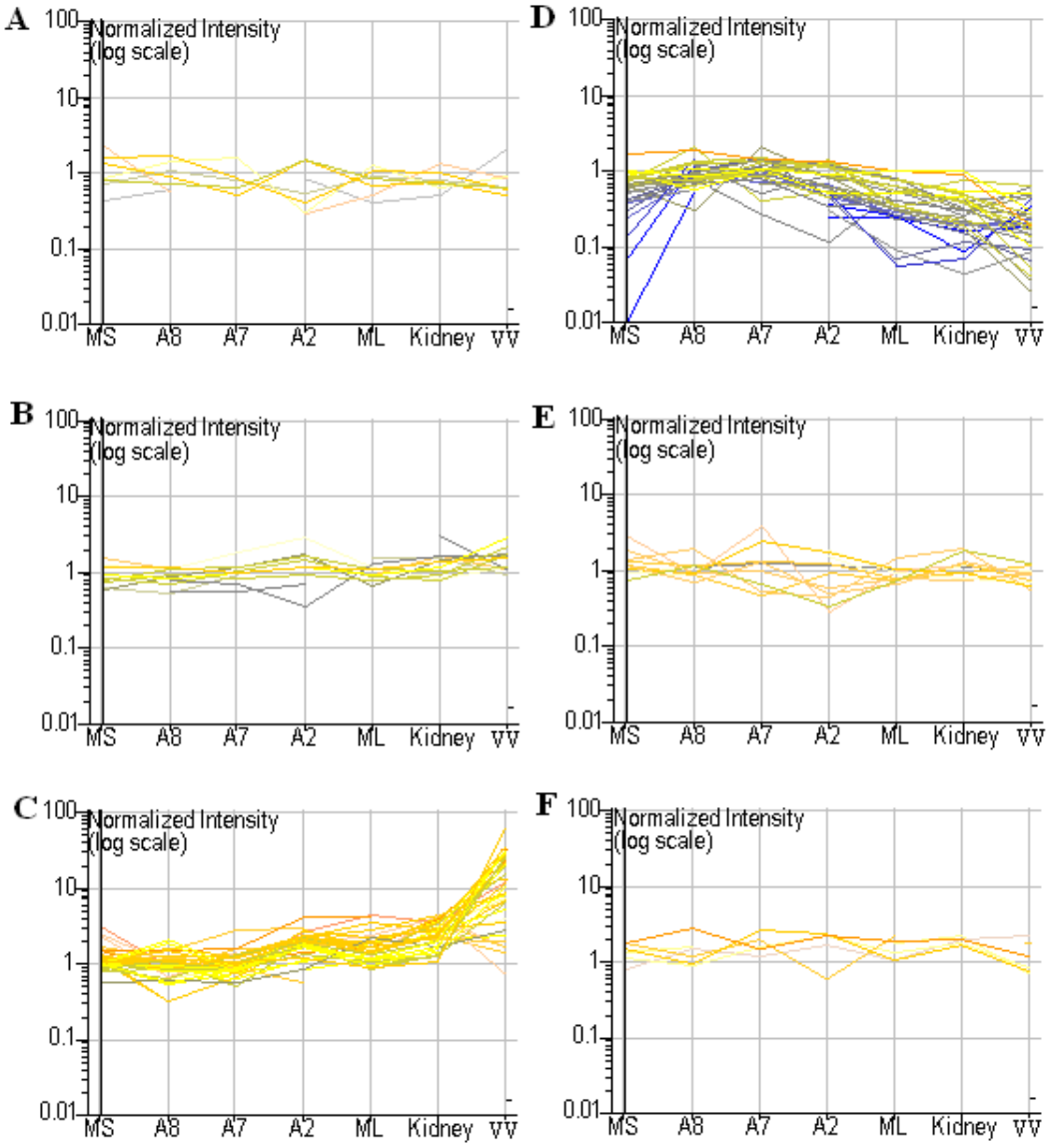

Figure A.4. Clones with similar methylation profiles in the donor cells and the *in vivo*-produced blastocysts were clustered by using Self Organizing Map analysis. Representative clones from each of the clusters are listed in Table A.3. Hypermethylation levels of the donor cells in (C) are positively correlated decreasing blastocyst rates after SCNT.

Table A.3 Sequenced clones exhibiting similar methylation profiles in the donor cells and the *in vivo*-produced 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 A.3 (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 A.3 (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 A.3 (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		

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