Phanta Max Super-Fidelity DNA Polymerase

Catalog # P505

Version 5.1 Vazyme biotech co., ltd.

1 Introduction

Phanta Max Super-Fidelity DNA Polymerase is a new generation superior enzyme based on Phanta DNA Polymerase for robust PCR with higher fidelity. The unique extension factor, specificity-promoting factors and plateau-inhibiting factor newly added to Phanta Max greatly improve its long-fragment amplification ability, specificity, and PCR yield. Phanta Max is capable of amplifying long fragments such as 40 kb λ DNA, 40 kb plasmid DNA, 20 kb genomic DNA and 10 kb cDNA. The amplification error rate of Phanta Max is 53-fold lower than that of conventional Taq and 6-fold lower than that of Pfu. In addition, Phanta Max has a good resistance to PCR inhibitors and can be used for direct PCR amplifications of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. Phanta Max contains two monoclonal antibodies inhibiting the 5′→3′ polymerase activity and 3′→5′ exonuclease activity at room temperature, which enable Phanta Max to perform greatly-specific Hot-Start PCRs. The amplification generates blunt-ended products, which are compatible with ClonExpress II One Step Cloning Kit (Vazyme, Cat. No. #C112, #C113, #C114, #C115).

2 Package Information

Components	P505-d1 100 U 100 rxn (50 μl/rxn)	P505-d2 500 U 500 rxn (50 μl/rxn)	P505-d3 1,000 U 1,000 rxn (50 μl/rxn)
Phanta Max Super-Fidelity DNA Polymerase (1 U/μl)	100 μl	P505-d1 × 5	P505-d1 × 10
2×Phanta Max Buffer	2×1.25 μl		
dNTP Mix (10 mM each)	100 μl		
10× Loading buffer	1.25 μl		

3 Storage

Store at -20℃; avoid repeated freezing and thawing.

4 Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of whole dNTPs into acid-insoluble products in 30 minutes at 74℃ with activated salmon sperm DNA as the template / primer.

5 Quality Control

Residual Endonulease Test: Incubate 10 U of this enzyme and 0.3 μg of Supercoiled pBR322 DNA at 37℃ for 4h; the DNA electrophoresis bands remain unchanged.

Residual *E.Coli* gDNA Test: Detecting the residual nuclear acid in 10 U of this enzyme with *E.Coli* 16S rDNA-specific TaqMan qPCR, the genome DNA of *E.Coli* is less than 10 copies.

Function Assay: Load 1 U of this enzyme into a 50 μl PCR system and set extension time as 30 sec/kb, amplify 5 fragments with various lengths and different contents of GC respectively and use 10 ng of λ DNA, 50 ng of plasmid DNA, 100 ng of human genome DNA and 1 ul of cDNA from Hela cells respectively. After 35 cycles, use 1/10 of the PCR products to perform 1% agarose gel electrophoresis and EB staining, then there shall be a specifically single band responding to expect.

6 Experimental Process

6.1 For Conventional PCR

Recommended PCR System

Keep all components on ice during the experiment. All components need to be mixed up thoroughly after thawing and put back to -20℃ immediately for storage after using.

ddH_2O	up to 50 μl
2× Phanta Max Buffer [a]	25 μl
dNTP Mix (10 mM each)	1 μl
Primer 1 (10μM)	2 μl
Primer 2 (10μM)	2 μl
Phanta Max Super-Fidelity DNA Polymerase (1 U/μl)	1 μl
Template DNA [b]	x μl

a. 2× Phanta Max Buffer contains Mg^{2+}. The final concentration of Mg^{2+} is 2 mM.

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

Templates	Input Template DNA
Genomic DNA	50 - 400 ng
Plasmid or Virus DNA	10 pg - 30 ng
cDNA	1 - 5 μl (≤ 1/10 of the total volume of PCR system)

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation [a]	95℃	30 sec / 3 min	1
Denaturation	95℃	15 sec	25 - 35
Annealing [b]	56℃ - 72℃	15 sec	
Extension [c]	72℃	30 - 60 sec / kb	
Final Extension	72℃	5 min	1

a. For pre-denaturation, the recommended temperature is 95℃, and the recommended time is 30 sec for plasmid / virus DNA and 3 min for genomic DNA / cDNA.

b. For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72℃, the annealing step can be removed (two-step PCR). If necessary, annealing temperature can be further optimized in a gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising annealing temperature is helpful to improve poor amplification specificity.

c. Longer extension time is helpful to increase the amplification yield.

