Ultra High-Fidelity PCR Master Mix

Cat. no. TTC-PE15
Storage: Store at -20 °C
Product Size: 1 mL

Introduction

TOOLS Ultra High-Fidelity PCR Master Mix is a new generation of superior enzyme based on Ultra High-Fidelity DNA Polymerase for robust polymerase chain reaction (PCR) with higher fidelity. The unique extension factor, specificity-promoting factors, and plateau-inhibiting factor that have been added to 2× Ultra High-Fidelity PCR Master Mix greatly improve its long-fragment amplification ability, specificity, and PCR yield. The 2× Ultra High-Fidelity PCR Master Mix 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 the 2× Ultra High-Fidelity Super-Fidelity DNA Polymerase is 53-fold lower than that of conventional Taq and sixfold lower than that of nfu. In addition, the 2× Ultra High-Fidelity Super-Fidelity DNA Polymerase 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. The 2× Ultra High-Fidelity DNA Polymerase contains two monoclonal antibodies that inhibit the 5'→3' polymerase activity and 3'→5' exonuclease activity at room temperature, enabling the 2× Ultra High-Fidelity DNA Polymerase to perform highly specific Hot-Start PCRs. The 2× Ultra High-Fidelity PCR MasterMix contains 2× Ultra High-Fidelity Super-Fidelity DNA Polymerase, dNTP, and an optimized buffer system. The amplification can begin only with the addition of a primer and template, thereby easing PCR setup and improving reproducibility. Protective agents in this product are capable of repeated freeze–thaw cycles.

Protocol

Unit definition

One unit (U) is defined as the amount of enzymes that incorporate 10 nmol of dNTPs into acid-insoluble products in 30 min at 74°C with activated salmon sperm DNA as the template or primer.

Primer Design

1. Choose C or G as the last base of the 3'-end of the primer;
2. Avoid continuous mismatching at the last eight bases of the 3'-end of the primer;
3. Avoid a hairpin structure at the 3'-end of the primer;
4. The temperature of the primers should be within the range of 55°C–65°C;
5. Additional sequences should not be included when calculating the temperature of the primers;
6. GC content of the primers should be within the range of 40%–60%;
7. The temperature and GC content of forward and reverse primers should be as similar as possible.

Recommended PCR Reaction

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

Template	Volume	Final concentration
Genomic DNA Plasmid or Virus DNA cDNA	X µL (50 – 400 ng) Y µL (10 pg ~ 30 ng) 1 – 5 µL (≤ 1/10 of the total volume of PCR system)	-
Primer 1(10 µM)	2 µL	0.4 µM
Primer 2(10 µM)	2 µL	0.4 µM
2x Master Mix	25 µL	-
ddH_2O	Up to 50 µL	-

PCR Cycle Setup

Temperature	Time	Cycles
95°C[a]	30 sec/3 min	-
95°C	15 sec	25-35 cycles
56-72°C[b]	15 sec	
72°C[c]	30-60 sec/kb	
72°C	5 min	-

1. For predenaturation, the recommended temperature is 95°C, and the recommended time is 30 s for plasmid or virus DNA and 3 min for genomic DNA or cDNA.
2. For annealing, the recommended temperature is the temperature of the primers. If the temperature of the primers is higher than 72°C, the annealing step can be removed (two-step PCR). If necessary, the annealing temperature can be further optimized as a gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising the annealing temperature in 3°C-increments is helpful for improving poor amplification specificity.
3. Longer extension time is helpful for increasing the amplification yield.

For Long-Fragment PCR

Use high-quality templates and improve the template usage. Use long primers. When the recommended PCR program does not work, try the touchdown two-step PCR as follows:

Temperature	Time	Cycles
95°C[a]	3 min	-
95°C	15 sec	5
74°C[b]	60 sec/kb	
95°C[c]	15 sec	5
72°C	60 sec/kb	
95°C[a]	15 sec	5
70°C	60 sec/kb	
95°C[b]	15 sec	25
68°C[c]	60 sec/kb	
68°C	5 min	-

For PCR Using Crude Material as the Template

TOOLS 2× Ultra High-Fidelity PCR Master Mix has a good resistance to PCR inhibitors and can be used for direct PCR amplification of bacteria, fungi, plant tissues, animal tissues, and whole blood samples. Crude materials that have been successfully amplified with Phanta Max Master Mix 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 amounts 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: Submerge a small amount of the sample in a lysis buffer with a final concentration of 200 μg/mL of Proteinase K (self-provided). Heat the mixture to 60°C for 10 min and then 95°C 10 min. Mix well and spin at room temperature. Collect the supernatant as a lysate (Lysis buffer: 20 mM Tris-HCl, 100 mM EDTA, 0.1% SDS, pH 8.0).

Application Examples

A. Suitable for Amplification of Fragments with Various Samples

1. Taking human genomic DNA as the template, 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. The temperature of all the primers was approximately 60°C (calculated using Primer Premier 5).

