2 × Phanta Flash Master Mix

NB-54-0210-01
NB-54-0210-02
NB-54-0210-03

2 × Phanta Flash Master Mix

Cat# NB-54-0210-01 size: 1 ml

Cat# NB-54-0210-02 size: 5 x1ml

Cat# NB-54-0210-03 size: 15 x 1 ml

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 that inhibit the 5'→3' polymerase activity and 3'→5' exonuclease activity at room temperature, enabling 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 (Neo Biotech NB-54-0002 NB-54-0003/NB-54-0004) and TOPO cloning kit (NB-54-0009).

02/Storage

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

03/Applications

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

04/Notes

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

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. To improve amplification success and yield, please use high-quality templates.
3. Phanta Flash Super-Fidelity DNA Polymerase has strong proof-reading activity. If TA cloning needs to be performed, it is recommended to purify the DNA before adding poly(A) tails.
4. Primer Design Guidance
   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 ~ 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

05/Experiment Process

05-1/PCR System

Keep all components on ice during the experiment. Thaw, mix and briefly centrifuge each component before use. And put back to -20℃ 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)

05-2/PCR Program

Standard program

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

Fast program[C]

Temperature	Time	Cycles
98℃	10 sec	28 - 35 cycles
Tm[a]	5 sec	
72℃	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℃, 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.

06/Examples

06-1/Extensive Template Compatibility

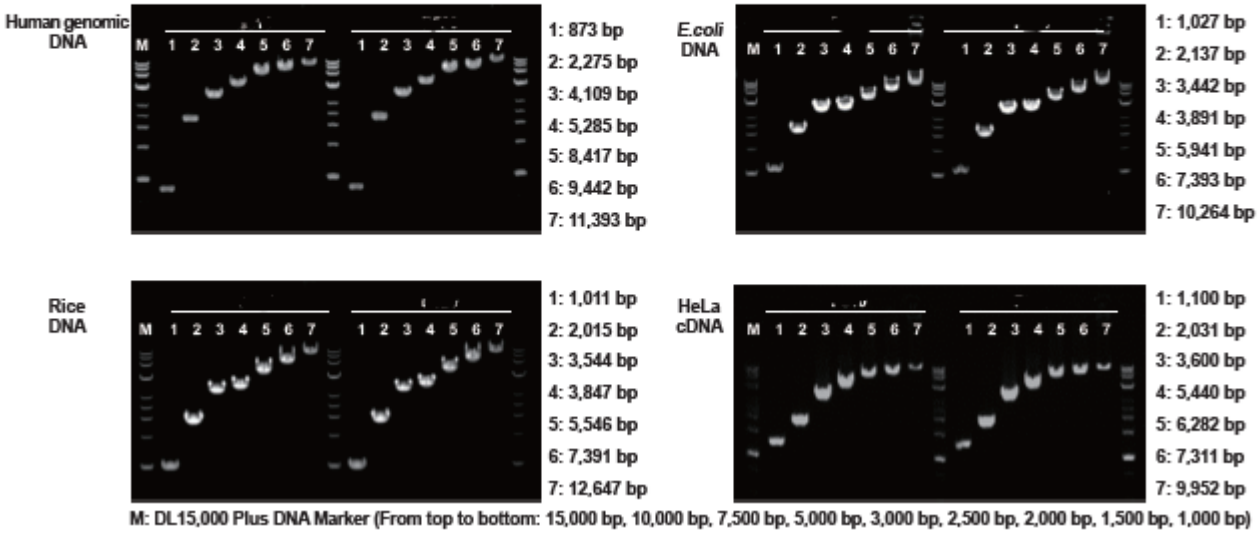
2 × Phanta Flash Master Mix (NB-54-0210) and 2 × Phanta Flash Master Mix (Dye Plus) (NB-54-0208) were used to amplify DNA fragments (1 - 12 kb) with four different types of templates (human genomic DNA, E.coli genomic DNA, rice genomic DNA and HeLa cDNA), respectively. The input amounts for human, E.coli and rice genomic DNA were 10 ng and for HeLa cDNA was 1 µl (Perform reverse transcription according to the standard operation process of NB-54-0182 and the input RNA amount was 1 µg). The PCR system and reaction program were as follows. The results showed that when the extension time was set at 5 sec/kb, clear product bands could be obtained by using NB-54-0210 or NB-54-0208 to amplify the target fragments with different lengths of 1 - 12 kb.

