2 × Rapid Taq Master Mix

Catalog # P222

Version 7.1

GeneBio Systems, Inc.™

Vazyme

Vazyme biotech co., ltd.

1. Introduction

The product contains Taq DNA Polymerase, Extension Enhancer, dNTP and optimized buffer. Its amplification speed is up to 15 sec/kb, and is suitable for Rapid PCR reaction. Its extreme amplification speed is 1 sec/kb within 1 kb, reducing PCR time dramatically. PCR reaction can start directly after addition of primers and templates with the Pre-mixed 2 × Master Mix, and thus the omitted pipetting procedure increases the throughput and reproducibility significantly. The product enables high efficient and stable amplifications, and is suitable for amplification of fragments < 5 kb with genomic DNA as templates; < 10 kb with plasmid/λDNA as templates. The added protective agent keeps the activity of 2 × Master Mix after multiple freezing and thawing cycles. The product contains electrophoresis loading buffer and dye and can be analyzed with gel electrophoresis directly after reaction. PCR products contain a 3'-A overhang, and is applicable for T Vector cloning and compatible with ClonExpress Cloning Kit (C112/C113/C114).

2. Package Information

Components	P222-01	P222-02	P222-03
2 × Rapid Taq Master Mix	5 × 1 ml	15 × 1 ml	50 × 1 ml

Components	P222-w1	P222-w2	P222-w3
2 × Rapid Taq Master Mix	5 × 1 ml	15 × 1 ml	50 × 1 ml
ddH_2O	5 ml	3 × 5 ml	10 × 5 ml

3. Storage

Stored at -20℃. Stable for 3 months at 4℃ after thaw.

4. Workflow

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 74℃, using activated salmon sperm DNA as template.

5. Protocol

Reaction system:

ddH_2O	To 50.0 μl
2 × Rapid Taq Master Mix	25.0 μl
Primer1 (10 μM)	2.0 μl
Primer2 (10 μM)	2.0 μl
Template DNA*	x μl

* The optimized concentration of template differs from different template types. For a 50 μl reaction system, the recommended input amount of template is as follows:

Genomic DNA of animals and plants	0.1-1 μg
Genomic DNA of *E. coli*	10-100 ng
cDNA	1-5 μl (< 1/10 of the reaction volume)
Plasmid DNA	0.1-10 ng
λDNA	0.5-10 ng

Program for the PCR reaction:

95℃	3 min (Pre-denaturation)*[a]	
95℃	15 sec	30-35 cycles
60℃*[b]	15 sec	
72℃	15 sec/kb*[c]	
72℃	5 min (Complete extension)	

a. The pre-denaturation condition is suitable for most PCR reaction, and it can be adjusted according to complexity of template. For complex templates, please increase the pre-denaturation time to 5-10 min.

b. The optimal annealing temperature depends on the Tm of the primers, and the recommended temperature is 3-5℃ lower than Tm. For complex templates, annealing temperature and extension time should be optimized to achieve high efficient amplification.

c. To obtain higher yield, for 1 kb products, extension time of 2-5 sec is recommended. For >1 kb products, increase extension time to 20-30 sec/kb.

6. Notes

Gel electrophoresis: The blue dye and yellow dye stand at 4 kb and 50 bp in 1% agarose gel electrophoresis, respectively.

Primers Design

1. Choose C or G as the last base of the 3' end of the primer;
2. Avoid primer sequences that form continuous mismatch at the last 8 bases of the 3' end;
3. Avoid primer sequences that form hairpin loops, especially at the 3' end;
4. The difference of Tm between forward primer and reverse primer is no more than 1 ℃. It is recommended to choose primers with Tm around 55℃~65℃ (Tm is recommended to be calculated by Primer 5);
5. Unpaired sequence to template should not be included when calculating Tm of the primers;
6. Choose primers with GC content around 40~60%;
7. A, G, T and C should be distributed evenly through the primer. Avoid sequences with high GC or AT content;
8. Avoid >5 bp complementary sequences internally or between primers. Avoid >3 bp complementary sequences at 3' end of two primers;
9. Check the specificity of primers by NCBI BLAST, to avoid nonspecific amplifications.

7. Trouble shooting

	No products or low yield	Nonspecific or smeared bands
Primers	Optimize primer design	Optimize primer design
Annealing temperature	Set temperature gradient and find the proper annealing temperature	Gradually increase temperature to 65℃ with a 2℃ interval.
Primer concentration	Increase the primer concentration properly	Decrease primer concentration to 0.2 μM.
Extension time	Increase extension time to 30 sec/kb	Decrease extension time when the nonspecific band is larger than target band.
Cycles	Increase cycle number to 35-40	Decrease cycle number to 25-30
Template purity	Use template with high purity	Use template with high purity
Template amount	Decrease the input for crude extractions; increase input for other types of samples	Adjust the input according to the recommended amount for the reaction system.