ChamQ Geno-SNP Probe Master Mix

Q811

Version 22.1

Introduction

ChamQ Geno-SNP Probe Master Mix is specially designed for single nucleotide polymorphism (SNP) typing by probe-based qPCR, which can be performed simply by only adding additional primers, Taqman MGB probes and templates. This master mix uses Champagne Taq DNA Polymerase as the core enzyme, with carefully optimized Buffer, the success rate of typing on low-concentration templates and complex templates has been increased. This product contains a unique ROX Passive Reference Dye that is suitable for all qPCR instruments. The concentration of ROX does not need to be adjusted on different instruments.

Compents

Components	Q811-02 (500 rxns/20 μl reaction)	Q811-03 (2,500 rxns/20 μl reaction)
2 × ChamQ Geno-SNP Probe Master Mix*	4 × 1.25 ml	5 × Q811-02

* It contains dNTP Mix, Mg^{2+}, Champagne Taq DNA polymerase, Specific ROX Reference Dye.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃. Keep away from light.

Applications

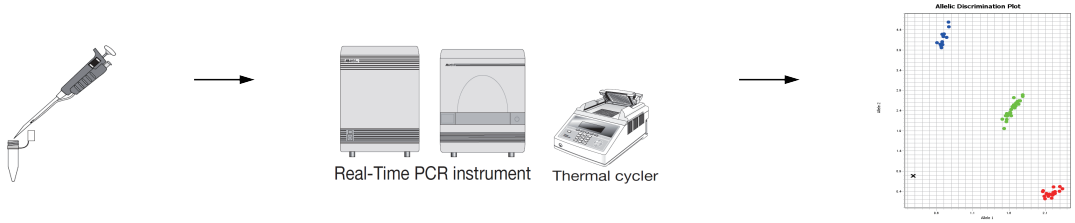
This product is suitable for DNA amplification from various type of templates such as genomic DNA, cDNA, plasmid DNA and λDNA.

Notes

For research use only. Not for use in diagnostic procedures.

Experiment Process

Prepare PCR reaction solution: PCR Terminal signal acquisition and result analysis



1. Prepare PCR reaction solution:

2 × ChamQ Geno-SNP Probe Master Mix	10 μl
Primer F (10 μM)	1.8 μl
Primer R (10 μM)	1.8 μl
TaqMan MGB Probe A (10 μM)	0.4 μl
TaqMan MGB Probe B (10 μM)	0.4 μl
gDNA	1 - 10 ng
ddH_2O	Up to 20 μl

▲ 1. For convenience, mix primers and probes into a 20 × assay (for example, 100 μM Primer F 18 μl, 100 μM Primer R 18 μl, 100 μM Probe A 4 μl, 100 μM Probe B 4 μl, filling up to 100 μl using TE). It is recommended that the final concentration of primer reaction is 900 nM, and the final concentration of probe reaction is 200 nM.

2. Do not use ROX-labeled probes because 2 × ChamQ Geno-SNP Probe Master Mix contains a special ROX.

3. Purchase Taqman genotyping assay to obtain primers and probes or design primers and probes through professional software such as Primer Express Software.

4. Each experiment requires a certain number of no template controls (NTCs) and positive controls of known genotypes

5. If the amplification reaction cannot be performed immediately after mixing, the mixed samples can be stored in a dark environment at 2 ~ 8°C for a maximum storage time of 72 h.

2. Run the PCR program as follows:

Amplification	Initial Denaturation	Rep: 1	95℃	30 sec
	Cycles	Reps: 45	95℃	10 sec
			60℃	30 sec
Acquisition	Terminal signal acquisition	Rep: 1	60℃	30 sec

▲ After the completion of PCR amplification, the end point signal cannot be collected immediately. The sample can be stored in a dark environment at 2 ~ 8℃ for up to 72 h.

FAQ & Troubleshooting

FAQ	Reason		Solution
No signal or low signal	Template	1. Template degradation	Confirm whether the DNA is degraded through agarose gel electrophoresis analysis.
		2. DNA concentration is incorrect	Re-measure the DNA concentration.
		3. The presence of inhibitors in the template	Dilute the DNA template.
		4. The input amount of DNA template is too low	Increase the DNA template input or the PCR cycle number.
	Reagent	1. Reagent expired	Repeat the test with the new batch reagent.
		2. Evaporation	Ensure that the wells are sealed, and avoid long-term storage and collect signals as soon as possible.
		3. The sample was not added to the well.	Make sure that the primer, probe, template and the amplification reagent are all in the wells.
		4. The SNP sites are included in the primer sequence	Confirm if there are SNP sites in the primer region by BLAST sequence alignment and redesigning if necessary.
	Instrument	1. Reporter group selection error	Confirm that the signal acquisition channel of the reporter group is correct and re-collect the end point signal.
The signals are too jumbled to form clusters	Template	1. The presence of inhibitors in the template	Dilute the DNA template.
		2. DNA template input is too low	Increase the DNA template input or the PCR cycle number.
	Instrument	1. Reporter group selection error	Confirm that the signal acquisition channel of the reporter group is correct and re-collect the end point signal.
		2. ROX signal is not selected	Select the ROX signal on the instrument that requires ROX correction.
The signals between the clusters are too close	Template	1. Template degradation	Confirm whether the DNA is degraded through agarose gel electrophoresis analysis.
	Reagent	1. Probe degradation	Repeat the test with a new batch of probes and ensure the storage conditions of primers, probes and reagent are correct.
		2. Probe design	Make sure the probe Tm value is in the good range.
	Instrument	1. Too many cycles	The number of reaction cycles does not exceed 45, and reduce it if exceeds 45.
The clustering effect is poor, and the signal has tail dragging	Template	1. DNA concentration is incorrect	Re-measure the DNA concentration.
		2. The presence of inhibitors in the template	Dilute the DNA template.
		3. Inconsistent template input	Re-determine the DNA concentration to ensure that the DNA template input is among 1 - 10 ng.
	Reagent	1. Reagent expired	Repeat the test with the new batch reagent.
		2. Evaporation	Ensure that the PCR wells are sealed, and avoid long-term storage and collect signals as soon as possible.
		3. The sample was not added to the well.	Make sure both the primer probe template and reagent are in the PCR reaction well.
		4. Sample was not fully mixed before PCR	Make sure the reagents are mixed thoroughly and repeat the test.
	Instrument	1. The instrument is not calibrated	Ensure that the PCR instrument is regularly calibrated.
		2. ROX signal is not selected	Select the ROX signal on the instrument that requires ROX correction.
NTC signal is too high	Reagent	1. Reagent contamination	Replace the primers, probes, amplification reagents, and all consumables, and repeat the experiment.
	Instrument	1. The instrument has fluorescent substance contamination	Clean the instrument.