Supplemental Data

Heterozygous Embryonic Stem Cell Lines Derived from Primate Parthenotes

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Supplemental Tables

Supplemental Table 1. Primer sequences, product size, and Tm for RT-PCRs and qPCRs

IMPRINTED GENES				
GENE	**5' PRIMER**	**3' PRIMER**	**bp**	**Tm**
TP73	ACTTCGAGGTCACTTTCCAGCAGT	TATTGCCTTCCACACGGATGAGGT	285	60
PPP1R9A	TAAGACCACCCTGCTGTGGAAACA	GTTTGCCAGTGTTGCTGAGATGCT	317	60
DLX5	ACCATCCTTCTCAGGAATCGCCAA	TGTGTTTGTGTCAATCCCAGCGAG	457	60
CPA4	TCTCTTTCCACCTTCAATCGGCCT	CAGGAAAGTCTGCGGCAATGTTGT	259	56
CDKN1C	AGCACATCTACGATGGAGCGTCTT	AGTCGCTGTCCACTTCGGTCCACT	235	60
SLC22A18	GCCACTTCTCGGAGGAGGTGCT	GGAGCAGTGGTTGTACAGAGGCG	230	55
GNAS	ATGGTGAGAAGGCAACCAAAGTGC	GAAGGTGCATGCGCTGAATGATGT	912	60
UBE3A	ATGCACTTGTCCGGCTAGAGATGA	AGCCAGACCCAGTACTATGCCAAT	263	60
ATP10A	AACCGGCTCAAGACCACCAAGTA	TGTAGTCCTCCCATAGGTCCCTGAA	211	60
PHLDA2	CTTCCAGCTATGGAAGAAGAAGCG	CGGCGGTTCTGGAAATCGATGA	255	55
H19	AGCTAGAGGAACCAGACCTCATCA	ATGGAATGCTTGAAGGTTGCCC	525	62
PEG3	TCGCTGAGGACAGGAAACCT	ACTCCCTTGCTCTTCCCGAT	303	56
MAGEL2	TAAAGAGCGCAGGACCTCCTCAAA	TGCCTTTGAGGCATTCATGTTGGG	346	60
MKRN3	GCGGCATTTGGACAAAGCAGATCA	ATCACAGGCAAGGAAAGGGAGGAT	238	60
MEST	ATGGGATAACGCGGCCATGGTG	TTCCAACCACACCCACAGAGTCTT	218	56
PEG10	ACAACAACAACTCCAAGCACACCG	TCTGGGTTGCCATCGAACTTCTCT	322	60
PLAGL1	GCCACGTTTCCCTGCCAATTATGT	TCTCTTCCGCATGGGATTTGAGGT	433	60
DIRAS3	TCCCAACGATGGGTAACGTCAGTT	TCCAGGGTTTCCTTCTTGGTGACT	382	60
SGCE	TACCCATCAGCAGGTGTCCTCTTT	AGGTGCGCCTATTGTAGGCAGTTA	265	60
ZIM2	TGTACCAACCGGAAGACGACAACA	TCGCCATCACAGGAAGGGAAAGAT	228	60
IGF2	CCAAAGTCCCGCTAAGATTCTCCA	GCAAAGATGATCCCTAGGTGTGCT	471	65
NDN	GAGCCGCCCGAATACGAGTT	GCAGGAGCAGTCTACCCCAA	564	60
SNPRN	AGTTACTGTGGATGAGGGTGATGC	CACCCAGGACCTTCCACTCATTTA	474	58
STEMNESS GENES				