6.2 For Long-fragment PCR

Phanta Max Super-Fidelity DNA Polymerase can extraordinarily perform a long-fragment amplification with high specificity and yields. If the recommended program is failure to work, the following Touch Down two-step PCR may be helpful:

Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	3 min	1
Denaturation	92℃	15 sec	5
Extension	74℃	60 sec / kb	
Denaturation	95℃	15 sec	5
Extension	72℃	60 sec / kb	
Denaturation	95℃	15 sec	5
Extension	70℃	60 sec / kb	
Denaturation	95℃	15 sec	25
Extension	68℃	60 sec / kb	
Final Extension	68℃	5 min	1

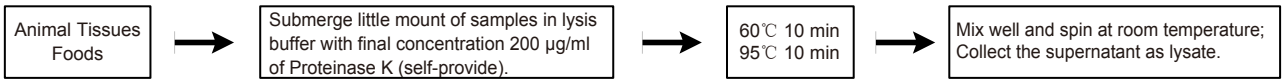
It is recommended to use high-quality templates and long primers . Increasing the input of template DNA may be helpful to improve the amplification yield.

6.3 For PCR Using Crude Material as Template

Phanta Max has a good resistance to PCR inhibitors and can be used for direct PCR amplifications of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. Crude materials that have been successfully amplified with Phanta Max are as follows:

Sample Type	Amplification Method	Template Recommendation (for a 50 μl PCR system)
Whole Blood	Direct PCR	1 - 5 μl
Filter Paper Dry Blood	Direct PCR	1 - 2 mm^2 filter paper
Cultured Cells	Direct PCR	Little amount of cells
Yeast	Direct PCR	A monoclone or 1 μl suspension
Bacteria	Direct PCR	A monoclone or 1 μl suspension
Mold	Direct PCR	Little amount of sample
Sperm	Direct PCR	Little amount of sample
Plankton	Direct PCR	Little amount of sample
Plant Tissue	Direct PCR	1 - 2 mm^2 tissue
Mouse Tail	PCR with lysate	1 - 5 μl lysate*
Food	PCR with lysate	1 - 5 μl lysate*

* Lysate Preparation:



Lysis Buffer: 20 mM of Tris-HCl, 100 mM of EDTA, 0.1% SDS, pH 8.0 (not included in this kit).

7 Application Examples

7.1 Suitable for Amplification of Fragments with Various Lengths

Taking human genomic DNA as templates, the target fragments of 0.6 kb, 1.0 kb, 2.6 kb, 3.0 kb, 4.0 kb, 5.1 kb, 6.2 kb, 7.1 kb, 8.5 kb, 10.6 kb, 17.8 kb, 20.3 kb, and 21.4 kb were amplified, respectively. The Tm of all primers are approximately 60℃ (calculated in Primer Premier 5). The reaction system and program are as follows:

