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2×PCR Super MasterMix, with blue dye

Product Number: PCM10B

Storage condition

-20℃

Component

Component	PCM10B
2×PCR Super MasterMix, with blue dye	1ml
ddH_2O	1ml

Description

This product is a premixed system composed of a new and efficient fast DNA Polymerase, Mg2+, dNTPs, PCR stabilizers and enhancers, with a concentration of 2×.This product is an innovative new type of fast DNA polymerase with extremely high amplification speed and stability. The extension speed can reach 5 s/kb, and the PCR can be completed in as little as 15 minutes. Long fragments (greater than 3 kb) or complex templates can use an extension speed of 10-30 s/kb or a large number of cycles. The original MasterMix formula makes the entire reaction system very stable, while complex templates can also be effectively amplified, with over 98% of PCR amplification being successful at once. When in use, only DNA templates and primers need to be added, and sufficient water can be added to react, which can minimize human error, reduce pollution, and save time.

This product has been added with a dye (blue) and can be directly subjected to electrophoresis detection after the reaction is completed. The amplified PCR product has an "A" base attached to its 3 'end, making it suitable for T/A cloning and suitable for seamless cloning kits, T4 linking kits, and receptive products.

This product is mainly suitable for experiments such as ultra fast PCR, complex templates, complex secondary structures, gene cloning with high fidelity requirements, and large-scale gene detection.

Quality control

After testing, there was no exogenous nuclease activity; PCR method for detecting non host residual DNA; Can effectively amplify single copy genes from multiple genomes.

Protocol

The following example is a PCR reaction system and reaction conditions for amplifying a 1 kb fragment using human genome DNA as a template. In practical operation, corresponding improvements and optimizations should be made based on the template, primer structure, and target fragment size.

1. PCR Reaction system

Reagent	50 μL	25 μL	20 μL	final concentration
2×PCR Super MasterMix, with blue dye	25 μL	12.5 μL	10 μL	1×
Forward Primer, 10 μM	2 μL	1 μL	0.8 μL	0.4 μM
Reverse Primer, 10 μM	2 μL	1 μL	0.8 μL	0.4 μM
Template DNA	<0.5 μg	<0.25 μg	<0.2 μg	<0.5 μg/50 μL
ddH_2O	up to 50 μL	up to 25 μL	up to 20 μL	

Note: Please use the final concentration of 0.1-1.0μM as a reference for setting the primer concentration range. In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the concentration of primers can be reduced to optimize the reaction system.

2. PCR reaction conditions

Step	temperature	time

Pre denaturation	98 ℃	30 s	
Denaturation	94 ℃	10 s	30-35 cycles
Annealing	55-65 ℃	15 s	
Extension	72 ℃	5-15 s/kb	
Final extension	72 ℃	1 min	

Note: 1) For simple templates, the pre denaturation time can be controlled at 30s-1 min, while for complex templates such as bacterial solution, the pre denaturation time can be increased to 2 min.

Optimize parameter settings

1. Template DNA quantity setting:

Excessive templates may lead to non-specific amplification or smear. The recommended usage of template DNA in the 50 μL PCR reaction system is as follows:

-Human Genomic DNA 5 ng -500 ng

-Escherichia coli genomic DNA 50 pg-100 ng

-Plasmid DNA 10 pg-1 ng

2. Primer concentration setting:

Primer concentration can be set to 0.1 μM-1.0 μM. Low primer concentration may result in fewer amplification products. Excessive primer concentration can inhibit specific amplification and may lead to non-specific amplification.

3. Annealing temperature setting:

In general experiments, the annealing temperature is 5 ℃ lower than the melting temperature Tm of the amplification primer. When ideal amplification efficiency cannot be achieved, the annealing temperature can be appropriately reduced; When non-specific reactions occur, the annealing temperature can be appropriately increased. For complex templates, it is necessary to adjust the annealing temperature to achieve efficient amplification.

4. Extension time setting:

The extension time should be set based on the size of the amplified fragment. The following are recommended extension times:

Simple templates such as plasmids: 5-15 s/kb;

Conventional genome and cDNA templates: 10-15 s/kb;

Complex template and rough extraction template: 20-30 s/kb;

The extension time should not be too short and should be at least 5 s/kb or more, nor should it exceed 30 s/kb.

5. Cycle number setting:

The number of cycles can be set based on the downstream application of the amplification product. If the number of cycles is too small, the amplification amount is insufficient; If there are too many cycles, the probability of mismatch will increase, and the non-specific background will be severe. So, while ensuring product yield, the number of cycles should be minimized as much as possible.