OCT4	TGGAGAAGGAGAAGCTGGAGCAAA	GAAGCTAAGCTGCAGAGCCTCAAA	212	60
NANOG	CAGAACTGTGTTCTCTTCCACCCA	CGCTGATTAGGCTCCAACCATACT	801	60
TDGF	CATATTGGCCAGTCTGGTCTCGAA	ATTCCTTCAGCACTCTGGTTCCTC	303	60
TERT	AGCTATGCCCGGACCTCCAT	GCCTGCAGCAGGAGGATCTT	185	60
LEFTYA	CCTGTGTCCTTCCATTTCCTGTCT	AAGCCCTTCATCCTTCCTCTTAGC	341	60
SOX2	CCCCCGGCGGCAATAGCA	TCGGCGCCGGGGAGATACAT	448	60
CARDIAC GENES				
GAPDH	GTGGTCTCCTCCGACTTCAACA	GTCTCTCTCTTCCTCTTGTGCTCT	217	61
Alpha-MHC	GTCATTGCTGAAACCGAGAATG	GCAAAGTACTGGATGACACGCT	413	61
hANP	GAACCAGAGGGGAGAGACAGAG	CCCTCAGCTTGCTTTTTAGGAG	406	61
NKX2.5	TGGCTACAGCTGCACTGCCG	GGATCCATGCAGCGTGGAC	167	57
Mlc2a	AAGGTGAAGTGTCCCAGAGG	ACAGAGTTTATTGAGGTGCCCC	377	61
cTnT	GGCAGCGGAAGAGGATGCTGAA	GAGGCACCAAGTTGGGCATGAACGA	152	64
qPCR				
GENE	**5' PRIMER**	**3' PRIMER**	**PROBE**	
PLAGL1	AGTACAACACCATGCTGGGCTAT	TGCTGGCCGCATGGA	AGAGGCACCTGGCC	
SGCE	ACCCAAAACCTGGCGAGAT	TCCAGGTCGGTCTGGGTAAC	AGTAATGATCCCATAACATT	
SNRPN	AAGCAACCAGAGCGTGAAGAA	TCCCCACGCAGCAACAC	AGCGGGTTTTGGGTCT	
H19	CCTCCCCGACTCTGTTTCC	CACAACTCCAACCAGTGCAAA	CCGTCCCTTCTGAATT	
IGF2	GTCGGCCCAGCCAGAGT	CGGCTACCATCATCTCCATTG	AGGAAGGAGTTTGGCC	
NECDIN	TGTCTCCGAGGACTAGCCAAGT	GCCCTGGTGAGGATCAGAAA	TGGAGGCAGATGAAT	
UBE3A	GAAGGAGAACAAGGAGTTGATGAAG	CCTCCACAACCAGCTGAAAAA	AGGTGTTTCCAAAGAA	
PEG10	CCCTTCGAGAGCAAGTGGAA	GCGGAGCTCGATGTCATCAT	CCACCCCTGAGGATG	
GAPDH	GGTGGTCTCCTCCGACTTCA	ACCAGGAAATGAGCTTGACAAAG	CCCACTCTTCCACCTTCGACGCTG	
DIRAS3	CGGCAACTTCCGTCATGAGT	CCAGCAACTGGCAGTAGGTATTT	CCTGCCGACCATTG	
OCT4	CCCACTGGTGCCGTGAA	TTGGCAAATTGCTCGAGTTCT	GGACTCCTCCGGGTTTTGCTCCAG	
BISULFITE SEQUENCING PRIMERS				
NAME	**5' PRIMER**	**3' PRIMER**	**bp**	**Tm**
BIS H19	GAGGTGTTTTTATTTTTTGGATGAT	ACTCCTAAATTAACATCTCAAACCTAC	637	51
H19 SNP	CAGTTGTAGGTGTGGAATCAGAAGTGGC	GTTAATGTCTGGCCACTTAGGGCT	845	69

