**Blood sampling and DNA extraction**

Peripheral blood was drawn in EDTA containing vacutainer (BD, Oxford, UK) Tube. Erythrocytes of the samples were lysed by osmotic shock using 20 mM Tris-HCl (pH 8.0). DNA was extracted using Genomic DNA isolation kit (FavorPrep, Favorgen, Taiwan). The DNA concentration was determined using a Nanodrop spectrophotometer (Thermo Scientific, USA). Acceptable DNA samples (A260/A280 of 1.8-2.0) were diluted to 50 ng/μl and stored at -80°C until used.

**Genotyping**

Genotype of the polymorphism (CDH1) was determined by allele specific polymerase chain reaction method using the following primers 5′GCCTTATGATTCTCTGCTCG-3′ (Wild Type forward), 5′GCCTTATGATTCTCTGCTCA-3′ (Mutant Type forward) and 5′-AACCACCAGCAACGTGATTT-3′ (reverse). Briefly, the PCR mixture contained 1x PCR master mix (Thermo Fisher Scientific, Waltham, MA USA ), 10 picomoles of each primer, 50- 100 ng of genomic DNA in a total volume of 25 μl. Amplification was accomplished by following thermal cycling condition: 95°C for 5 minutes (one cycle); 95°C for 30 Seconds, 57°C for 20 Seconds and 72°C for 30 Seconds (30 cycles); 72°C for 7 minutes (one cycle), and hold at 4°C. The amplification product (241 bp) was visualized and documented using Gel documentation (protiensimple, Santa Cara, CA USA) after resolving the product along with DNA size markers (1 Kb+ DNA ladder, Invitrogen, USA) in 2% agarose gel and staining the gel with SYBR Safe DNA gel stain (Thermo Fisher Scientific, USA).

**Sequencing**

The two of the samples which showed heterozygous genotype were subjected to sequencing using Sanger method (ABI 3500).