**RhD and ABO genotyping.** RhD genotyping of hES cell lines by PCR was reported by Arce et al and Simsek et al\(^1\):\(^2\) with minor modifications. Since all hES cells were maintained on MEF, we designed a pair of human DNA specific PCR primers that only amplified human DNA sequences. PCR primers were: RhD-F, 5′'-tgaccctgagatgcgtccac-3′ and RhD-R, 5′'-agcaacgataccacgttgtct-3′, which amplify intron 4 between exons 4 and 5, and generate only a 1,200 bp fragment with DNA from RhD negative individuals, whereas in RhD positive individuals, 100 bp and 1,200 bp (which is weak due to the fragment size of amplification) are generated. This strategy has been confirmed to be in complete agreement with serologically determined phenotypes.\(^2\) In brief, genomic DNA was isolated from hES cells using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA), and 200 ng DNA per reaction in 50μl was used for PCR amplification. PCR conditions: 94°C for 45 sec, 60°C for 1.5 min, and 72°C for 2.0 min for 35 cycles with final extension at 72°C for 7 min. PCR products were separated on a 1.2% agarose gel and visualized by ethidium bromide staining. DNA from mononuclear cells of normal human blood with RhD positive and negative individuals was used as positive and negative controls.

Genotyping of ABO blood group was developed based on the polymorphism of glycosyltransferase among ABO blood group individuals.\(^3\) First, human specific PCR primers were designed to amplify a DNA fragment surrounding nucleotide 258, in which O allele contains one nucleotide (G) deletion at this site and generates a cutting site for restriction enzyme Kpn I, but eliminates a cutting site of restriction enzyme Bst EII. PCR products were then subjected to restriction digestion by Kpn I and Bst EII: PCR product from O/O genotype can only be digested by Kpn I to generate two new shorter fragments, but is resistant to the digestion of Bst EII; while PCR product from A/A, B/B and A/B genotypes is resistant to Kpn I digestion, and is only cut by Bst EII; whereas PCR product from genotypes of A/O or B/O can be digested partially by both enzymes. Therefore, the first PCR amplification and restriction digestion is able to distinguish O blood type and non-O blood type. Based on the results, the second set of PCR primers were designed to amplify the region of nucleotide 700, where both A and O alleles contain a G nucleotide that can be digested by Msp I, while the B allele has an A nucleotide at this position that generates an Alu I cutting site. The combination of two separate PCR amplification at two diagnostic positions of the glycosyltransferase and four restriction enzyme digestions can clearly distinguish A, B or O alleles. In brief, the PCR reaction was carried out with a set of primers amplifying the region of nucleotide 258 (primers: O-type-F, 5′'-gccgttgccagggccagctg-3′, O-Type-R, 5′'-aatgtcagagtcactgac-3′, PCR product, 268 bp), the PCR product was purified by a Qiagen Kit, digested by Kpn I and Bst EII, and separated on a 2% agarose gel and visualized by ethidium bromide staining. For the O/O genotype, Kpn I generates 174 bp and 93 bp fragments, and Bst EII does not cut the PCR product; for the A/A, B/B and A/B genotypes, Kpn I does not cut the PCR product, Bst EII generates 174 bp and 93 bp fragments; for A/O or B/O genotypes, both Kpn I and Bst EII partially cuts the PCR product and generates 267 bp (original), 174 bp and 93 bp fragments. Second PCR amplification using primers amplifying the region of nucleotide 700 was carried out (primers: AB-Type-F, 5′'-tgctggaggtgcgctcaagc-3′, AB-Type-R, 5′'-gtgaaaatcgccctgcctcttg-3′, PCR product, 278 bp), PCR product was purified by a Qiagen Kit, digested by Alu I and Msp I and separated as above. For the B/B genotype, Alu I digestion generates 187 bp + 91 bp fragments, and Msp I digestion generates 206 bp + 47 bp. For A/A, A/O and O/O genotypes, Alu I does not cut the PCR product, Msp I generates 187 bp + 47 bp fragments. For the A/B or B/O genotypes, Alu I generates 278 bp (no cut) + 187 bp + 91 bp fragments; and Msp I generates 206 bp and 187 bp + 47 bp fragments.
References