2 × Vazyme LAmp Master Mix

P311/P312

Version 23.1

Product Description

Vazyme LAmp DNA Polymerase is a mixed enzyme of *Taq* DNA Polymerase and a protein containing 3'→5' exonuclease activity (proofreading activity). The fidelity is 6-fold higher than Taq DNA Polymerase. With a specially optimized buffer system, Vazyme LAmp DNA Polymerase is suitable for Long-range PCR (up to 21 kb when using the genome as a template). It has high amplification efficiency for templates of different origins and lengths. 2 × Vazyme LAmp Master Mix contains Vazyme LAmp DNA Polymerase, dNTP and optimized buffer system. It only needs to add primers and templates to perform amplification reaction, thereby reducing pipetting operations and improving detection throughput and reproducibility of results. The system contains protective agents that keep 2 × Master Mix stable in activity after repeated freezing and thawing. This product is available in a version containing loading buffer and tracking dye, so PCR products can be directly loaded for electrophoresis after the reaction, which is convenient to use. The PCR product has an adenine at the 3' end that can be cloned into the T vector. It is compatible with ClonExpress and TOPO cloning kits (Vazyme #C112/C113/C115/C601).

Components

Components	P311-01	P311-02	P311-03
2 × Vazyme LAmp Master Mix	1 ml	5 × 1 ml	15 × 1 ml

Components	P312-01	P312-02	P312-03
2 × Vazyme LAmp Master Mix (Dye Plus)	1 ml	5 × 1 ml	15 × 1 ml

Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Applications

It is applicable for the amplification of DNA samples such as genomic DNA, cDNA, plasmid DNA, and λDNA.

Notes

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

Primer Design Guidance

1. It is recommended that the last base at the 3' end of the primer should be G or C.
2. Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer.
3. Avoid hairpin structures at the 3' end of the primer.
4. Differences in the Tm value of the forward primer and the reverse primer should be no more than 1°C and the Tm value should be adjusted to 55 ~ 65°C (Primer Premier 5 is recommended to calculate the Tm value).
5. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value.
6. It is recommended that the GC content of the primer to be 40% - 60%.
7. 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.
8. Avoid the presence of complementary sequences of 5 or more bases either within the primer or between two primers. Avoid the presence of complementary sequences of 3 or more bases at the 3' end of two primers.
9. Use the NCBI BLAST function to check the specificity of the primer to prevent nonspecific amplification.

Experiment Process

Reaction System

Components	Volume
ddH_2O	To 50 μl
2 × Vazyme LAmp Master Mix	25 μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Template DNA*	x μl

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

Template Types	Amount
Animal & Plant Genomic DNA	0.1 - 1 μg
E. coli Genomic DNA	10 - 100 ng
cDNA	1 - 5 μl (≤1/10 of the total volume of PCR system)
Plasmid DNA	0.1 - 10 ng
λDNA	0.5 - 10 ng

Reaction Program

Target fragment <5 kb

Temperature	Time	Cycles
94°C	5 min (Initial Denaturation)	
94°C	30 sec	30 - 35
55°C*	30 sec	
72°C	30 sec/kb	
72°C	7 min (Final Extension)	

* The annealing temperature needs to be adjusted according to the Tm value of the primer, generally set to be 3 ~ 5°C lower than the Tm value of the primer; For complex templates, it is necessary to adjust the annealing temperature and extend the extension time to achieve efficient amplification.

Target fragment >5 kb

Temperature	Time	Cycles
94°C	5 min (Initial Denaturation)	
94°C	30 sec	30 - 35
68°C*	30 - 60 sec/kb	
68°C	7 min (Final Extension)	

* For fragments >5 kb, it is recommended to use long primers with a Tm value of 68 ~ 70°C, and combine the annealing/extension temperature to 68°C. This can significantly improve amplification specificity. In addition, extending the extension time can help to increase the amplification yield.

FAQ & Troubleshooting

	No amplification products or low yield	Nonspecific products or smeared bands
Primer	Optimize primer design	Optimize primer design
Annealing temperature	Set temperature gradient and find the optimal annealing temperature	Try to increase the annealing temperature to 65°C at 2°C intervals
Primer concentration	Increase the concentration of primers properly	Decrease the final concentration of primers to 0.2 μM
Extension time	Increase the extension time properly	Shorten the extension time properly, when there are nonspecific bands larger than the target band
Cycles	Increase the number of cycles to 35 - 40 cycles	Decrease the number of cycles to 25 - 30 cycles
Template purity	Use templates with high purity	Use templates with high purity
Input amounts of template	Decrease the amount of crude samples; adjust the amount of other samples according to the recommended amount and increase it properly	Adjust the template amount according to the recommended amount