2 × Phanta® Flash Master Mix

P510

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Instruction for Use

Version 21.1

Contents

01/Product Description

2 × Phanta Flash Master Mix is a new generation superior enzyme based on Phanta Flash Super-Fidelity DNA Polymerase. Through directed optimization of Phanta DNA Polymerase, Phanta Flash Super-Fidelity DNA Polymerase has the characteristics of rapid amplification (4 - 5 sec/kb) while maintaining high fidelity and yield. Matched with optimized buffer system, this kit can achieve high amplification specificity. And it has excellent compatibility with crude samples, templates with uracil and GC-rich system (primer/template). This kit contains two types of monoclonal antibodies inhibiting the 5'→3' polymerase activity and 3'→5' exonuclease activity at room temperature, which enable it to perform hot start PCR with great specificity. It contains all required reaction components (Phanta Flash Super-Fidelity DNA Polymerase, dNTP and optimized buffer), except primers and templates, thereby simplifying the operation process and improving the detection throughput and repeatability. Amplification will generate blunt-ended products, which are compatible with ClonExpress kits (Vazyme #C112/C113/C115) and TOPO cloning kit (Vazyme #C601).

02/Components

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

03/Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

04/Applications

This product is suitable for PCR with various templates such as genomic DNA, cDNA, plasmid DNA, dU-containing DNA, crude samples, etc.

05/Notes

1. For fragments ≤10 kb, the recommended extension time is 4 - 5 sec/kb. For fragments >10 kb, the recommended extension time is 10 sec/kb.
2. High quality templates should be used to ensure successful amplification and products yield.
3. Phanta Flash Super-Fidelity DNA Polymerase has strong proof-reading activity. If TA cloning needs to be performed, please perform purification before dA-tailing.
4. Primer Design
   a. It is recommend that the last base at the 3' end of 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℃ and the Tm value should be adjusted to 55℃ to 65℃ (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. Control 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 and 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 non-specific amplification.

06/Experiment Process

06-1/PCR System

Keep all components on ice during the experiment. Thaw, mix and briefly centrifuge each component before use. And put back to -20˚C 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*	x µl

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

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

06-2/PCR Program

Standard program

Temperature	Time	Cycles
98˚C	30 sec	
98˚C	10 sec	
Tm[a]	5 sec	28 - 35 cycles
72˚C	4 - 5 sec/kb[b]	
72˚C	1 min	

Fast program[c]

Temperature	Time	Cycles
98˚C	10 sec	
Tm[a]	5 sec	28 - 35 cycles
72˚C	4 - 5 sec/kb[b]	

a. Set the annealing temperature according to the Tm value of the primers. If the Tm value of the primers is higher than 72˚C, the annealing step can be removed (two-step PCR). If necessary, annealing temperature can be further optimized through setting temperature gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising annealing temperature is helpful to improve amplification specificity.

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

Target fragment size	Extension time
≤10 kb	4 - 5 sec/kb
>10 kb	10 sec/kb

c. Through experimental verification, there is no significant difference in performance when adopting either standard program or fast program. You can choose according to your operating habits.

