

Product components

Components	Component number	Size-1	Size-2
		1 mL	5 mL
2× FASA™ Genotyping Master Mix (Standard ROX)	RM30252	1 mL	5 mL

Product Description

2× FASA™ Genotyping Master Mix is a specialized kit for genotyping using Fluorescent allele specific amplification based on Genotyping Systems (FASA). The FASA genotyping system consists of 2 allele-specific forward primers (3' terminal sequence is different, 5' terminal fusion detection probe sequence), 1 allele-universal reverse primer and 2 different fluorescent-labeled probes. Competitive allele-specific amplification is achieved through specific matching of the 3' terminal base of the forward primer, and the amplified products are detected by the corresponding probe and discriminated by different fluorophore labels, achieving rapid typing of SNP and InDel (Insertion and Deletion). This product is convenient to use at a low cost, which is very suitable for high-throughput allele detection, such as molecular breeding, genomics research and other fields.

Storage

-20°C

Features

Simple and flexible

This product consists of PCR buffer, dNTP, MgCl₂, DNA polymerase, ROX (Passive Reference Dye) and two fluorescent-labeled probes. Only the allele-specific primers need to be designed to achieve genotyping. Product operation is simple and flexible.

Low cost

Users can use universal detection probes without the need for probe design and synthesis for each detection site, greatly reducing detection costs, especially suitable for high-throughput allele typing detection.

Good compatibility

Alternative to similar gene typing kits.

Precautions

1. 2× FASA™ Genotyping Master Mix contains fluorophores-labeled probes and ROX, please avoid strong light exposure when storing this product or preparing PCR reaction.
2. Avoid repeated freeze-thaw, which may degrade product performance; This product can be stored at -20°C or -80°C for a long time, if frequently used in the short term, please temporarily store it at 2~8°C to reduce the repeated freeze-thaw circles.
3. This product is premixed with Standard ROX. Please check that the ROX level of the reagent is compatible with your qPCR machine. If you use Microplate Reader, all three reagents premixed with different levels of ROX can be matched.
4. Gently vortex to mix the reagent and avoid foaming. After brief centrifugation, place on ice.
5. Prior to experiment, allelic typing primers should be separately designed and synthesized according to SNP or InDel sequence. (Allelic typing primers include 2 specific forward primers and 1 universal reverse primers, among which the 5' ends of 2 forward primers should be added with corresponding detection probe sequences (see "FAQs" for detection probe sequence information)).

Operation Description

Preparations

- Instrument and reagent selection: Select Microplate Reader or qPCR machine that can read FAM, HEX, and ROX fluorescence. Prior to use 2x FASA™ Genotyping Master Mix, please check that the ROX level of the reagent is compatible with your qPCR machine, and the Microplate Reader can match all three reagents premixed with different levels of ROX.
- PCR primers preparation: Dilute the genotyping primer (freshly prepared) to 100 μM, and premix the primers according to the following table; The existing primers for genotyping detection can be directly used.

SNP Specific Primers	Final concentration (μM)	Volume (μL)
Allele-specific Primer 1-FAM (100 μM)	12	12
Allele-specific Primer 2-HEX (100 μM)	12	12
Common, Reverse Primer (100 μM)	30	30
Nuclease-Free Water	N/A	to 100

- DNA sample preparation and control setting: This product is suitable for detection of wet DNA and dry DNA under various reaction volumes. For wet DNA, the presence of PCR inhibitors such as EDTA should be avoided as much as possible. In general, to reduce influence of PCR inhibitors when using crude DNA sample, it is recommended to dilute it 3-5 times with H₂O before use. In order to obtain the best experimental results, it should be ensured that the quantity and quality of DNA samples in each reaction are relatively consistent, and 5-100 ng DNA as template input recommended in each reaction. In order to obtain reliable clustering pattern for analyzing, a minimum of 20 DNA samples are strongly recommended. For validating the genotyping results, at least two no-template controls (NTCs) should be included in each plate.
- Other reagents preparation: 2x FASA™ Genotyping Master Mix, Nuclease-Free Water, pure liquid paraffin oil (Prevent PCR reaction from evaporation).
- PCR consumables: PCR tubes or 96/384-well plate, PCR sealing films.

Reaction system

- PCR reagent preparation: Completely thaw the reagent at room temperature, vortex to fully mix prevent bubbles. After brief centrifugation, place on ice.
- DNA template preparation: Take out DNA sample, thaw it at room temperature, swirl to mix well, then centrifuge briefly. Add equal amount of DNA template into each well of 96/384-well plate (this step can be ignored for dry DNA).
- Preparation of reaction premix: According to DNA samples, calculate the volume of PCR reaction premix required; Take a clean 2.0 mL centrifuge tube, prepare PCR reaction mix according to the Table below, gently swirl and mix, and place on ice after brief centrifugation. **(Due to volume loss, it is recommended to prepare the premix at 1.1 times the required volume).**

Components	96-well plate	384-well plate	96-well plate	384-well plate
	(Wet DNA)	(Wet DNA)	(Dry DNA)	(Dry DNA)
DNA Template	5 uL	2.5 μL	N/A	N/A
2x FASA™ GMM	5 uL	2.5 μL	5 uL	2.5 μL
SNP Specific Primers	0.14 uL	0.07 μL	0.14 uL	0.07 μL
Nuclease-Free Water	N/A	N/A	5 uL	2.5 μL
Total	10 μL	5 μL	10 μL	5 μL

- Dispense reaction premix: Dispense the premix in step 3 into 96/384-well plate containing DNA template in step 2. Add 5~10 μL liquid paraffin oil, seal the PCR plate with the sealing plate film, gently swirl to mix, briefly centrifuge, and place it on ice.

