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2× LiTaq™ SuFi PCR Master Mix

Cat. #: M0031 Size: 1 ml

Introduction

2× LiTaq™ SuFi PCR Master Mix is a master mix of LiTaq™ SuFi DNA Polymerase which is hotstart and superfidelity, dNTP and optimized buffer system at 2× concentration. Therefore, amplification can be carried out as long as primers and templates are added, resulting in less frequent liquid relief operation and higher repeat ability of flux and results. The protective agent in the system guarantees that LiTaq™ SuFi PCR Master Mix can keep its activity even after many freezing and thawing cycles. This product is capable of amplifying 20kb genomic DNA, and will generate blunt ends compatible with LiClone™ One Step Cloning Kit (M0010).

Package Information

Components	M0031
2× LiTaq™ SuFi PCR Master Mix	1 ml

Storage

All materials should be stored at -20°C.

Quality Control

1. PCR reaction system:

Components	Reaction Volume
2× LiTaq™ SuFi PCR Master Mix	25 µl
DNA template (< 250 ng/50 µl)	variable
Upstream primer (0.5 µM)	2.5 µl
Downstream primer (0.5 µM)	2.5 µl
Distilled water (dH_2O)	To 50 µl
Total reaction volume	50 µl

2. PCR reaction program:

Step	Temperature	Time
Initialization	98°C	30 sec - 3 min
Denaturation	98°C	5-10 sec
Annealing	45-72°C	10-30 sec
Elongation	72°C	2-4 kb/min
Final Elongation	72°C	5-10 min

Protocol

1. **Denaturation**: For simple DNA templates, the pre-denaturation temperature is 98°C and the pre-denaturation time is 30 sec to 1 minutes. For more complicated templates, the pre-denaturation time can be extendeed to 3 minutes.

2. **Annealing**: the annealing temperature should be the 3-5°C lower than the Tm of primer. If the ideal amplification efficiency cannot be obtained, the annealing temperature should be changed in a gradient to optimize. When non-specific reactions occur, the annealing temperature should be appropriately increased. Two-step PCR can be used for primers with high Tm.

3. **Elongation**: The extension time should be set according to the length of the amplified fragment and the complexity of the template. The amplification efficiency of the LiTaq™ SuFi DNA Polymerase is 4-6 kb/min. For simple templates, the rate can be 6 kb/min.

4. **Cycles**: The number of cycles can be set based on the downstream applications of the PCR product. If the number is too low, the amount of PCR product is insufficient; if the number is high, the probability of mismatch and the non-specific background are increased. Therefore, the number of cycles should be reduced as much as possible yet ensuring the yield of the product.

Trouble Shooting

No product at all or low yield

1. High quality or purified DNA templates are preferred to enhance the success of PCR.
2. Repeat and make sure that there are no pipetting errors.
3. Use fresh high quality dNTPs.
4. Do not use dNTP mix or primers that contain dUTP or dITP.
5. Sample concentration may be too low. Use more templates.
6. Template DNA may be damaged. Use carefully purified template and make sure template is not fragmented.
7. Increase extension time.
8. Increase the number of cycles.
9. Optimize annealing temperature
10. Optimize enzyme concentration.
11. Optimize the denaturation time.
12. Check the purity and concentration of the primers.
13. Check primer design.

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