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Product Description

This product contains Taq DNA Polymerase, dNTP and an optimized buffer system, except primers and templates, thereby simplifying the operation process and improving the detection throughput and repeatability. The protector in the mix enables the maintenance of enzyme activity after repeated freezing and thawing. Dye plus version is also provided, which enables direct electrophoresis. The PCR products contain A at the 3'-end and can be directly cloned into T-Vector.

Components

Components	ATR-P509
2X Taq Master Mix (Dye Plus)	1 mL

Components	ATR-P510
2X Taq Master Mix (Dye Plus)	5 × 1 mL

Storage

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

Applications

It is suitable for conventional PCR.

Notes

Notes for Operation

Prepare the reaction system on ice due to Taq DNA Polymerase has activity at room temperature.

Primer Design

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 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 to 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. 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.

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Experiment Process

Prepare the reaction system on ice as follows.

ddH_2O	To 50 µl
2 × Taq Master Mix	25 µl
Primer1 (10 µM)	2 µl
Primer2 (10 µM)	2 µl
Template DNA*	x µl

*The recommended amount of DNA template for a 50 µl reaction system is as follows:

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

Run the following PCR program.

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

a. The initial denaturation condition is applicable for most PCR. It can be changed according to the complexity of templates. If the template structure is complex, please extend the initial denaturation time up to 5 - 10 min;

b. Set the annealing temperature according to the Tm value of the primes. Generally, the annealing temperature should be 3 ~ 5°C lower than the Tm value of the primes. For complex template, adjust the annealing temperature and extend the extension time to achieve excellent amplification.