ALLSHENG

How to Achieve SNP Genetyping Detection on Fluorescence Microplate Reader

Genotyping is the process of comparing an individual's target DNA sequence with another individual's DNA sequence or reference sequence to determine the genotype differences of the individual. Single nucleotide polymorphism (SNP) refers to the DNA sequence polymorphism caused by a single nucleotide variation at the genomic level. As a third-generation molecular marker, SNP is widely used for genotype identification of multiple species and is an important basis for studying genetic variation in human families, animal and plant strains. Therefore, it is widely used in population genetics research and disease-related gene research, playing an important role in pharmaco-genomics, diagnostics, and biomedical research.

TaqMan probe is a double labeled, self quenching hydrolysis probe that adds two specific probes with different fluorescent labels at both ends in PCR reaction to identify different alleles. TaqMan SNP genotyping analysis includes two allele specific TaqMan MGB probes, containing different fluorescent dyes and a pair of PCR primers to detect specific SNP targets.

We conduct SNP experiments using known DNA samples and compare the genotypes of PCR products on real-time PCR system and fluorescence microplate reader.

Experimental Material

- Kit name: Human ALDH2 Gene Polymorphism Detection Kit (rs671) Code: FYG-rs671
- Instrument: Gene amplification instrument, Allsheng Feyond-F100 fluorescence microplate reader
- PCR tube: White PCR 8-strip tube (Code: Roche 6612601001)

Experimental Method

A total of 28 known samples validated, including 24 DNA samples and 4 no template control (NTC).

Dilute allele AA, allele GG, and allele AG at 1:100, 1:500, and 1:1000, respectively, as unknown samples.

After PCR amplification, the samples are directly placed on the fluorescence microplate reader with a white PCR 8-strip tube and tube holder for detection.

01 PCR Amplification System

Component	Volume
RealFAST probe PCR mix	10 μL
Primer&Probe premix	2 μL
ddH2O	6 μL
DNA	2 μL
Total	20 μL

Table 1 PCR Amplification System (20 μL)

02 PCR Instrument Parameter Settings

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95 °C	5 min	1
Denaturation	95 °C	10 sec	40
Annealing / Elongation	60 °C	30 sec	40
Low Temperature Preservation	4 °C	∞	1

Table 2 PCR Reaction Condition

03 Fluorescence Microplate Reader Parameter Settings

Detection Mode	Fluorescence
Detection Type	Endpoint method
Detection Wavelength	FAM channel, HEX channel
PMT Increase	Automatic gain
Integral Time	40 us

Table 3 Microplate Reader Detection Parameter

Figure 1 Layout of the Test Sample

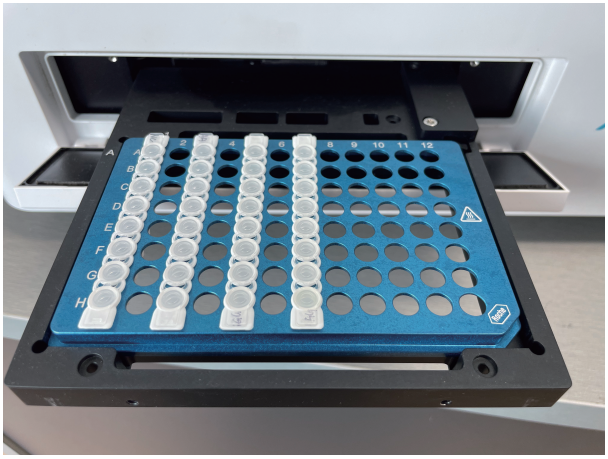

Figure 2 Direct Detection of PCR Products

Experimental Result

The detection results of the fluorescence microplate reader show that the sample distribution of homozygotes and heterozygotes is consistent with that of Roche480. The position of FAM homozygotes is close to the X-axis, while the position of HEX homozygotes is close to the Y-axis. The cluster formed by heterozygotes containing FAM and HEX is between the two homozygote clusters.

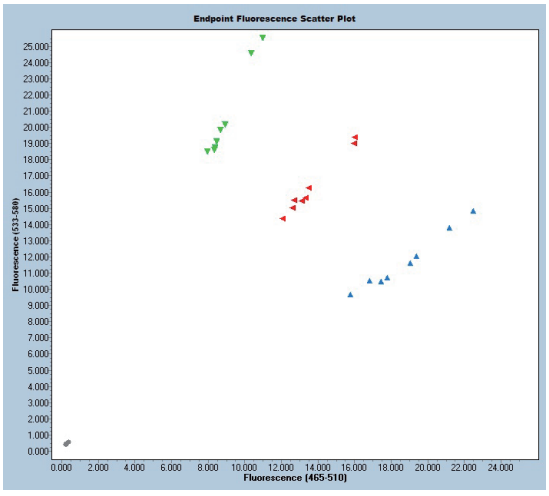

Figure 3 Genotyping Diagram of Roche

Figure 4 Genotyping Diagram of Feyond-F100

Feyond-F100 fluorescence microplate reader can be equipped with customized filters that comply with SNP genetyping experiments. The independent algorithm analysis software can meet the fluorescence detection requirements of three genetyping, making it convenient and fast to achieve data analysis of gene clusters, and providing intuitive results display.

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