PCR System

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

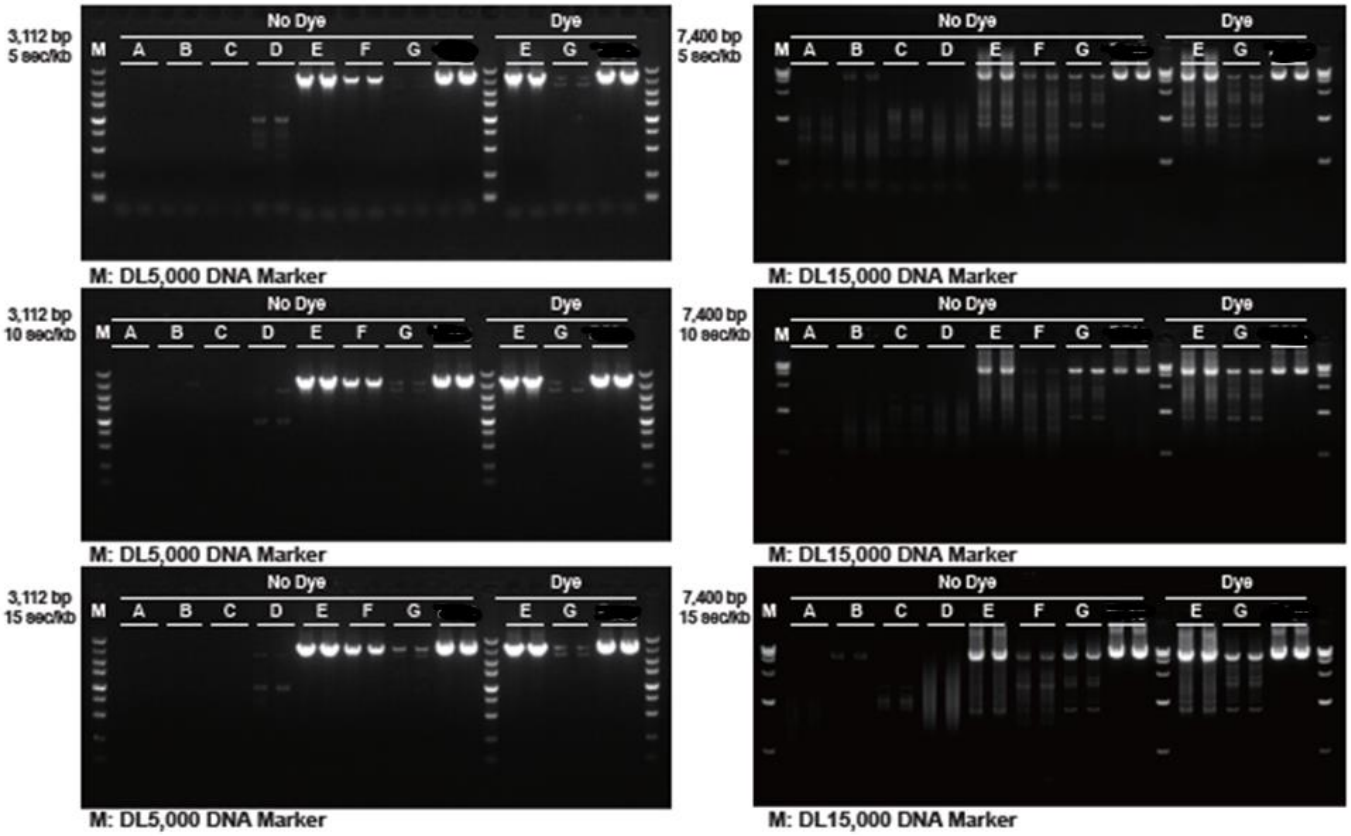
Temperature	Time	Cycles
98℃	10 sec	35 cycles
Tm	5 sec	
72℃	5 sec/kb	



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)

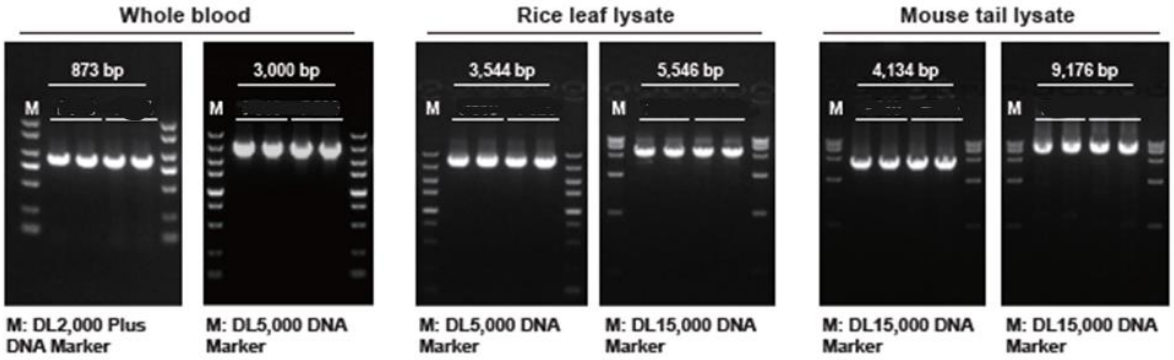
06-2/Excellent Reaction Speed and Yield

NB-54-0210 or NB-54-0208 and high fidelity DNA polymerase from other companies (Supplier A, Supplier B, Supplier C, Supplier D, Supplier E, Supplier F and Supplier G) were used to amplify from mouse genomic DNA. The input DNA amount is 5 ng. PCR amplification was performed according to the instruction manual. The results showed that NB-54-0210 and NB-54-0208 had the significant advantages of rapid amplification, high yield and good specificity.



