Genotyping by Agena MassARRAY ® platform

The studied SNPs were genotyped using the Agena MassARRAY ® platform (Agena Bioscience, Sequenom, San Diego, CA) according to the manufacturer’s protocol available at (<https://doi.org/10.1007/978-1-4939-6442-0_5>), using recommended reagents in the iPLEX Gold SNP genotyping kit. Briefly, specific assays were designed using the MassARRAY Assay Design software package (v3.1). The primers, extended sequences, and calls of genotypes are shown in **Table 1**. The process involved a locus-specific PCR reaction, with 20 ng DNA, 0.5 U Hotstar Taq, 0.5pmol primers, and 0.5 mM dNTPs in a 5μl PCR reaction mix. The condition of locus-specific PCR reaction was: ① 94℃ 4 minutes; ② 45 cycles of 20 seconds at 94℃, 30 seconds at 56℃, and 1 minute at 72℃; ③ more 3 minutes at 72℃. Before undertaking the following iPLEX GOLD primer extension reactions, residual nucleotides were dephosphorylated with SAP enzymes by adding 2μl SAP Mix containing 0.5U SAP into the PCR mix, and incubating at 37℃ for 40 minutes and 85℃ for 5 minutes.

Then single-base extension reactions were performed by adding 2μl EXTEND Mix at the following conditions: I. 94º C for 30 seconds, II. 94º C for 5 seconds, III. 52º C for 5 seconds, IV. 80º C for 5 seconds, V. GOTO III, 4 more times, VI. GOTO II, 39 more times, VII. 72º C for 3 minutes. The products were desalinated with Spectro CLEAN resin (Sequenom) by adding 16μl water, vertical rotation for 30 minutes, and centrifuge. A 10 nL aliquot of the desalinated product was spotted onto a 384-format Spectro CHIP with the MassARRAY Nanodispenser. Mass determination was carried out with the MALDI-TOF mass spectrometer and MassARRAY Type 4.0 software was used for data acquisition.

SNP genotypes were named using cluster analysis with a default parameter setting. Genotypes were further reviewed manually to correct classification errors caused by clustering artifacts.

**Table 1.** The primers and extended sequences of rs369065 and rs314280

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| SNP\_ID | 2nd-PCRP | 1st-PCRP | AMP\_LEN | Tm(NN) | UEP\_SEQ | EXT1\_CALL | EXT1\_SEQ | EXT2\_CALL | EXT2\_SEQ |  |
| rs369065 | ACGTTGGATGAACCACAGAATGACTCCTGC | ACGTTGGATGGGGATTTGCAGAGGGTTAAG | 98 | 45.3 | ccttTTTCTGATGGCATCCA | T | ccttTTTCTGATGGCATCCAA | C | ccttTTTCTGATGGCATCCAG |  |
| rs314280 | ACGTTGGATGATGGAGACAAATGCAAGGCG | ACGTTGGATGGGAAACAAAAGCAAAGCGAC | 113 | 51.5 | AGCAGAGGCACTTCAGAATA | G | AGCAGAGGCACTTCAGAATAC | A | AGCAGAGGCACTTCAGAATAT |  |

Notes: 2nd-PCRP, Secondary amplification primer (includes secondary tag). 1st-PCRP, Primary amplification primer (includes primer tag). AMP\_LEN, Amplicon length in bases. Tm(NN), Extend primer melting temperature, calculated by Nearest Neighbor method. UEP\_SEQ, Extend primer sequence. EXT1\_CALL, SNP sequence alternative producing the analyte 1. EXT1\_SEQ, Sequence of analyte 1. EXT2\_CALL, SNP sequence alternative producing the analyte 2. EXT2\_SEQ, Sequence of analyte 2.