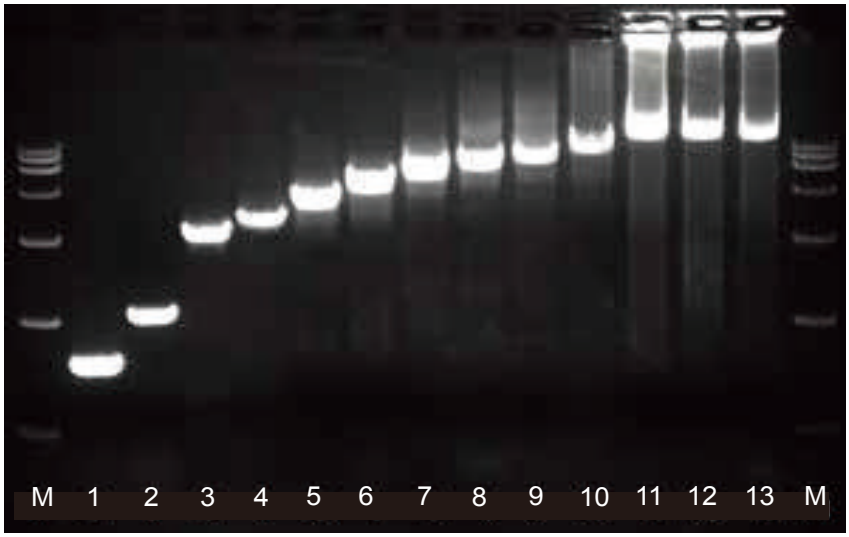
Recommended PCR System

ddH_2O	up to 50 μl
2× Phanta Max Buffer	25 μl
dNTP Mix (10 mM each)	1 μl
Primer 1 (10μM)	2 μl
Primer 2 (10μM)	2 μl
Phanta Max Super-Fidelity DNA Polymerase (1 U/μl)	1 μl
Human Genomic DNA (100 ng/μl)	2 μl

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	3 min	
Denaturation	95℃	15 sec	} 35
Annealing	60℃	15 sec	
Extension	72℃	30 sec / kb	
Final Extension	72℃	5 min	

Electrophoresis Results of the PCR Products



M: DL15,000 DNA Marker
1: 0.6 kb
2: 1.0 kb
3: 2.6 kb
4: 3.0 kb
5: 4.0 kb
6: 5.1 kb
7: 6.2 kb
8: 7.1 kb
9: 8.5 kb
10: 10.6 kb
11: 17.8 kb
12: 20.3 kb
13: 21.4 kb

7.2 Stable Amplification of Crude Materials

1.Taking the human whole blood as template, a target fragment of 1,295 bp was amplified with Phanta Max Super-Fidelity DNA Polymerase, a high fidelity DNA polymerase from company A, and a high fidelity DNA polymerase from company B, respectively. The Tm of all primers are around 60℃ (calculated in Primer Premier 5). The reaction system and program are as follows:

Recommended PCR System

ddH_2O	up to 50 μl
2× Phanta Max Buffer	25 μl
dNTP Mix (10 mM each)	1 μl
Primer 1 (10μM)	2 μl
Primer 2 (10μM)	2 μl
Phanta Max Super-Fidelity DNA Polymerase (1 U/μl)	1 μl
Whole Blood *	x μl

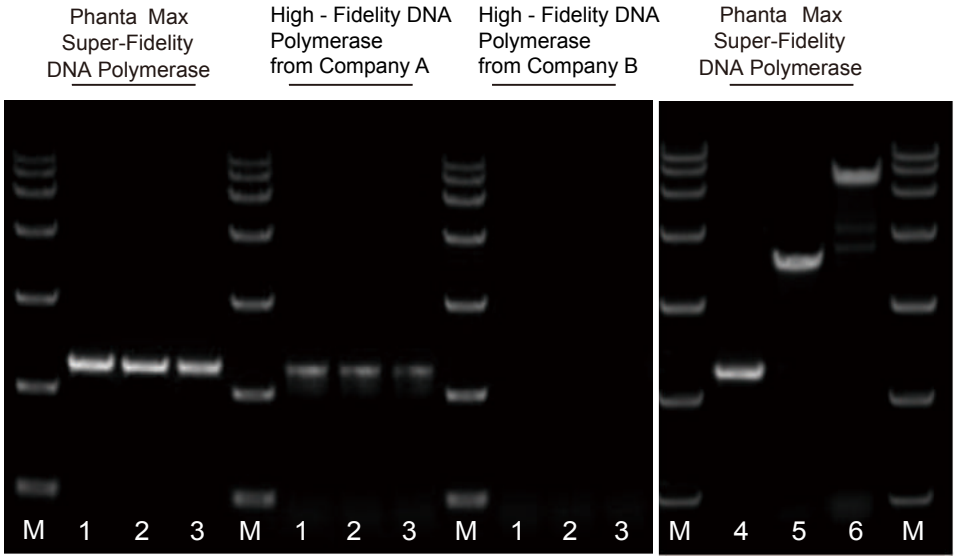
*The inputs of the whole blood are 1μl, 2μl, 4μl, respectively.

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	3 min	
Denaturation	95℃	15 sec	} 35
Annealing*	60/63/70℃	15 sec	
Extension	72℃	30 sec / kb	
Final Extension	72℃	5 min	

* The annealing temperatures for 1.3 kb, 3.6 kb and 8.5 kb of target fragments are 60℃, 63℃, and 70℃, respectively.

