2 × Phanta Flash Master Mix

P510

Version 24.1

Product Description

2 × Phanta Flash Master Mix utilizes the new generation of Phanta Flash Super-Fidelity DNA Polymerase. Through the directed evolution of Phanta DNA Polymerase, Phanta Flash Super-Fidelity DNA Polymerase has the characteristics of high fidelity (mismatch rate is 1/106 of conventional *Taq* DNA polymerase) and rapid amplification (5 sec/kb). With the application of the latest hot-start technology and the addition of specific factors, its amplification specificity and success rate are significantly improved. 2 × Phanta Flash Master Mix contains Phanta Flash Super-Fidelity DNA Polymerase, dNTP and optimized buffer. It only requires the addition of primers and templates for amplification, thereby reducing pipetting operations and improving detection throughput and reproducibility of results. The amplification products of 2 × Phanta Flash Master Mix are blunt-ended, which are subsequently applicable for ClonExpress Ultra One Step Cloning Kit V2 (Vazyme #C116) and Ultra-Universal TOPO Cloning Kit (Vazyme #C603).

Components

Components	P510-01	P510-02	P510-03
2 × Phanta Flash Master Mix	1 ml	5 × 1 ml	15 × 1 ml

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

It is applicable for amplification reaction of genomic DNA, cDNA, dU-containing DNA and crude samples as templates.

Notes

For research use only. Not for use in diagnostic procedures.

1. For fragments ≤10 kb, the recommended extension time is 5 sec/kb. For fragments >10 kb, the recommended extension time is 10 sec/kb.
2. Please use high-quality templates to improve amplification success rate and yield.
3. Phanta Flash Super-Fidelity DNA Polymerase has strong proof-reading activity. If the DNA product needs to perform TA cloning, it is recommended to purify it before adding an A-tail.
4. Primer Design Guidance
   a. It is recommended that the last base at the 3' end of the primer should be G or C.
   b. Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer.
   c. Avoid hairpin structures at the 3' end of the primer.
   d. Differences in the Tm value of the forward primer and the reverse primer should be no more than 1°C and the Tm value should be adjusted to 55 ~ 65°C (Primer Premier 5 is recommended to calculate the Tm value).
   e. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value.
   f. It is recommended that the GC content of the primer to be 40% - 60%.
   g. The overall distribution of A, G, C, and T in the primer should be as even as possible. Avoid using regions with high GC or AT contents.
   h. Avoid the presence of complementary sequences of 5 or more bases either within the primer or between two primers. Avoid the presence of complementary sequences of 3 or more bases at the 3' end of two primers.
   i. Use the NCBI BLAST function to check the specificity of the primer to prevent nonspecific amplification.

Experiment Process

Reaction System

Perform all operations on the ice during the experiment. Thaw, mix, and briefly centrifuge each fraction before use. After use, please return it to -20°C in time for storage.

Components	Volume
ddH_2O	up to 50 μl
2 × Phanta Flash Master Mix	25 μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Template DNA*	x μl

* Optimal reaction concentration varies in different templates. In a 50 μl system, the recommended template usage is as follows:

Template Types	Amount
Genomic DNA	10 - 500 ng
Plasmid or Virus DNA	10 pg - 20 ng
cDNA	1 - 5 μl (≤1/10 of the total volume of PCR system)

Reaction Program

Standard program

Steps	Temperature	Time	Cycles
Initial Denaturation	98°C	30 sec	
Denaturation	98°C	10 sec	
Annealing[a]	Tm + 5°C	5 sec	28 - 35
Extension[b]	72°C	5 - 10 sec/kb	
Final Extension	72°C	1 min	

Fast program[c]

Steps	Temperature	Time	Cycles
Denaturation	98°C	10 sec	
Annealing[a]	Tm + 5°C	5 sec	28 - 35
Extension[b]	72°C	5 - 10 sec/kb	

a. It is recommended that the annealing temperature is the Tm value of primers + 5°C. The highest annealing temperature should not exceed 68°C.

b. Set the extension time according to the following table:

Target fragment size	Extension time
≤10 kb	5 sec/kb
>10 kb	10 sec/kb

c. It has been verified through experiments that there is no significant difference in performance regardless of whether using the standard program or the fast program. You can choose according to your operating habits.

FAQ & Troubleshooting

◇ No amplification products or low yield

① Primer: Optimize primer design.

② Annealing temperature: Set temperature gradient and find the optimal annealing temperature.

③ Primer concentration: Increase the concentration of primers properly.

④ Extension time: Increase the extension time to 10 - 15 sec/kb properly.

⑤ Cycles: Increase the number of cycles to 36 - 40 cycles.

⑥ Template purity: Use templates with high purity.

⑦ Template amount: Adjust the template amount according to the recommended amount and increase it properly.

◇ Nonspecific products or smeared bands

① Primer: Optimize primer design.

② Annealing temperature: Try to increase the annealing temperature and set temperature gradient.

③ Primer concentration: Decrease the concentration of primers properly.

④ Cycles: Decrease the number of cycles to 25 - 30 cycles.

⑤ Template purity: Use templates with high purity.

⑥ Template amount: Adjust the template amount according to the recommended amount and decrease it properly.

◇ Products plugged agarose wells

① Experimental environment: To avoid aerosol pollution, the experimental environment needs to be thoroughly cleaned, or the operating environment, experimental reagents and consumables should be replaced before re-amplification.

② Template amount: Decrease the amount of templates.

③ Cycles: Decrease the number of cycles to 25 - 30 cycles.

④ Annealing temperature: Set temperature gradient and find the optimal annealing temperature.