2 × CYA9 LongHiFi PCR Master Mix

Product Description	Size	
2×CYA9 LongHiFi PCR Master Mix	CYPC8401	CYPC8402
	1ml	5ml

Store: −20℃, 2 years

Product Description

The kit contains CYA9 LongHiFi 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×.

CYA9 kits are the mixture of super-fidelity enzymes and a variety of elongation factors which enables CYA9 to greatly improve the amplification length, speed, fidelity and the yield. It is the better choice for the amplification of long fragments, high-speed and super-fidelity.

The Mix contains red tracer dye for quicker electrophoresis. And the PCR amplification products are with blunt 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: 0.5 or one times of Pfu;
- Fast amplification: 3-4kb/min;
- Super Fidelity: Up to 54 times of traditional Taq DNA polymerase;
- Powerful amplification capacity: suitable for the amplification of less than 10kb fragments (complicated genome DNA template) and less than 15kb sequences (simple gDNA, plasmid and son on);

Recommended PCR system settings:

Component	25 μl Reaction	50 μl Reaction	Final Concentration
2× CYA9 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	95°C	3 minutes	
Denaturation	95°C	10 seconds	30 cycles
Annealing	55°C	10-15 seconds	
Extension	72°C	15-20 seconds / kb	
Final Extension	72°C	2-5 minutes	
	4-8°C	Hold	

Precautions:

1. Due to CYA9 fast amplification capacity, 10-15s/kb extension time are recommended for simple or plasmid templates; 20s-25s/kb extension time for complicated template;

2. If the things of main belt being blurred up and down are observed when electrophoresis, this shows that the extension time is too long. Decreasing the extension time is recommended.

3. For templates with high GC content, the pre-denaturation and denaturation temperatures can be increased to 98°C, a temperature which has no clear influence to CYA9 activity.

4. 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

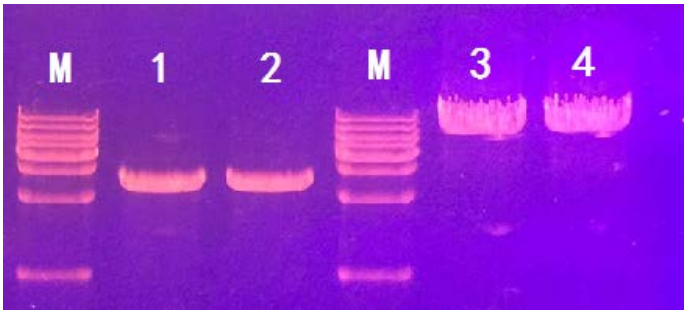

Figure.1. Amplification of DNA fragment from 50 ng human genomic DNA in 50μl reaction mixture.
Lane M: 1kb ladder;
Lane 1-2: Hα1AT, 2.6Kb, extension 20 sec/kb;
Lane3-4: ß-globin, 6kb, extension 20 sec/kb.

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