Electrophoresis Results of the Amplification Products



M : DL15,000 DNA Marker
1 : 1.3 kb （1 μl of blood)
2 : 1.3 kb （2 μl of blood）
3 : 1.3 kb （4 μl of blood）
4 : 1.3 kb （2 μl of blood）
5 : 3.6 kb （2 μl of blood）
6 : 8.5 kb （2 μl of blood）

*Use the high-fidelity polymerase of company A and B according to their own protocols.

2. Taking the tomato leaf, rice leaf, polished rice as templates, and the purified genomic DNA from rice leaf as positive control, target fragments of 1.3 kb was amplified with Phanta Max Super-Fidelity DNA Polymerase from Vazyme, a high fidelity DNA polymerase from company A, and a high fidelity DNA polymerase from company B ,respectively. The Tm of all primers are approximately 60℃ (calculated in Primer Premier 5).
The reaction system and program are as follows:

Recommended PCR System

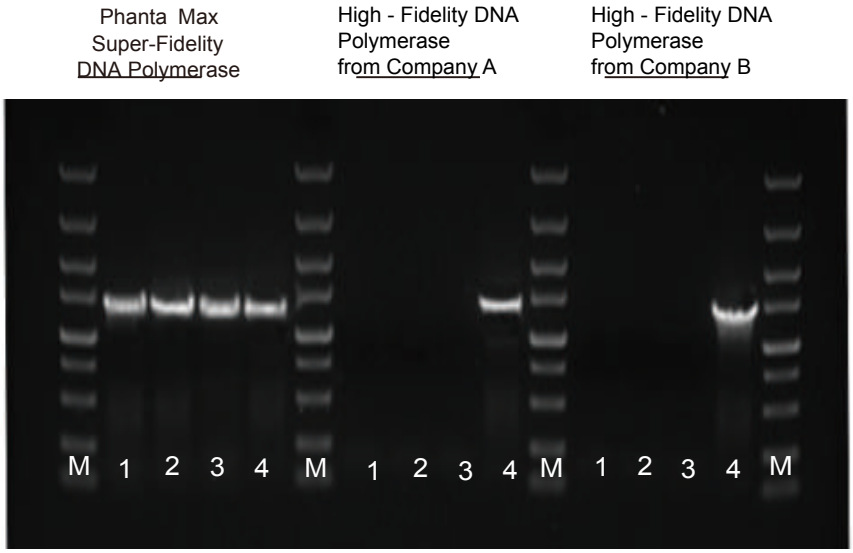
ddH_2O	up to 50 μl
2× Phanta Max Buffer	25 μl
dNTP Mix (10 mM each)	1 μl
Primer 1 (10μM)	2 μl
Primer 2 (10μM)	2 μl
Phanta Max Super-Fidelity DNA Polymerase (1 U/μl)	1 μl
Plant Tissues*	x μl

*The recommended diameter of the plant tissues is 0.3 - 3 mm.

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	3 min	
Denaturation	95℃	15 sec	} 35
Annealing	60℃	15 sec	
Extension	72℃	30 sec / kb	
Final Extension	72℃	5 min	

Electrophoresis Results of the Amplification Products



M: DL 5,000 DNA Marker

1: Tomato leafs

2: Rice leafs

3: Polished rice

4: Purified genomic DNA from rice leaf

*Use the high-fidelity polymerase of company A and B according to their own protocols.

3. Using the lysate of mouse tails as templates, a target fragment of 2.5 kb was amplified with Phanta Max Super-Fidelity DNA Polymerase from Vazyme, a high fidelity DNA polymerase from company A, and a high fidelity DNA polymerase from company B, respectively. The Tm of all primers are approximately 60℃ (calculated in Primer Premier 5). The reaction system and program are as follows:

