Phanta Flash Super-Fidelity DNA Polymerase

P521

Version 24.1

Product Description

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), by the directed evolution of Phanta DNA Polymerase. With the application of the latest hot-start technology and the addition of specific factors, its amplification specificity and success rate are significantly improved. The amplification products of this product 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	P521-d1 (100 U)	P521-d2 (500 U)	P521-d3 (1,000 U)
Phanta Flash Super-Fidelity DNA Polymerase	100 µl	5 × 100 µl	10 × 100 µl
2 × Phanta Flash Buffer	2 × 1.25 ml	10 × 1.25 ml	20 × 1.25 ml
10 × DNA Loading Buffer	1.25 ml	5 × 1.25 ml	10 × 1.25 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 target fragments ≤10 kb, it is recommended to set the extension time to 5 sec/kb; when target fragments >10 kb, it is recommended to set the extension time to 10 sec/kb.
2. Please use high-quality templates to increase the success rate and yield of amplification.
3. Phanta Flash Super-Fidelity DNA Polymerase has the strong proofreading activity. If TA cloning needs to be performed, it is recommended to purify the DNA before adding the adenine.
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. 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 component 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 Buffer[a]	25 μl
Forward primer (10 μM)	2 μl
Reverse primer (10 μM)	2 μl
Phanta Flash Super-Fidelity DNA Polymerase	1 μl
Template DNA[b]	x μl

a. 2 × Phanta Flash Buffer already contains Mg^{2+} (final concentration - 2 mM) and dNTP (final concentration - 0.4 mM).

b. 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	28 - 35
Annealing[a]	Tm + 5°C	5 sec	
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	28 - 35
Annealing[a]	Tm + 5°C	5 sec	
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.