07/Examples

07-1/Extensive Template Compatibility

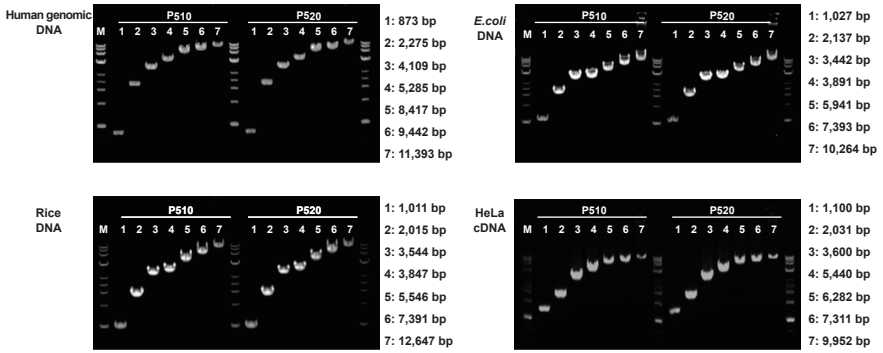
2 × Phanta Flash Master Mix (Vazyme #P510) and 2 × Phanta Flash Master Mix (Dye Plus) (Vazyme #P520) were used to amplify DNA fragments (1 - 12 kb) with 4 types of templates (human genomic DNA, *E.coli* genomic DNA, rice genomic DNA and HeLa cDNA), respectively. The input amounts for human, *E.coli* and rice genomic DNA were 10 ng and for HeLa cDNA was 1 µl (Perform reverse transcription according to the standard operation process of Vazyme #R312 and the input RNA amount was 1 µg). Reaction mix and program were as follows. As a result, clear bands were obtained.

Reaction Mix

Components	Volume
ddH_2O	up to 50 µl
P510 (or P520)	25 µl
Primer 1 (10 µM)	2 µl
Primer 2 (10 µM)	2 µl
Template	x µl

PCR Program

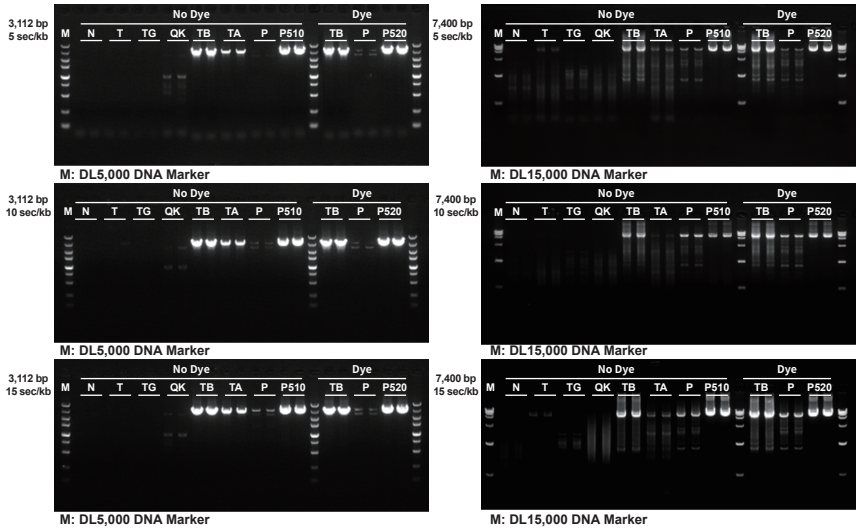
Temperature	Time	Cycles
98˚C	10 sec	
Tm	5 sec	35 cycles
72˚C	5 sec/kb	



M: DL15,000 Plus DNA Marker (From top to bottom: 15,000 bp, 10,000 bp, 7,500 bp, 5,000 bp, 3,000 bp, 2,500 bp, 2,000 bp, 1,500 bp, 1,000 bp)

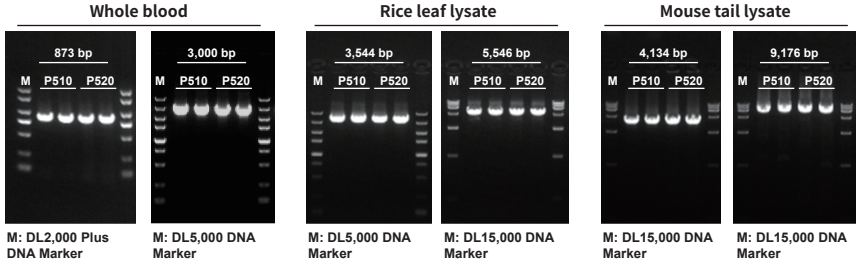
07-2/Excellent Reaction Speed and Productivity

P510, P520 and high fidelity DNA polymerase from other companies (N company, T company, TG company, QK company, TB company, TA company and P company) were used to amplify from mouse genomic DNA. The input DNA amount is 5 ng. PCR amplification was performed according to the instruction manual. As a result, P510 and P520 performed better in amplification speed, products yield and specificity.



