PrimeSNP™ Genotyping Kit

User's Instruction

Description

PrimeSNP™ Genotyping Kit uses Competitive Allele Specific PCR method for genotyping of purified DNA samples. This method does not need to synthesize specific probes for each SNP and indel. Instead, it only requires two unique pairs of probes to achieve accurate genotyping of genomic DNA samples. By analyzing the intensity and ratio of terminal fluorescence signal, genotype is automatically discriminated, and the effect of clustering is intuitive. This kit has the advantages of less detection time, lower reagent cost and higher detection accuracy.

Kit Contents

	5,000 preps
1. 2 × PrimeSNP™ Genotyping Mix	50 ml
2. DNase-Free ddH_2O	10 ml

	50,000 preps
1. 2 × PrimeSNP™ Genotyping Mix	50 ml × 10
2. DNase-Free ddH_2O	100 ml

- 2 × PrimeSNP™ Genotyping Mix: including modified Taq DNA polymerase, probe, dNTPs, $MgCl_2$, buffer, enhancer, optimizer, and stabilizer. In PCR reaction, only the purified DNA samples, primers and ddH_2O need to be added to the 2 × PrimeSNP™ Genotyping Mix.

Features

- High accuracy: the accuracy rate of positive control is more than 98%.
- Low cost: no need to synthesize many expensive dual-labeled probes.
- Optimized PCR system: the system has good stability and high amplification specificity.
- Anti-pollution PCR system: it can effectively eliminate the aerosol pollution caused by PCR products, without worrying about the interference of environmental pollution on the fluorescence signal of negative control. This can ensure the specificity and

genotyping accuracy of amplification.

- Application: this kit can be used in molecular biology experiments such as molecular marker-assisted breeding, quantitative trait locus (QTL) mapping and genetic marker identification with large sample size.

Protocol

Important Notes Before Starting:

- According to the primer design principle of fluorescence quantitative PCR, users need to design two upstream primers with 3' ends falling at the detection site and a common downstream primer. The 5' ends of two upstream primer are connected to one tag sequence, respectively. Primers need to be validated before large-scale detection.
    - Tag sequence A: 5'-GAAGGTGACCAAGTTCATGCT-3' (for FAM signal)
    - Tag sequence B: 5'-GAAGGTCGGAGTCAACGGATT-3' (for HEX or VIC signals)
- Requirements for purified DNA: Dissolve DNA with water or TE. The purity test results should meet OD260/280=1.7-1.9 and OD260/230=1.7-2.0. Gradient experiments should be set up above the recommended minimum amount of DNA added, according to the table below. After the optimization, add DNA to the tubes at the optimum amount.

Species	DNA (ng)
Homo sapiens	10
Mus musculus	8.9
Brassica campestris	2
Oryza Sativa	1.5
Triticum aestivuml	53
Zea mays	8

- The qPCR reaction volume is 20 μL.
- Requirements for single test: the number of samples per primer for single test should not be less than 20, and the number of negative controls should not be less than 2. Positive controls for different genotypes should be set.

- 2 × PrimeSNP™ Genotyping Mix should thaw on ice, invert, and mix before use. However, please note that both DNA template and 2 × PrimeSNP™ Genotyping Mix should avoid repeated freeze-thaw.
- Setting requirements: Users need to refer to the user's guide of fluorescent quantitative PCR instrument to set up the program under Genotyping module. The reaction time required in each step of the PCR reaction procedure should not be shortened. The 2 × PrimeSNP™ Genotyping Mix does not contain ROX so the reference fluorescence should be set to "None".

Preparations

1. Prepare primer premix. Please refer to the final concentration of PCR primers recommended in the table below.

Component	Final concentration
Allele-specific forward primer 1(100μM)	12 μM
Allele-specific forward primer 2(100μM)	12 μM
Common reverse primer (100μM)	30 μM
DNase-Free ddH_2O	-

2. If the ambient temperature is high, 2 × PrimeSNP™ Genotyping Mix may become turbid. Please keep it on ice for 1-2 min, until the mix becomes clear before use.

Setting Up Genotyping PCR System

1. According to the table below, add 2 × PrimeSNP™ Genotyping Mix, primer premix and ddH_2O into tube.

   a) 2 × PrimeSNP™ Genotyping Mix should not be placed at room temperature for a long time.

Component	Volume
2 × PrimeSNP™ Genotyping Mix	10 μl
Primer premix	0.28 μl
Template (DNA)	X μl
DNase-Free ddH_2O	(9.72-X) μl
Total Volume	20 μl

Thermocycling Conditions for Genotyping PCR

Note: Please refer to the user's guide for the corresponding model of fluorescent quantitative PCR and set it according under the genotyping module. For ABI series models, FAM and VIC are selected as fluorescence channels, FAM and hex are selected for other models.

1. Initial denaturation: 94°C for 5 min
2. 10 cycles:
    a) 94°C for 20 sec
    b) 55-65°C for 1 min(-1°C/cycle) (The temperature range should be optimized according to the target fragment size, GC content, primer sequence, etc. According to the optimized temperature range, the specific cooling range is adjusted.)
3. 26 cycles:
    a) 94°C for 20 sec
    b) 55°C for 1 min (Fluorescence signal acquisition needs to be checked, and fluorescence signal acquisition should be checked in the early test stage to determine the amount of template added and whether negative controls are amplified. After the amplification conditions are optimized, there is no need to check the fluorescence signal acquisition in this step.)
4. Fluorescence acquisition: 30°C for 1 min (For ABI series models, "Pre-read stage" and "Post-read stage" are set to 30°C, and "Post-read stage" is taken as the final signal acquisition step.)

Increase Amplification Reaction Program

Under the premise that there is no obvious amplification curve for negative controls, if the clustering is not obvious and the fluorescence signal of the sample is low, the amplification reaction can be increased by running the following program under Genotyping module.

1. 3 cycles (Increase amplification reaction program should not run more than 2 times, and the total number of reaction cycles should not be more than 42.):
    a) 94°C for 20 sec
    b) 57°C for 1 min

2. Fluorescence acquisition: 30°C for 1 min (For ABI series models, there is no need to set "Pre-read stage" when running the increase amplification reaction program, just set "Post-read stage" for fluorescence acquisition.)

Transportation

Transport with ice bags to ensure that the kit is at <4°C.

Storage

If used frequently, 2 × PrimeSNP™ Genotyping Mix can be stored at 4°C for a short time (within 7 days) and away from light. For long term storage, please keep it at - 20°C and away from light.