Supplemental Table 2. RT-PCR expression analysis of cardiac-specific genes in contracting aggregates derived from PESCs (rPESC lines) and a bi-parental control ESCs (ORMES-22)

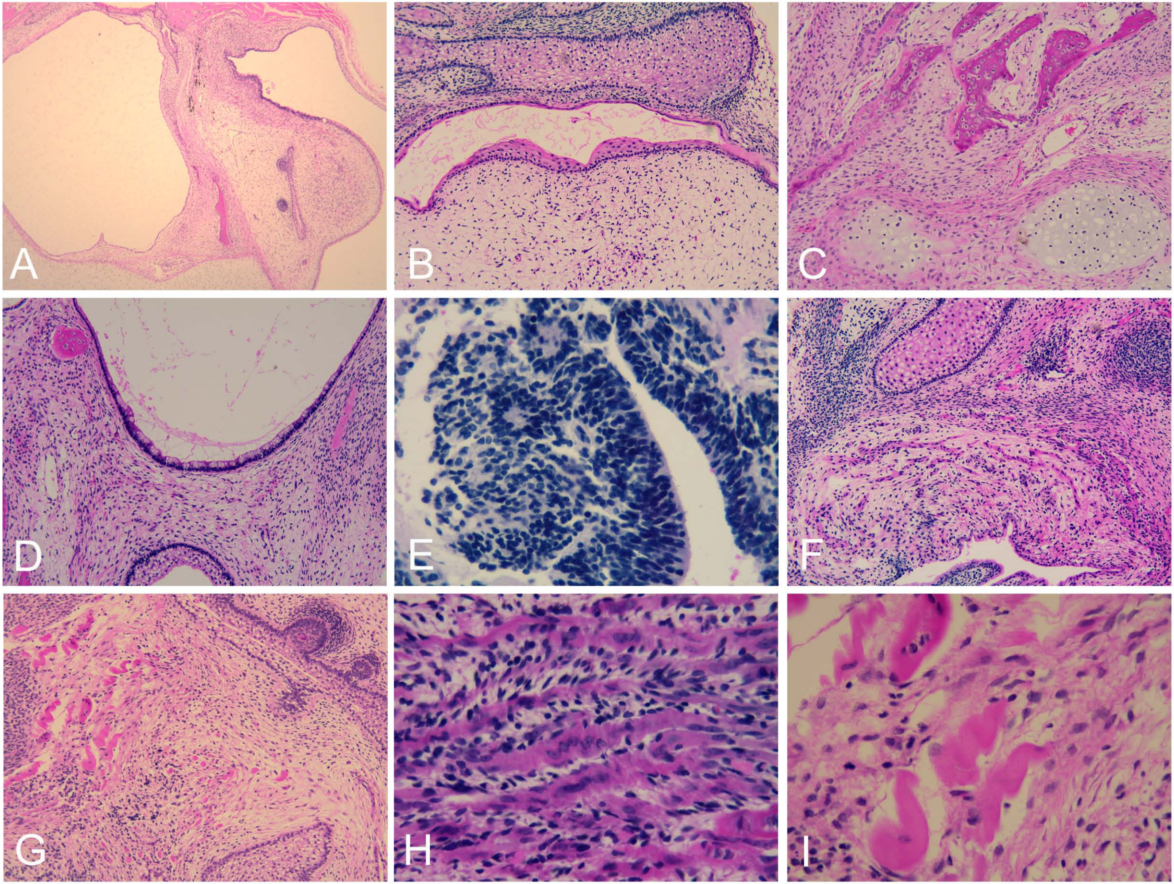
Gene	Adult heart tissue	ORMES-22 undifferentiated	ORMES-22 cardiac differentiation	rPESC-2 cardiac differentiation	rPESC-3 cardiac differentiation	rPESC-4 cardiac differentiation
GAPDH	+	+	+	+	+	+
ALPHA-MHC	+	-	+	+	+	-
hANP	+	-	+	+	+	+
NKX2.5	+	-	+	+	+	+
TROPONIN T	+	-	+	+	+	+
MLC-2A	+	-	-	+	+	-

Presence (+) or absence (-) of PCR cDNA products generated from probes for each gene

Supplemental Figure legends

Supplemental Figure 1. Cytogenetic analysis of rhesus monkey PESCs by G-banding demonstrating normal euploid karyotypes (42, XX) for rPESC-2, -3, -4 and -5 that were indistinguishable from fertilized female rhesus karyotypes. G-banding analysis of rPESC-1 revealed that the majority of analyzed cells (24/32) displayed cytogenetically normal XX female chromosome complement. However, remaining cells exhibited unbalanced chromosomal abnormalities: two of 32 analyzed cells were characterized by the presence of three copies of chromosome 18 and six cells were characterized by the presence of three copies of chromosomes 14 and 18. It is unclear at this point, whether these abnormalities were inherited from the parental embryo or occurred during ESC culture.