Template	Volume	Final concentration
Human Genomic DNA (100 ng/μL)	1 μL	-
Primer 1(10 μM)	2 μL	0.4 μM
Primer 2(10 μM)	2 μL	0.4 μM
2x Master Mix	25 μL	-
ddH_2O	Up to 50 μL	-

Recommended PCR Program

Temperature	Time	Cycles
95°C[a]	3 min	-
95°C	15 sec	35 cycles
60°C[b]	15 sec	
72°C[c]	30 sec/kb	
72°C	5 min	-

2. Taking human whole blood as the template, a target fragment of 1,295 bp was amplified with TOOLS 2× Ultra High-Fidelity PCR Master Mix. The temperature of all the primers was approximately 60°C (calculated using Primer Premier 5).

The reaction system and program are as follows:

Template	Volume	Final concentration
Whole blood	1 - 4 μL	-
Primer 1(10 μM)	2 μL	0.4 μM
Primer 2(10 μM)	2 μL	0.4 μM
2x Master Mix	25 μL	-
ddH_2O	Up to 50 μL	-

Recommended PCR Program

Temperature	Time	Cycles
95°C[a]	3 min	-
95°C	15 sec	35 cycles
60/63/70°C[b]	15 sec	
72°C[c]	30 sec/kb	
72°C	5 min	-

The annealing temperatures for 1.3 kb, 3.6 kb, and 8.5 kb of the target fragments are 60°C, 63°C, and 70°C, respectively.

3. Taking a tomato leaf, a rice leaf, and polished rice as templates and the purified genomic DNA from the rice leaf as a positive control, target fragments of 1.3 kb were amplified with TOOLS 2× Ultra High-Fidelity PCR Master Mix. The temperature of all the primers was approximately 60°C (calculated using Primer Premier 5).

The reaction system and program are as follows:

Template	Volume	Final concentration
Plant tissues *	X μL	-
Primer 1(10 μM)	2 μL	0.4 μM
Primer 2(10 μM)	2 μL	0.4 μM
2x Master Mix	25 μL	-
ddH_2O	Up to 50 μL	-

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

Recommended PCR Program

Temperature	Time	Cycles
95°C[a]	3 min	-
95°C	15 sec	35 cycles
60°C[b]	15 sec	
72°C[c]	30 sec/kb	
72°C	5 min	-

4. Using the lysate of mouse tails as the template, a target fragment of 2.5 kb was amplified with TOOLS 2× Ultra High-Fidelity PCR Master Mix. The temperature of all the primers was approximately 60°C (calculated using Primer Premier 5). The reaction system and program are as follows:

Template	Volume	Final concentration
Lysate of Mouse Tails	2 μL	-
Primer 1(10 μM)	2 μL	0.4 μM
Primer 2(10 μM)	2 μL	0.4 μM
2x Master Mix	25 μL	-
ddH_2O	Up to 50 μL	-

Recommended PCR Program

Temperature	Time	Cycles
95°C[a]	3 min	-
95°C	15 sec	35 cycles
60°C[b]	15 sec	
72°C[c]	30 sec/kb	
72°C	7 min	-

B. Amplification of Fragments with High GC Content

1. Taking human genomic DNA as the template, the GC content of the amplicons was higher than 68%. The temperature of all the primers was approximately 60°C (calculated using Primer Premier 5).

The PCR reaction system and the PCR program are as follows:

Template	Volume	Final concentration
Lysate of Mouse Tails	2 μL	-
Primer 1(10 μM)	2 μL	0.4 μM
Primer 2(10 μM)	2 μL	0.4 μM
2x Master Mix	25 μL	-
ddH_2O	Up to 50 μL	-

Recommended PCR Program

Temperature	Time	Cycles
95°C[a]	3 min	-
95°C	15 sec	35 cycles
72°C[c]	45 sec/kb	
72°C	5 min	-

Troubleshooting

No or Low Yield of PCR Products

Primers	Optimize primer design
Annealing Temperature	Set gradient annealing temperature to determine the optimal temperature
Concentration of Primers	Appropriately improve the concentration of primers
Extension Time	Appropriately increase the extension time from 30 s/kb to 1 min/kb
Cycle Numbers	Increase number of cycles to 35–40
Purity of Templates	Use high-purity templates
Template Input	Refer to the recommended reaction system and increase the input properly

Unspecific or Smear Bands in Electrophoresis

Primers	Optimize primer design
Annealing Temperature	Try to improve annealing temperature and set gradient annealing temperature
Concentration of Primers	Decrease the concentration of primers to a final concentration of 0.2 μM
Extension Time	Appropriately decrease the extension time when blend bands longer than the target bands appear
Cycle Numbers	Decrease number of cycles to 25–30
PCR Programs	Use two-step PCR or touchdown PCR
Purity of Templates	Use high-purity templates
Template Input	Modify or decrease template inputs referring to the recommended reaction system

This product is for research only, not for diagnostic or clinical use.