ChamQ Geno-SNP Probe Master Mix

Q811

Version 9.1

Vazyme Biotech Co., Ltd.

Introduction

ChamQ Geno-SNP Probe Master Mix is specially designed for single-nucleotide polymorphism (SNP) typing by probe method. SNP typing can be performed directly after primers, probes, and templates are added, which makes it easy to use. Champagne Taq DNA Polymerase contained in this master mix as the core enzyme, along with optimized buffer, increases the success rate of typing of low-concentration templates and complex templates. The UTG/UDG anti-pollution system included in this master mix, which can function at room temperature to remove the pollution existing in the system, ensures the accuracy of the typing. At the same time, the special ROX Passive Reference Dye contained in this product, makes the product applicable to all qPCR instruments without the need to adjust the ROX concentration on different instruments.

Package Information

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

* Contain dNTP/dUTP Mix , Mg2+, Champagne Taq DNA polymerase, Heat-labile UDG, Specific ROX Reference Dye.

Applicable qPCR Instrument

Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOnePlus™ 7500, 7500 Fast, ViiA™7;

Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon™, Opticon®, Opticon 2, Chromo4™;

Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000;

Stratagene MX4000™, MX3005P™, MX3000P™;

Eppendorf Mastercycler® ep realplex, realplex 2 s;

Roche Applied Science LightCycler™ 480 and other instrument;

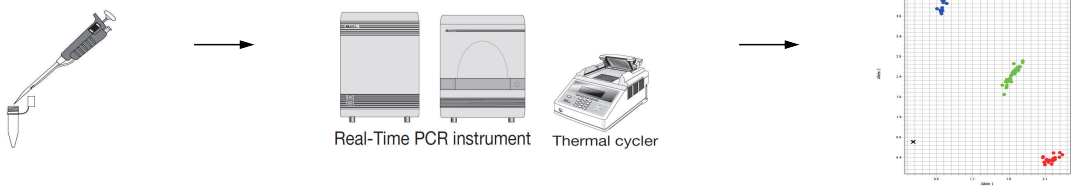
▲This product contains special ROX Reference Dye, applicable to all QPCR instrument, do not need to adjust the Rox concentration for different instruments.

Storage

Store at -30˚C ~ -15˚C, and protected from light. Shipped at -20˚C to 4˚C.

Protocol

Prepare the reaction solution → PCR reaction → Terminal signal collection and result analysis



1. Prepare a reaction solution in a qPCR tube as follows:

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

a. 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, that is 20 × assay), the recommended final concentration of the primer is 900 nM, and the final concentration of the probe is 200 nM.

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

c. Primers and probes can be purchased from Taqman genotyping assay or designed through specialized software such as Primer Express Software.

d. Each trial requires a certain number of template-free controls (NTC) and positive controls for known genotypes.

e. If the PCR reaction 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 hours.

2. Perform qPCR reaction and collect the terminal signal:

Amplification	Pre-denaturation	Reps: 1	95℃	30 sec
	Cycling reaction	Reps: 45	95℃	10 sec
			60℃	30 sec
Collection	Terminal signal collection	Reps: 1	60℃	30 sec

a.The thermosensitive UDG enzyme can function at room temperature, it is work before the PCR program is set. And it is inactivated during the pre-denaturation step PCR at 95℃.
b.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 hours.

Trouble Shooting

FAQ	Reason		Solution
No signal or low signal	Template	1.Template degradation	Agarose gel electrophoresis to confirm whether the DNA was degraded.Re-measure the DNA concentration
		2. DNA concentration is incorrect	
		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 increase the PCR cycle number.
	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 PCR well.	Make sure both the primer probe template and the amplification reagent are in the PCR reaction well.
		4. The SNP site is included in the primer sequence	Confirm if there is a SNP site in the primer region by BLAST sequence alignment and redesigning if necessary.
	Instrument	1. Report (Reporter) group selection error	Confirm that the collection channel of the reporting 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 increase the PCR cycle number.
	Instrument	1. Report group selection error	Confirm that the collection channel of the reporting 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 to each other	Template	1. Template degradation	Agarose gel electrophoresis to confirm whether the DNA was degraded.
	Reagent	1. Probe degradation	Repeat the test with a new batch of probes and ensure the storage conditions of primer probe 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 cycles, 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 PCR well.	Make sure both the primer probe template and reagent are in the PCR reaction well.
		4. Sample is mixed Insufficient before PCR reaction	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.