Recommended PCR Program

- Set the reaction program on the PCR instrument, and set the heated lid to 105 °C; load the PCR reaction tubes in the

instrument and start the program.

Step	Temp	Time	Cycles
Pre-denaturation	95°C	15 min	1
Specific amplification	95°C	10 s	10
	65°C, -1°C/cycle	60 s	
Enrichment amplification	95°C	10 s	30*
	57°C	60 s	
Reading plate	30°C	30 s	1

2. The cycle number of the "Enrichment amplification" step in the reaction program is for reference only, and the actual reaction cycle number will be affected by the quality of DNA samples and the efficiency of primer amplification; For primers used for the first time, the optimal number of cycles for the "Enrichment amplification" step should be explored by users. For example, if the genotyping result is not obvious to analyze by 30 cycles, user can repeat the amplification of the obtained product in plate according the program shown below till having an obvious plate reading. In general, the number of amplification cycles of the "Enrichment amplification" step does not exceed 42.

Step	Temp	Time	Cycles
Pre-denaturation	95°C	10 s	4
Annealing and Extention	57°C	60 s	

Plate reading

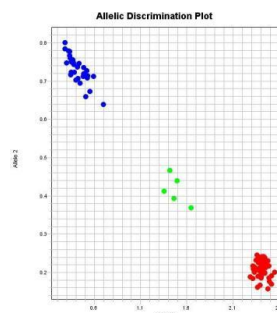
1. Plate Reading: After PCR amplification, read the fluorescence signal value below 30°C in the end-point fluorescence mode, where FAM and HEX are set to discriminate the genotype of the DNA sample, and ROX is set to correct the well-to-well error.
2. Plate Reading on Microplate Reader: When using the Microplate Reader, please set the excitation wavelength and emission wavelength of the three fluorophores as shown in the following table.

Fluorophore	Excitation (nm)	Emission (nm)
FAM	485	520
HEX	535	556
ROX	575	610

3. Data analysis: User can take the built-in "genotyping software" or Excel sheet to analyze the data document collected from qPCR instrument or Microplate Reader

Data analysis method: After signal normalization and multicomponent analysis, the bulid-in software graphs the normalized data from each well as a single datapoint on the scatterplot. For example, FAM and HEX fluorescence signal values of each reaction well (or the ratio to ROX of the well) are plotted on x - and y - axes, respectively.

The genotyping results can be seen in the following figure.



Sample marked red datapoints represent homozygous (aa) (FAM), those marked blue are homozygous (AA) (HEX), and those marked green are heterozygous (Aa) (FAM/HEX).

FAQs

1. Genotyping failed

It is recommended to perform agarose gel electrophoresis to confirm whether the target products were successfully amplified and identify whether there were non-specific amplification products. Amplification failure or non-specific amplification will lead to genotyping failure or unreliable results. Unreasonable primer design, primer degradation, PCR inhibitors, low quantity or quality of DNA are common problems that should be solved prior to each experiment. User could refer to following specific solutions:

2. Unreasonable primer design

It is recommended to use Primer3 (<https://bioinfo.ut.ee/primer3-0.4.0/>) for genotyping Primer design (Parameters Settings: Product size is 70 ~ 150 bp; Primer size is 18 ~ 28 nt; Primer Tm is 60 ~ 62 °C). After the primer design is completed, the detection probe sequence should be added to the 5' end of the forward primer, and the detection probe sequence information is as follows: 5'-GAAGGTGACCAAGTTCATGCT-3' (FAM), 5'-GAAGGTCGGAGTCAACGGATT-3' (HEX); When primers are synthesized, it is recommended to purify them by PAGE or HPLC to obtain high purity PCR primers and improve PCR specificity.

3. PCR inhibitors

This product is suitable for the detection of wet DNA and dry DNA samples under various reaction volumes. For wet DNA sample, PCR inhibitors such as EDTA should be avoided as much as possible. In general, to reduce influence such PCR inhibitors when using crude DNA sample, it is recommended to dilute it 3-5 times with H₂O prior to experiment. For DNA samples that cannot be amplified after dilution, it is recommended to purify or reextract DNA.

4. Low DNA quantity or degraded DNA

Using Nanodrop or Qubit to detect the concentration of DNA samples. It is recommended to use DNA with a concentration >5 ng/μL. In order to obtain the best experimental results, it should be ensured that the quantity and quality of DNA samples in each reaction are relatively consistent, and 5-100 ng DNA as template input recommended in each reaction..

5. The number of DNA samples is too small or the genotype is monomorphic

6. In order to obtain reliable clustering pattern for analyzing, a minimum of 20 DNA samples are strongly recommended. For validating the genotyping results, at least two no-template controls (NTCs) should be included in each plate.

When DNA samples smaller than 20, a relatively single genotype or similar genetic background would easily lead to the inability to obtain three different genotypes (AA, Aa, aa), resulting in genotyping failure.

6. Evaporation of reaction system

FASA genotyping system requires high signal uniformity, so it is recommended to use pure liquid paraffin oil to prevent reaction mix from evaporation. It is recommended to add 5 μL liquid paraffin oil into the 5 μL reaction volume and add 10 μL liquid paraffin oil into the 10 μL reaction volume.

7. Contamination of reaction system

It is necessary to avoid contamination caused by the experimental environment and operation, and it is recommended to establish NTC (No Template Control) to evaluate experiment contamination.