untitled protocol

Xinping Wang,Heng Guo,Yu Li,Haixia Wang,Jia He,Lati Mu,Yunhua Hu,Jiaolong Ma,Yizhong Yan,Shugang Li,Yusong Ding,Mei Zhang,Qiang Niu,Jiaming Liu,Jingyu Zhang,Rulin Ma,Shuxia Guo,Xinping Wang,Heng Guo,Yu Li,Haixia Wang,Jia He,Lati Mu,Yunhua Hu,Jiaolong Ma,Yizhong Yan,Shugang Li,Yusong Ding,Mei Zhang,Qiang Niu,Jiaming Liu,Jingyu Zhang,Rulin Ma,Shuxia Guo

Abstract

This is a SNP Genotyping protocol

Citation: Xinping Wang,Heng Guo,Yu Li,Haixia Wang,Jia He,Lati Mu,Yunhua Hu,Jiaolong Ma,Yizhong Yan,Shugang Li,Yusong Ding,Mei Zhang,Qiang Niu,Jiaming Liu,Jingyu Zhang,Rulin Ma,Shuxia Guo untitled protocol. **protocols.io**

dx.doi.org/10.17504/protocols.io.mmtc46n

Published: 15 Jan 2018

Protocol

Step 1.

DNA ExtractionFasting venous blood (200 µL) was taken from each study subject and a non-centrifugal columnar blood genomic DNA isolation kit (Tiangen, Beijing, China) was used to extract the whole blood genomic DNA. Extracted DNA was verified by gel electrophoresis (0.7% agarose). A NanoDrop spectrophotometer (NanoDrop technologies, Inc.: Wilmington, DE, USA) was used for quantitative determination of DNA concentration and purity: concentration□30 ng/µL and purity levels (OD260/OD280) of 1.7–2.0 were considered acceptable. Samples that met these criteria were diluted to 10–30 ng/µL using double-distilled water and stored at -80°C until use.

Step 2.

PCR AmplificationPrimers were designed using the Mysequenom tool (www.mysequenom.com/Home) and Assay Designer 3.0 software (SEQUENOM, Inc.: San Diego, CA, USA). Final PCR reaction volumes were 15 µL, which included 1µL DNA samples, 0.3 µL dNTPs, 7.4 µLwater, 1.5 µL10□PCR buffer, 1.5 µL MgCl2, 0.3 µLTaq enzymes, and 3 µL PCR amplification primer mixture. Cycling conditions were as follows: predegeneration at 94°C for 4min; followed by 35 cycles of denaturation at 94°C for 20s, annealing at 56°C for 30s, and extension at 72°C for 1min. A final extension step was carried out at 72°C for 3min, after which samples were maintained at 4°C. Reactions were set up in an ice bath and each PCR experiment included a negative control reaction.

Step 3.

PCR Products PurificationShrimp alkaline phosphatase (SAP) was used to remove excess dNTPs from samples after PCR. This step served to ensure the accuracy of single-base extension. The final SAP reaction volumes were 5.0 mL, which included 0.5 µL 10 □ SAP buffer, 2 µL PCR product, 2 µL double-distilled water, and 0.5 µL SAP enzyme. Reactions were carried out by incubation at 37°C for 40 min, followed by incubation at 85°C for 5 min. The reaction products were stored at 4°C.

Step 4.

Single-Base ExtensionFor single-base extension reactions, final reaction volumes were 6.0 µL, which

included 0.5 µL Snapshot reagent, 2.5 µL water, 1 µL primer mix, 2 uL purified PCR products. Reaction conditions were as follows: denaturation at 94°C for 30s; followed by 40 cycles of 94°C for 5 s, 52°C for 5s, and finally 52°C for 5s. Reaction products were stored at 4°C.

Step 5.

Genotyping AnalysisTake 1 µL reaction product plus 9 µL HIDI, 95°C denaturation 3min, immediately ice-water bath, all representative SNP genotyping experiments were done using TaqMan technology on an ABI3730XL system (Applied Biosystems: Carlsbad, CA, USA). T gene-mapper was used to complete the classification and output the results.