3G Taq Master Mix (Red Dye)

P115

Version 22.1

Product Description

3G Taq Master Mix (Red Dye) contains 3G Taq DNA Polymerase, dNTP Mix, visualization red dye and an optimized buffer system. It is convenient to use and causes less pollution, which improves the detection throughput and the results reproducibility. 3G Taq DNA Polymerase has higher inhibitor tolerance and amplification efficiency than wild-type *Taq* DNA Polymerase. The product contains a visualization red dye, which can directly perform polyacrylamide gel electrophoresis and agarose gel electrophoresis after PCR, thereby simplifying the operation.

Components

Components	P115-01	P115-02
2 × 3G Taq Master Mix (Red Dye)	5 ml	10 × 5 ml

Storage

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

Applications

It is applicable for amplification reaction of animal, plant and microbial DNA.

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℃ and the Tm value should be adjusted to 55 ~ 65℃ (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

ddH_2O	To 50 µl
2 × 3G Taq Master Mix (Red Dye)	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 follow:

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

95℃	3 min (Initial Denaturation)[a]	
95℃	15 sec	30 - 35 cycles
60℃[b]	15 sec	
72℃	60 sec/kb	
72℃	5 min (Final Extension)	

a. The condition of initial denaturation is applicable for most amplification reactions and can be adjusted according to the complexity of the template structure. If the template structure is complex, the initial denaturation time can be extended to 5 - 10 min to improve its effect.

b. The annealing temperature needs to be adjusted according to the Tm value of the primer, generally set to be 3 ~ 5℃ 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.

FAQ & Troubleshooting

	No amplification products or low yield	Nonspecific products or smear 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℃ at 2℃ 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

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