

Genotyping

The genomic DNA was amplified by PCR using HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). Genotyping assays were carried out with 5 ng of genomic DNA. PCR primers are shown in Table. They were used at 167 nM final concentrations for a PCR volume of 6 μ l. The PCR condition was 95°C for 15 min for hot start, followed by denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec, extension at 72°C for 1 min for 45 cycles, and finally incubation at 72°C for 10 min. PCR products were first treated with shrimp alkaline phosphatase (SAP) (Amersham, Freiburg, Germany) for 20 min at 37°C to remove excess dNTPs followed by an incubation for 10 min at 85°C to inactivate SAP. ThermoSequenase (Amersham) was used for the base extension reactions. Extension primers were used at a final concentration of 5.4 μ M in 10 μ l reactions. The base extension reaction condition was 94°C for 2 min, followed by 94°C for 5 sec, 52°C for 5 sec, and 72°C for 5 sec for 40 cycles. All reactions (PCR amplification, base extension) were carried out in a Tetrad PCR thermal cycler (MJ Research, Ramsey, Minnesota). The final base extension products were treated with SpectroCLEAN resin (Sequenom) to remove salts in the reaction buffer. This step was carried out with a Multimek 96-channel autopipette (Beckman Coulter, Krefeld, Germany), and 16 μ l of resin-water suspension was added into each base extension reaction, making the total volume 26 μ l. After a quick centrifugation (2,000 rpm, 3 min) in an Eppendorf Centrifuge 5810, 10 nl of reaction solution was dispensed onto a 384 format SpectroCHIP (Sequenom) prespotted with a matrix of 3-hydroxypicolinic acid (3-HPA) by using a SpectroPoint nanodispenser (Sequenom). A modified Bruker Biflex matrix-assisted laser desorption ionisation time-of-flight mass spectrometer (Sequenom) was used for data acquisitions from the SpectroCHIP. Genotyping calls were made in real time with MassARRAY RT software (Sequenom). For analysis of sequence binding sites MatInspector release 7.0 (Genomatix, Munich, Germany) was used. Genotyping call rates were 96.8 % or higher.