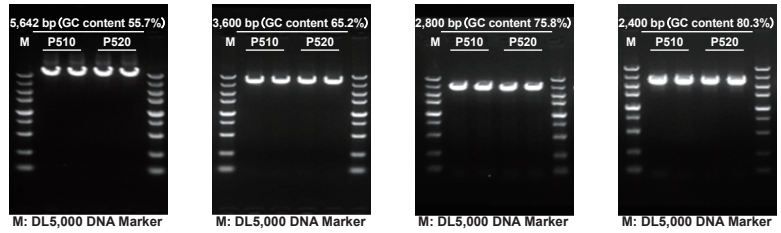
07-3/Stable Amplification Ability from Crude Samples

P510 and P520 are compatible with various PCR inhibitors. They can be used for direct PCR with bacteria, fungi, plant tissues, animal tissues and whole blood samples. P510 and P520 were used to amplify DNA fragments from human whole blood (2 μl), rice leaf lysate (Lysed with Vazyme #P073) and mouse tail lysate (Lysed with Vazyme #P073). The PCR extension time is 5 sec/kb. PCR amplification was performed according to 07-1. As a result, P510 and P520 have excellent performance.



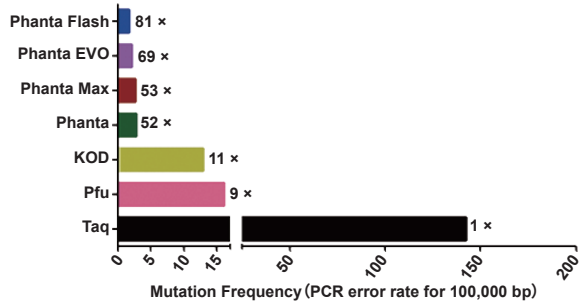
07-4/Perfect Compatibility with High GC Content

Phanta Flash Super-Fidelity DNA Polymerase can efficiently amplify DNA fragments with high GC content, which is unachievable by using conventional DNA polymerase. P510 and P520 were used to amplify DNA fragments with 55.7% (5.6 kb), 65.2% (3.6 kb), 75.8% (2.8 kb) and 80.3% (2.4 kb) GC contents from 10 ng human genomic DNA. Perform PCR amplification according to 07-1. As a result, P510 and P520 are compatible with various high GC content templates.



07-5/Reliable High Fidelity

LacI Assay (Cline, J. et al. *Nucleic Acids Research.* 24:3546-3551(1996)) was used to determine the fidelity of various polymerases. As a result, Phanta Flash Super-Fidelity DNA Polymerase has ultra-high fidelity. It is 81-fold superior than that of Taq DNA Polymerase



08/FAQ & Troubleshooting

No PCR product /low yield	Primer	Optimize the primers
	Annealing temperature	Set gradient annealing temperature and find the optimal annealing temperature
	Primer concentration	Increase the primer concentration appropriately
	Extension time	Increase the extension time up to 10 - 15 sec/kb appropriately
	Cycles	Increase the number of cycles up to 36 - 40 cycles
	Template purity	Use templates with high purity
	Template amount	Adjust the template amount according to the recommend amount and appropriately increase the amount
Smearing or extra band	Primer	Optimize the primers
	Annealing temperature	Increase annealing temperature and set gradient annealing temperature
	Primer concentration	Decrease the primer concentration appropriately
	Cycles	Decrease the number of cycles to 25 - 30 cycles
	PCR program	Use two-step or Touch down PCR program
	Template purity	Use templates with high purity
	Template amount	Adjust the template amount according to the recommend amount and appropriately decrease the amount