2 × Phanta UniFi Master Mix (Dye Plus)

P526

Version 24.1

Product Description

Phanta UniFi DNA Polymerase is a next-generation high-fidelity DNA polymerase carefully selected based on the BioSmart platform, featuring ultra-high fidelity and universal primer annealing. Combined with the latest hot-start technology and superior buffer system, this product has high success rates and specificity at specific universal annealing temperatures, enabling stable amplification of various targets. Its fidelity is 220-fold higher than that of *Taq* polymerase, providing an efficient and convenient solution for PCR experiments requiring highly precise amplification, such as molecular cloning, sequencing, and site-directed mutagenesis. 2 × Phanta UniFi Master Mix (Dye Plus) is a premix that contains Phanta UniFi DNA Polymerase, dNTPs, Mg^{2+}, and other necessary components for PCR amplification. It only requires the addition of primers and templates for amplification, thereby reducing pipetting operations and improving detection throughput and result stability. This product is applicable for standard templates, crude products, and GC-rich systems (including primers and templates). This product contains tracking dyes, and amplification products can be directly loaded for gel electrophoresis after the reaction. The amplification products are blunt-ended, subsequently applicable for ClonExpress Ultra One Step Cloning Kit V3 (Vazyme #C117) and Ultra-Universal TOPO Cloning Kit (Vazyme #C603).

Components

Components	P526-01	P526-02	P526-03
2 × Phanta UniFi Master Mix (Dye Plus)	1 ml	5 × 1 ml	15 × 1 ml

Storage

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

Applications

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

Notes

1. Please use high-quality templates to increase the success rate and yield of amplification.
2. If TA cloning needs to be performed, it is recommended to purify the DNA before adding the adenine.
3. Primer Design Guidance:
   a. It is recommended that the primer length be 21 - 25 nt, and 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, and also avoid the formation of hairpin structures.
   c. 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).
   d. The Tm value difference between multiple PCR primers should be as small as possible, while minimizing the complementary base pairing between primer pairs.
   e. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value; it is recommended that the GC content of the primer be 40% - 60%.
   f. The overall distribution of Primer A, G, C, and T should be as uniform as possible, avoiding regions with high GC or AT content.
   g. 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.
   h. When amplifying long fragments (≥5 kb), the length of the primer should be 25 - 35 nt and the Tm value should be >62°C.
   i. Primer cannot contain inosine (I) or uracil (U).
   j. After the primer design, please use NCBI BLAST function to check primer specificity to avoid nonspecific amplification.

Experiment Process

Reaction System

Thaw and mix each component thoroughly before use. Please return components to -20°C in time for storage.

Components	Volume
ddH_2O	up to 50 μl
2 × Phanta UniFi Master Mix (Dye Plus)	25 μl
Primer 1 (10 μM)[a]	2.0 μl
Primer 2 (10 μM)[a]	2.0 μl
Template DNA[b]	x μl

a. When amplifying fragments >10 kb or performing multiplex PCR, please reduce the final concentration of each primer to 0.2 μM.

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	5 - 200 ng
Plasmid or Virus DNA	10 pg - 50 ng
cDNA	1 - 5 μl (≤1/10 of the total volume of PCR system)
Crude samples	1 - 5 μl (≤1/10 of the total volume of PCR system)

Reaction Program

Steps	Temperature	Time	Cycles
Initial Denaturation	98°C	30 sec	
Denaturation	98°C	10 sec	25 - 35[c]
Annealing[a]	60°C	10 sec	
Extension[b]	72°C	15 - 30 sec/kb	
Final Extension	72°C	5 min	

a. ① When the Tm values of both forward and reverse primers are >50°C, the annealing temperature should be 60°C; ② When the Tm value of one of the forward or reverse primer is ≤50°C or both primers have Tm values ≤50°C, the annealing temperature should be 50°C; ③ It can also set the Annealing temperature according to the Tm value of the primer itself.

b. Extension time Please set according to the length of the target fragment using the following methods:

PCR Types	Target Fragment Size	Extension Time
Singleplex PCR	<10 kb	15 sec/kb
	≥10 kb	30 sec/kb
Multiplex PCR	≤2 kb (Maximum Length)	30 sec/kb

c. For multiplex PCR, it is recommended to use 25 cycles. Excessive cycle numbers may affect amplification uniformity.

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 30 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.