

## Product Description

Green Taq Mix is a ready-to-use 2 × PCR mix that contains Taq DNA Polymerase, dNTPs and an optimized buffer system. The amplification can start only with the addition of primer and template, thereby reducing pipetting steps and improving throughput, specificity and reproducibility. Protective reagents in the Green Taq Mix enable its resistance to repeated freezing and thawing. The mix contains green loading dye which enable direct loading PCR products onto agarose gels for electrophoresis. The PCR product has A-tailing at the 3' end, which can be cloned into T vector and is suitable for ClonExpress and TOPO cloning kits (Vazyme #C112/C113/C115/C601).

## Components

Components	P131-01	P131-02	P131-03
Green Taq Mix	5 × 1 ml	15 × 1 ml	50 × 1 ml

## Storage

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

## Applications

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

## Notes

### Primer Design Guidance

1. It is recommend that the last base at the 3' end of 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 T<sub>m</sub> value of the forward primer and the reverse primer should be no more than 1°C and the T<sub>m</sub> value should be adjusted to 55 ~ 65°C (Primer Premier 5 is recommended to calculate the T<sub>m</sub> value).
5. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer T<sub>m</sub> value.
6. Control 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 and 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 non-specific amplification.

## Experiment Process

### Reaction System

ddH <sub>2</sub> O	To 50 µl
Green Taq Mix	25 µl
Primer1 (10 µM)	2 µl
Primer2 (10 µM)	2 µl
Template DNA*	x µl

\* The optimal reaction concentration is different for different templates. The following table shows the recommended template usage for 50 µl reaction system:

Genomic DNA of Animals and Plants	0.1 - 1 µg
<i>Escherichia coli</i> genomic DNA	10 - 100 ng
cDNA	1 - 5 µl (not exceed 1/10 of the total volume of the PCR reaction)
Plasmid DNA	0.1 - 10 ng
λDNA	0.5 - 10 ng

### Reaction Program

95°C	3 min (Initial denaturation) <sup>a</sup>	} 30 - 35 cycles
95°C	15 sec	
60°C <sup>b</sup>	15 sec	
72°C	60 sec/kb	
72°C	5 min (Final extension)	

- a. The initial denaturation conditions are suitable 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 the initial denaturation effect.
- b. The annealing temperature needs to be adjusted according to the T<sub>m</sub> value of the primer, generally set to be 3 ~ 5°C lower than the T<sub>m</sub> 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	Unspecific 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°C at 2°C intervally
Primer concentration	Increase the concentration of primers properly	Decrease the final concentration of primer to 0.2 µM
Extension time	Increase the extension time properly	Reduces the extension time when there are unspecific bands larger than the target bands
Cycles	Increase the number of cycles to 35 - 40 cycles	Reduce the number of cycles to 25 - 30 cycles
Template purity	Use templates with high purity	Use templates with high purity
Input amounts of template	Crude samples may need to be reduced in usage; Other sample usage refers to the recommended amount of the reaction system and increases in moderation	Adjust the dosage according to the recommended amount of the reaction system

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