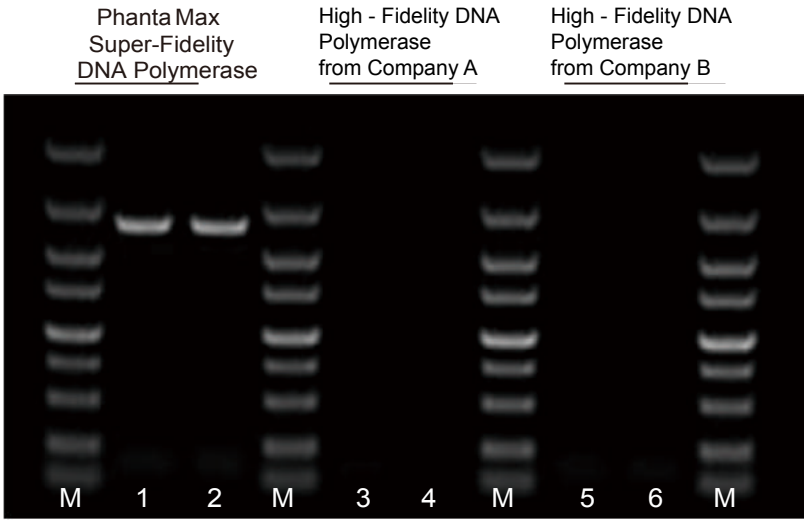
Recommended PCR System

ddH_2O	up to 50 μl
2× Phanta Max Buffer	25 μl
dNTP Mix (10 mM each)	1 μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Phanta Max Super-Fidelity DNA Polymerase (1 U/μl)	1 μl
Lysate of Mouse Tails	2 μl

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	3 min	
Denaturation	95℃	15 sec	} 35
Annealing	60℃	15 sec	
Extension	72℃	30 sec / kb	
Final Extension	72℃	5 min	

Electrophoresis Results of the Amplification Products



M: DL 5,000 DNA Marker

1-6: Target fragments is 2.5 kb in size

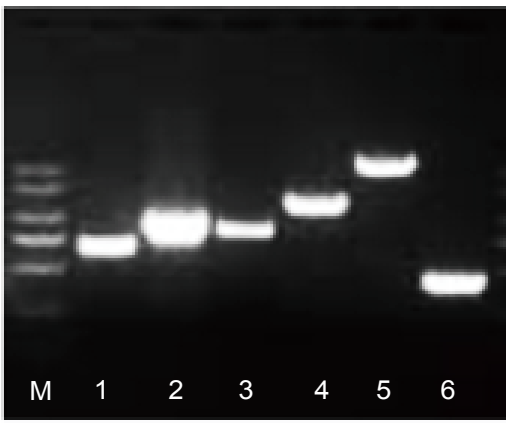
7.3 Excellent Amplification of Fragments with High GC Content

Phanta Max Super-Fidelity DNA Polymerase is capable of amplifying GC-rich fragments that conventional polymerase cannot amplify. Taking human genomic DNA as templates, target fragments of 654 bp, 900 bp, 800 bp, 1200 bp, 1400 bp, and 426 bp were amplified, respectively, The GC contents of all these amplicons are higher than 68%. High amplification efficiency is shown in the following figure. The Tm of all primers are approximately 60℃ (calculated in Primer Premier 5). The PCR reaction system refers to Section 6.1, and the PCR program is as follows:

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	3 min	
Denaturation	95℃	15 sec	} 35
Extension	72℃	45 sec / kb	
Final Extension	72℃	5 min	

