WebSite www.ebiocell.com
E-mall order@ebiocell.com
tech@ebiocell.com

Polymerase Chain Reaction(PCR)

Reagents

1） 5 μl 10X Standard Taq Reaction buffer
2） 1 μl 10 mM dNTPs
3） 1 μl 10 mM Forward Primer
4） 1 μl 10 mM Reverse Primer
5） Template DNA
6） 25 μl Taq DNA Polymerase
7） Make up to 50 μl Nuclease-free water

Procedure

The protocol is composed of 3 basic steps: denaturation, annealing, and extension.

1） Preheat the thermocycler to 95°C.
2） Gently mix the reaction components. If necessary, centrifuge briefly to collect the liquid to the bottom of the tube.
3） If using a PCR machine without a heated lid, overlay the sample with mineral oil.
4） Transfer PCR tubes from ice to the preheated PCR machine (95°C) for the initial denaturation step.
5） Begin thermocycling:
   a） Initial denaturation: 95 °C, 30 seconds
   b） 30 cycles of: 95 °C, 15-30 seconds; 45 °C – 68 °C, 15 – 60 seconds; 68 °C, 1 minute/kb
   c） Final extension: 68 °C, 5 minutes
   d） Hold: 4 – 10 °C
6） Validate the final reaction mixture by running a fraction on an agarose gel with an appropriate molecular marker. Directly sequence the amplified product or carry out restriction enzyme digests.

Recommendations

1） $MgCl_2$: For most Taq DNA Polymerase a $MgCl_2$concentration of 1.5 – 2.0 mM is optimal. The final concentration in standard 1X Standard Taq Reaction Buffer is 1.5 mM. If

another concentration is optimal, a reaction buffer that does not contain $MgCl_2$ can be used and the $MgCl_2$ can be added separately.

2） Additives: DMSO and formamide can be added to improve difficult targets such as GC-rich sequences.

3） Template DNA: Use high quality, purified DNA. For a 50 µl reaction, recommended amounts are:

a） Genomic DNA 1 ng – 1 µg

b） Plasmid or viral DNA 1 pg – 1 ng

4） Taq DNA Polymerase: 1.25 units/50 µl reaction is recommended, however this can vary from 0.25 – 2.5 units/50 µl reaction.

5） Denaturation: A longer initial denaturation step (2 – 4 minutes at 95 °C) can be employed for difficult templates such as GC-rich sequences. For colony PCR 5 minutes at 95 °C is recommended. During themocycling 15 – 30 seconds at 95 °C is recommended.

6） Annealing: The temperature is dependent on the T_M of the primer pair (usually 45 °C – 68 °C). Optimise by carrying out temperature gradient PCR starting 5 °C below the T_M.

7） Extension: The recommended temperature is 68 °C with the time generally 1 minute per kb. A final extension of 5 minutes at 68 °C is recommended.

8） Cycle number: Generally, 25 – 35 cycles yields sufficient product. Up to 45 cycles can be employed to detect low copy number targets.