Tinzyme Co., Limited

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2×Taq Plus Master Mix

Product Number: PCM007

Shipping and Storage

-20℃.

Components

Component	PCM007
2×Taq Plus Master Mix	1ml×5

Description

This product is a pre prepared mixture of Taq Plus DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer required for PCR reaction, with a concentration of 2 times. 2×Taq Plus Master Mix is designed for optimizing the PCR amplification reaction of large DNA fragments, with increased amplification length, good fidelity, fast amplification speed, and high reaction efficiency. It is suitable for amplifying fragments of 1-20kb length. When in use, only templates and primers need to be added and diluted to 1 time the concentration to perform PCR reaction, greatly simplifying the operation process and reducing contamination during PCR operation. The PCR product amplified using this product has a 3 '- end "A" protrusion, so it can be directly cloned into a T vector.

Features

1. More efficient: Using λDNA as a template, the amplification length can reach 20kb.
2. More sensitive: More sensitive than Taq enzyme amplification (Figure 1).
3. Stable: Repeated freeze-thaw for dozens of times, left at 4℃ for 30 days, and left at room temperature for one week, the amplification performance is not affected.
4. Quick: All necessary reagents for PCR reaction are collected in one tube, and the reaction system can be prepared in a few minutes.

Application

1. Can replace most uses of 2×Taq Master Mix;
2. High sensitivity PCR amplification is required;
3. Large fragment PCR amplification reaction (up to 20kb).

Quality control

After testing, there were no residual exogenous nucleases, and the qPCR method detected no residual E. coli DNA, which can effectively amplify single copy genes in the human genome.

Suggestion

The PCR product amplified using this product has a 3-terminal "A" protrusion and can be directly cloned into a T vector.

Note

1. It needs to be completely dissolved before use to prevent uneven ion concentration.
2. The appropriate number of cycles should be selected according to the experimental purpose. Too few cycles can lead to insufficient amplification. If there are too many cycles, the amplification amount will increase, but the mutation rate will increase and cause non-specific amplification.
3. Set an appropriate annealing temperature based on the Tm value of the primer. If the annealing temperature is too low, it will cause non characteristic amplification. If the annealing temperature is too high, the target band may not be amplified.

Protocol

1. Common reaction systems (50μl) :

2×Taq Plus Master Mix*	25μl
Upstream primer	0.2-1.0μM（final conc.）
Downstream primer	0.2-1.0μM（final conc.）
Template	1-50ng(Plasmid) 10ng-1μg(Genome)
ddH_2O	Up to 50μl

Note:The final concentration of Mg^{2+} is 2mM

2. Common PCR reaction programs:

2.1. When the amplified fragment is less than 3K:

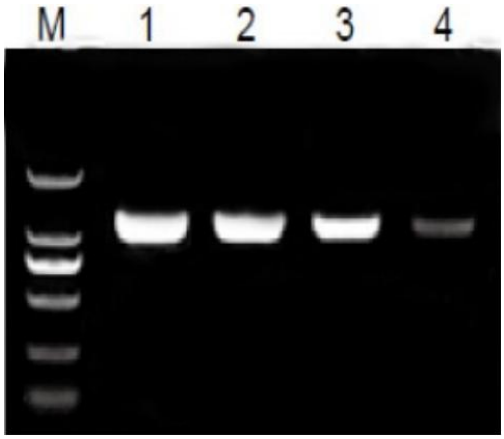
Step	Temperature	Time	Cycles
Pre denaturation	94℃	90s	1
Denaturation	94℃	20s	30
Annealing	50-60℃	20s	
Extend	72℃	1kb/60s	
Final extension	72℃	5min	1
Heat preservation	4℃	Heat preservation	1

2.2. When the amplified fragment is ≥ 3K (recommended primer length ≥ 30bp):

Step	Temperature	Time	Cycles
Pre denaturation	94℃	5min	1
Denaturation	94℃	5s	30
Annealing/Extension	68℃	1kb/60s	
Final extension	72℃	5min	1
Heat preservation	4℃	Heat preservation	1

Application instance

In the 50μl amplification system, using 100ng~0.1ng mouse genomic DNA as templates, 1.1kb DNA fragments can be well amplified.



(Figure 1)

Swimming lane M: DNA Ladder 2000;

Swimming lane 1:100ng;

Swimming lane 2:10ng;

Swimming lane 3:1ng;

Swimming lane 4:0.1ng.