

Introduction

2× LiTaq™ Plus PCR Master Mix is a mixture of LiTaq™ Plus DNA polymerase, dNTP, an optimized buffer system and an enzyme containing 3'→5' exonuclease activity. Its fidelity is 6 times greater than that of LiTaq™ DNA Polymerase. Compared with LiTaq™ DNA Polymerase, LiTaq™ Plus DNA polymerase has stronger amplification performance, higher sensitivity, and is more tolerant of impurities within 5 kb amplifying range. It can amplify up to 10 kb from human genomic DNA or up to 15 kb from λ DNA. Protective agents in the 2× LiTaq™ Plus PCR Master Mix enable the resistance to repeated freeze-thaw cycles. The obtained PCR products are compatible with **LiClone™ One Step DNA Assembly Kit** (Cat. #: M0010), and can be directly used for cloning into T-Vectors as most PCR products amplified with Taq DNA polymerase have one A at the 3'-terminus.

Package Information

| Components | M0025-05 | M0025-15 | M0025-50 |
|-------------------------------|----------|----------|----------|
| 2× LiTaq™ Plus PCR Master Mix | 5×1 ml | 15×1 ml | 50×1 ml |

Storage

All materials should be stored at -20°C.

Quality Control

Exonuclease Activity: A reaction containing 10 U of enzyme and 0.6 µg of λ-Hind III incubated for 16 hours at 37°C resulted in no visually discernible change to DNA as determined by agarose gel electrophoresis.

Endonuclease Activity: A reaction containing 10 U of enzyme and 0.6 µg of Supercoiled pBR322 DNA incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

Functional Assay: 30 cycles of PCR amplification of 100 ng human genomic DNA with 1.25 units of LiTaq™ DNA Polymerase results in the expected 360 bp α-1-antitrypsin gene product, as determined by agarose gel electrophoresis.

Protocol

1. General reaction mixture for PCR:

| | |
|-------------------------------|----------|
| 2× LiTaq™ Plus PCR Master Mix | 25 µl |
| Template DNA* | Optional |
| Primer 1 (10 µM) | 2 µl |
| Primer 2 (10 µM) | 2 µl |
| ddH ₂ O | to 50 µl |

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

| | |
|-----------------------|-----------|
| Human Genomic DNA | 0.1~1 µg |
| Bacterial Genomic DNA | 10~100 ng |
| λDNA | 0.5~5 ng |
| Plasmid DNA | 0.1~10 ng |

2. Thermocycling Conditions for a Routine PCR:

| | | |
|-------|--------------------------|-------------|
| 94°C | 5 min (Pre-denaturation) | |
| 94°C | 15 sec | } 35 cycles |
| 55°C* | 30 sec | |
| 72°C | 60 sec/ kb | |
| 72°C | 7 min (final extension) | |
| 4°C | Hold | |

* Annealing temperature is based on the T_m of the primer pair and is typically 1-2°C below the calculated T_m.

Handling Notes

LiTaq™ Plus DNA Polymerase also shows polymerase activity at room temperature. Thus, it is better to set up reaction systems on ice and immediately start the reaction when it is done, so as to reduce nonspecific amplification in the preparatory stage and get better PCR result.

Primers Designing Notes

1. Choose C or G as the last base of the 3' end of the primer;
2. Avoid continuous mismatch at the last 8 bases of the 3' end of the primer;
3. Avoid hairpin structure at the 3' end of the primer;
4. T_m of the primers should be between 55°C~65°C;
5. 5' adding sequence should not be included when calculating T_m of the primers;
6. GC content of the primers should be between 40%~60%;
7. T_m and GC content of forward and reverse primers should be as similar as possible.