ChamQ Geno-SNP Probe Master Mix

Q811

Version 21.1

Introduction

ChamQ Geno-SNP Probe Master Mix is specially designed for single nucleotide polymorphism (SNP) typing by probe-based qPCR. Only primers, probes, and templates need to be added additionally, which is convenient. Champagne Taq DNA Polymerase is the core component of this master mix. Matched with optimized buffer system, the success rate of SNP typing from low-concentration and complex templates is increased. The reagent incorporates a dUTP/UDG anti-contamination system, which works at room temperature to eliminate the amplification products contamination to ensure the accuracy of SNP typing. 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/dUTP Mix, Mg^{2+}, Champagne Taq DNA polymerase, Heat-labile UDG, Specific ROX Reference Dye.

Storage

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

Applications

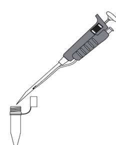
This product is suitable for DNA amplification from various type of templates such as genomic DNA, cDNA, plasmid DNA and λDNA.

Notes

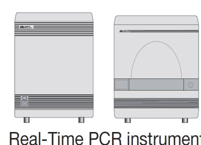
For research use only. Not for use in diagnostic procedures.

Experiment Process

Prepare the reaction solution



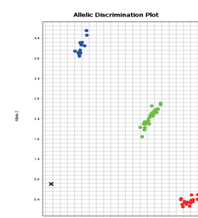
PCR



Real-Time PCR instrument

Thermal cycler

Terminal signal acquisition and result analysis



1. Prepare the reaction mix as follows:

Component	Volume
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. The primers and probes can be mixed into a 20 × assay (eg. 100 μM Primer F 18 μl, 100 μM Primer R 18 μl, 100 μM Probe A 4 μl, 100 μM Probe B 4 μl, fill up to 100 μl using TE). The recommended final concentration of the primer is 900 nM, and the final concentration of the probe is 200 nM.
2. Do not use ROX-labeled probes because the master mix contains a special ROX.
3. Purchase Taqman genotyping assay to obtain primers and probes or design primers and probes through specialized software such as Primer Express Software.
4. Each trial requires a certain number of template-free control (NTC) and positive control with known genotypes.
5. If the PCR cannot be performed immediately after the mixing, the mixed sample can be stored in a dark environment at 2 ~ 8°C for up to 72 h.

2. Run the qPCR program as follows:

Amplification	Initial-denaturation	Rep: 1	95˚C	30 sec
	Cycling reaction	Reps: 45	95˚C	10 sec
			60˚C	30 sec
Acquisition	Terminal signal acquisition	Rep: 1	60˚C	30 sec

▲ 1. The thermosensitive UDG can function at room temperature. It works before running the PCR program. And it is inactivated during the initial-denaturation step at 95˚C.

2. 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˚C 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 is 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.

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