Supplemental Figure 2. Analysis of differentiation potential of rPESC lines in teratomas. A: Cystic areas within a teratoma (x 40; H&E); B: Ectoderm-derived stratified squamous epithelium with some areas of keratinization (x100; H&E); C: Mesoderm-derived cartilage and bone (x 100; H&E); D: Endoderm-derived intestinal-type columnar epithelium (x 100; H&E); E: Immature neuroectodermal tissue (x 400; H&E); F-I: Muscle tissue (x100 and x400; H&E).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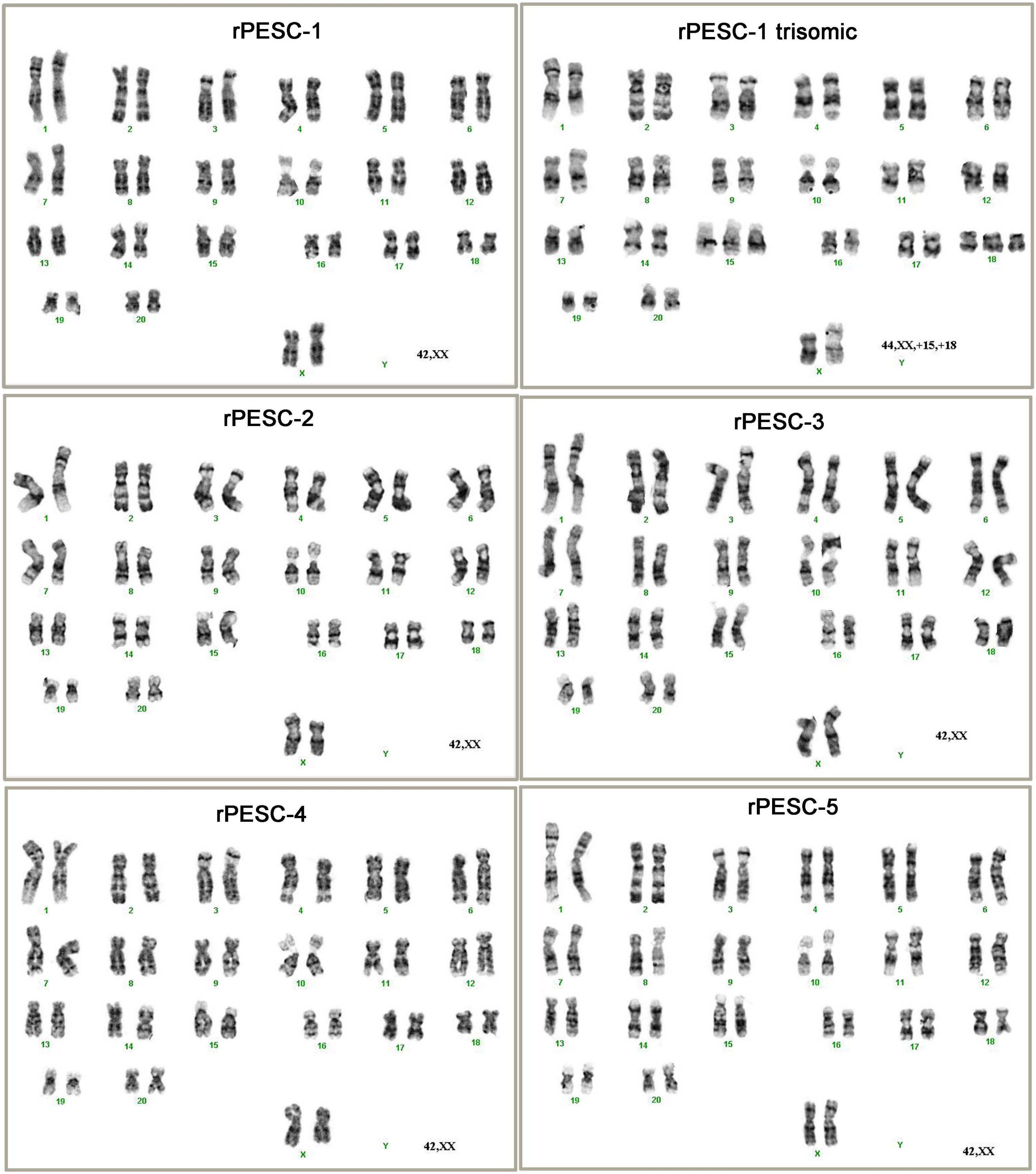
rPESC-1
42,XX
rPESC-1 trisomic
44,XX,+15,+18
rPESC-2
42,XX
rPESC-3
42,XX
rPESC-4
42,XX
rPESC-5
42,XX
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
X
Y