Detection of allele frequencies in the cDNA sample

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Abstract

This protocol provides an useful technique for detecting allele frequencies in the cDNA sample

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Protocol

Step 1.

Reverse transcription of total RNA

Reverse transcript total RNA to cDNA

Step 2.

Primer design

Design the PCR primers at conserved regions between alleles, and the amplified region includes target SNP site.

Step 3.

TaqMan probe design

Design two dye-labeled (FAM and VIC) TaqMan probes for allele-specific detection.

Step 4.

Preparation of the digital PCR chip

Prepare the reaction mix (Digital PCR Master Mix (Thermo Fisher Scientific), forward and reverse primers, TaqMan probes, diluted DNA, and water) and load it onto the digital PCR chip (Thermo Fisher Scientific).

Step 5.

PCR

Perform PCR with the following cycles: (1) 96°C, 10 min (2) 39 cycles of 56 °C, 2 min and 98°C, 30 sec and (3) 56°C, 2 min.

Step 6.

Detection of allele frequencies

Count the copy number of each allele by detecting fluorescence using the QuantStudioTM 3D (Thermo Fisher Scientific).