2 × CYF8 FastLong PCR MasterMix

Product Description	Size	
2×CYF8 FastLong PCR Master Mix	CYPC8001	CYPC8002
	1ml	5ml

Store: −20°C, 2 year

Product Description

The kit contains CYF8 FastLong DNA Polymerase, dNTPs and optimized reaction buffer at a concentration of 2x. Before reaction, DNA/cDNA template, specific primer, and ddH2O should be prepared to mix the final concentration of 1×.

The Mix contains red tracer dye for quicker electrophoresis. And the PCR amplification products are with polyA end, which can be directly employed to the clone reaction with our pTOPO vector. Of course, after purified, the amplification products also can be used for other follow-up experiment, such as digestion, ligation and fluorescence sequencing.

Product Features

- High yield;
- Fast and long amplification: 5s-10s/kb, about 10kb;
- Super Fidelity: Up to 3 to 6 times of traditional Taq DNA polymerase;
- Powerful amplification capacity: suitable for the amplification of complicated structure or high GC content template;

Recommended PCR system settings:

Component	25 μl Reaction	50 μl Reaction	Final Concentration
2×CYF8 PCR MasterMix	12.5 μl	25 μl	1 ×
Forward Primer (10 μM)	0.5 μl	1 μl	0.2 μM
Reverse Primer (10 μM)	0.5 μl	1 μl	0.2 μM
Template DNA	as required	as required	
ddH_2O	up to 25 μl	up to 50 μl	

Reference template dosage (50 μl reaction system)

Plasmid: 0.1-10ng; Bacterial genome: 10-100ng; Human genome: 50-150ng;

cDNA: 1-5μl from RT reaction;

Proposed PCR cycle conditions

Step	Temperature	Time	Cycle Number
Initial denaturation	94°C	2-3 minutes	
Denaturation	94°C	10 seconds	

Annealing	55°C	10-15 seconds	25-35 cycles
Extension	72°C	10 seconds/kb	
Final Extension	72°C	1-5 minutes	
	4-8°C	Hold	

Precautions:

1. Due to CYF8 super fast amplification capacity, 5s/kb extension and less cycles are recommended for simple or plasmid templates; 15s-20s/kb extension and more cycles for complicated template is recommended;

2. For templates with high GC content, the pre-denaturation and denaturation temperatures can be increased to 98°C, and/or extending the denaturation time; But for the long fragments, rising denaturation temperatures is not allowed to avoid the DNA damage, whereas extending pre-denaturation time (for example 5mins) and denaturation time (e.g. 5s-10s) is recommended.

3. If the amplification template GC content is high or the complex template amplification effect is poor, the DMSO can be added to the reaction mixture with the final concentration of 1%-8%, according to 1% gradient increasing to look for the best concentration. Or adding betaine to the final concentration of 1.0M-1.7M. And at the same time, using the Touchdown PCR.

Example Amplification

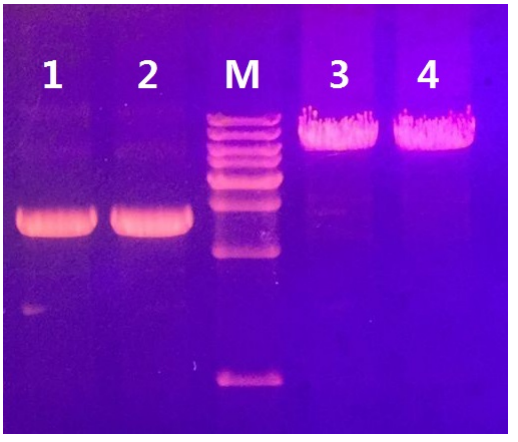

Figure1. Amplification of DNA fragment from 50 ng human genomic DNA in 50μl reaction mixture.
Lane M: 1kb ladder;
Lane 1-2: Hα1AT, 2.6Kb, extension 10 sec/kb;
Lane3-4: ß-globin, 6kb, extension 15 sec/kb.

This product is furnished for LABORATORY RESEARCH USE ONLY.
Not for diagnostic or therapeutic use.