2 × LongFast PCR MasterMix

User's Instruction

Description

2 × LongFast PCR MasterMix provides greater efficiency and elongation capabilities than conventional Taq DNA Polymerase. The PCR product has an "A" base at the 3' terminal, thus it is very convenient to clone to T- vector. This mastermix contains red tracking dye, which can be directly loaded for electrophoresis without adding a loading buffer.

Kit Contents

2 × LongFast PCR MasterMix	5 ml

Features

- **Fast amplification speed:** The extension rate can reach 6-12 kb/min.
- **Excellent fidelity:** The fidelity is more than 3 times that of Taq DNA Polymerase. For higher fidelity (>55 times of Taq), we recommend 2 × LongFastHiFi PCR MasterMix.
- **Ideal for long sequences:** When using complex genomic DNA as a template, the amplification products can be up to 10kb. When using simple genomic, plasmid, and phage DNA as templates, the amplification products can be up to 15kb.
- **Strong amplification capacity:** Ideal for some complex templates with special structure and templates with high GC content.

Quality Control

Each batch of 2 × LongFast PCR MasterMix must pass the amplification detection of the following two sizes of fragments before shipment:

- *Hα1AT*, 2.6 kb, extension 20 sec/kb

- *ß-globin*, 6 kb, extension 20 sec/kb.

Protocol

1. Set up the PCR reaction on ice as the following table:

Reagent	Volume	Final Concentration
2 × LongFast PCR MasterMix	25 μL	1 ×
Forward Primer (10 μM)	1 μL	0.2 μM
Reverse Primer (10 μM)	1 μL	0.2 μM
Template	X μL	-
ddH_2O	up to 50 μL	-

Recommended template amount:
Plasmid: 0.1-10ng. Bacterial genome: 10-100ng. Human genome: 50-150ng. cDNA: 1-5 μL from RT reaction.

2. Mix the reaction system (gently blow several times with a pipette to fully mix or gently mix with a vortex mixer at a lower speed), and then centrifuge to precipitate the liquid.

Thermocycling Conditions for PCR

Step	Temperature	Time	Cycle Number
Initial Denaturation	94°C	2-3 min	
Denaturation	94°C	10 sec	25-35 cycles
Annealing	55°C	10-15 sec	
Extension	72°C	10 sec/kb	
Final Extension	72°C	1-5 min	
	4-8°C	Hold	

Notes

- *Simple templates such as plasmid and simple genome can be extended at 5 sec/kb. Complex templates, such as the human genome, can be extended at 15-20 sec/kb.*
- *When the yield is low, extension time and cycle number can be increased.*
- *For short templates with high GC content, the initial denaturation and denaturation temperature can be increased to 98°C and extension time can be increased. For long templates, to avoid the DNA damage caused by high temperature, initial denaturation time can be extended to 5 min and denaturation time can be extended to 15-20 sec.*

- *If the GC content of the amplification template is high or the template is complex and the amplification result is not good, DMSO can be added to the final concentration of 1%-8%. Increase the concentration of DMSO at the gradient of 1% to optimize. Or add betaine to the final concentration of 1.0-1.7 M and use Touchdown PCR.*

Storage

The minimum shelf life is 2 years at -20°C or 1 month at 4°C.

Only for research and not intended for treatment of humans or animals