Electrophoresis Results of the Amplification Products



M : DL 2,000 DNA Marker

1 : 654 bp 68.1% GC Content

2 : 900 bp 69.4% GC Content

3 : 800 bp 71.3% GC Content

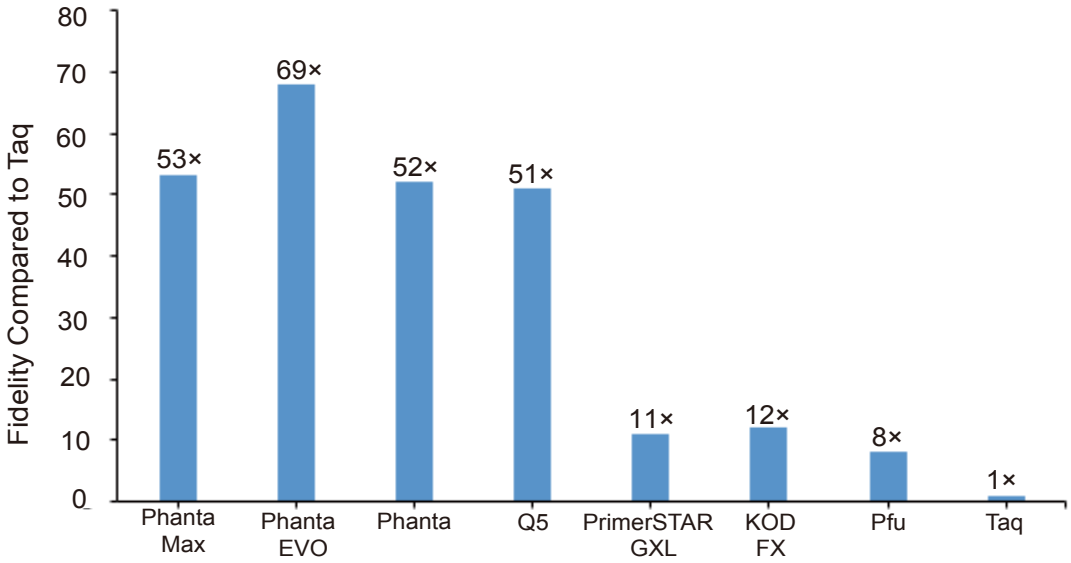
4 : 1,200 bp 73.5% GC Content

5 : 1,400 bp 74.7% GC Content

6 : 426 bp 76.8% GC Content

7.4 Reliable High Fidelity

The amplification fidelity of Phanta Max Super-Fidelity DNA Polymerase is 53-fold superior than that of Taq DNA Polymerase and 6-fold higher than that of Pfu, which is significantly superior to other products of the same kind. The following figure shows a comparison of amplification fidelity among various polymerases detected by a LacI Assay (Cline et al. ,Nucleic Acids Research,24: 3546-3551(1996).



8 Attentions

1. Use high-quality templates.
2. DO NOT use dUTP or any primers or templates that contain uracil.
3. Properly improve the input of Phanta Max Super-Fidelity DNA Polymerase according to the experiment demands, but no more than 2 U in a 50 μl reaction system.
4. The Phanta Max Super-Fidelity DNA Polymerase has strong proofreading activity. Therefore, the PCR products must be purified before adding A-Tailing when TA cloning.
5. To prevent the strong proofreading activity of the Phanta Max Super-Fidelity DNA Polymerase degrading primers, the polymerase should be loaded lastly when making up the reaction system.
6. Primers design notes:
   - Choose C or G as the last base of the 3'-end of the primer.
   - Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer.
   - Avoid hairpin structure at the 3'-end of the primer.
   - Tm of the primers should be within the range of 55℃ - 65℃ (recommend to calculate in Primer Premier 5), and the Tm difference between F and R primers should be less than 1℃.
   - Additional sequence should not be included when calculating Tm of the primers.
   - GC content of the primers should be within the range of 40% - 60%.
   - The general distribution of A, G, T, C in the primers should be uniform, and avoid using regions with rich GC and rich AT.
   - Keep complementary sequence less than 5 bases within the primers or between two primers, and complementary sequence less than 3 bases at the 3'-end of the primers.
   - Please search the specificity of the designed primers by NCBI BLAST to avoid non-specific amplification.

9 Troubleshooting

No or Low Yield of PCR Products

Primers	Optimize primer design
Annealing Temperature	Set gradient annealing temperature to find out the optimal one
Concentration of Primers	Appropriately improve the concentration of primers
Extension Time	Appropriately increase the extension time to 30 sec/kb-1 min/kb
Cycle Numbers	Increase cycle numbers to 35 - 40
Purity of Templates	Use high - purity templates
Template Input	Refer to the recommended reaction system and increase the input properly
Enzymes Input	Appropriately adjust the input of high-fidelity polymerase

Unspecific or Smear Bands in Electrophoresis

Primers	Optimize primer design
Annealing Temperature	Try to improve annealing temperature and set gradient annealing temperature to optimize
Concentration of Primers	Decrease the concentration of primers to final concentration as 0.2 μM
Extension Time	Appropriately decrease the extension time when blend bands longer than target bands appears
Cycle Numbers	Decrease cycle numbers to 25 - 30
PCR Programs	Use Two-Step PCR or Tough down PCR
Purity of Templates	Use high purity templates
Template Input	Modify or decrease templates input refering to the recommended reaction system
Enzymes Input	Appropriately adjust or decrease the input of high - fidelity polymerase