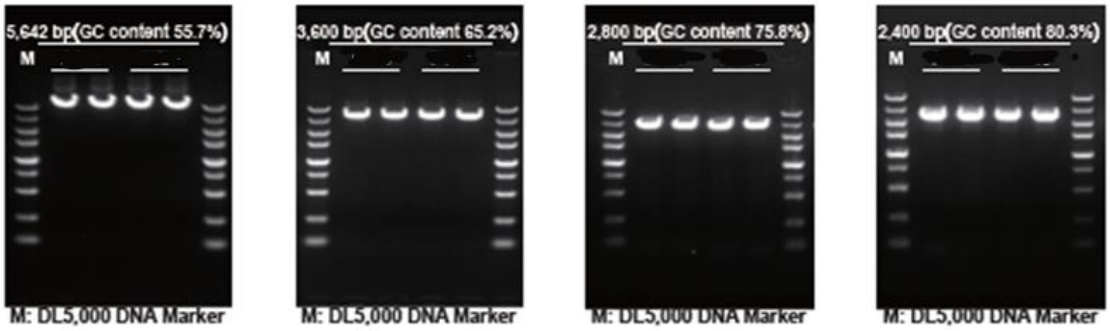
06-3/Stable Amplification Ability from Crude Samples

NB-54-0210 and NB-54-0208 have good resistance to various PCR inhibitors and can be used for direct PCR of bacteria, fungi, plant tissues, animal tissues or whole blood samples. NB-54-0210 and NB-54-0208 were used to amplify DNA fragments from human whole blood (2 µl), rice leaf lysate (Lysed with Neo Biotech NB-54-0133) and mouse tail lysate (Lysed with Neo Biotech NB-54-0133). The extension time of PCR is 5 sec/kb. The PCR system and reaction process refer to 06-1/Extensive Template Compatibility. The results showed that NB-54-0210 and NB-54-0208 could effectively amplify the above crude products, and had the characteristics of high yield and good specificity.



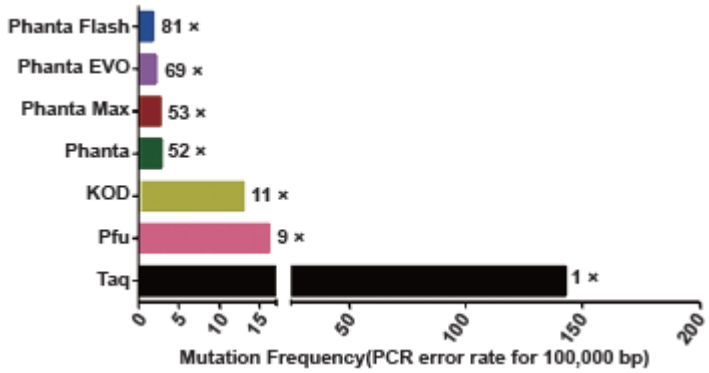
06-4/Perfect Compatibility with High GC Content

Phanta Flash Super-Fidelity DNA Polymerase can efficiently amplify DNA fragments with high GC content, which is unachievable by using conventional DNA polymerase. NB-54-0210 and NB-54-0208 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. The PCR system and reaction process refer to 06-1/Extensive Template Compatibility. The results showed that NB-54-0210 and NB-54-0208 were widely compatible with different high GC content templates, and the amplified products had high-specific bands.



06-5/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. The results show that Phanta Flash Super-Fidelity DNA Polymerase has ultra-high fidelity, and its fidelity is 81 times higher than Taq DNA Polymerase.



07/FAQ & Troubleshooting

No amplification products or low yield	Primer	Optimize the primers
	Annealing temperature	Set temperature gradient and find the optimal annealing temperature
	Primer concentration	Increase the concentration of primers properly
	Extension time	Increase the extension time to 10 - 15 sec/kb properly
	Cycles	Increase the number of cycles 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
Nonspecific bands or smear bands	Primer	Optimize primer design
	Annealing temperature	Try to increase the annealing temperature and set temperature gradient
	Primer concentration	Decrease the concentration of primer properly
	Cycles	Reduce 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 dosage according to the recommended amount of the reaction system and decrease it properly