PCR Protocol for *Taq* DNA Polymerase with Standard *Taq* Buffer (NEB #M0273)

Materials Required but not Supplied

Taq DNA Polymerase with Standard *Taq* Buffer

- Template DNA and associated forward and reverse primers
- Deoxynucleotide (dNTP) Solution Mix (NEB #N0447)
- Nuclease-free Water (NEB #B1500)

Overview

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (1). *Taq* DNA Polymerase is an enzyme widely used in PCR (2). The following guidelines are provided to ensure successful PCR using NEB's *Taq* DNA Polymerase. These guidelines cover routine PCR. Amplification of templates with high GC content, high secondary structure, or low template concentrations, may require further optimization. The NEB Tm Calculator is highly recommended to estimate an appropriate annealing temperature when using NEB products for PCR.

Protocol

Reaction setup:

1. Assemble all reaction components on ice. Each component should be gently mixed before adding to the reaction in a sterile thin-walled PCR tube. Mix the entire reaction to ensure homogeneity. Collect all liquid to the bottom of the tube with a quick centrifuge spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Component	25 µl reaction	50 µl reaction	Final Concentration
10X Standard *Taq* Reaction Buffer	2.5 µl	5 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
Template DNA	variable	variable	<1,000 ng
Taq DNA Polymerase	0.125 µl	0.25 µl	1.25 units/50 µl PCR
Nuclease-free water	to 25 µl	to 50 µl	

2. Quickly transfer the reactions to a thermocycler preheated to the denaturation temperature (95°C) and begin thermocycling.

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME

Initial Denaturation	95°C	30 seconds
25-30 Cycles	95°C 45-68°C* 68°C	15-30 seconds 15-60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

*Use of the Tm Calculator is highly recommended.

General Guidelines:

1. **Template**:
   Use high-quality, purified DNA templates to maximize success with PCR. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	Amount
Genomic	1 ng–1 µg
Plasmid or Viral	1 pg–10 ng

2. **Primers**:
   Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 can be used to design or analyze primers. The final concentration of each primer in a reaction may be 0.05–1 µM, typically 0.1–0.5 µM.

3. **Mg^{++} and additives**:
   Mg^{++} concentration of 1.5–2.0 mM is optimal for most PCR products generated with *Taq* DNA Polymerase. The final Mg++ concentration in 1X Standard *Taq* Reaction Buffer is 1.5 mM. This supports satisfactory amplification of most amplicons. However, Mg++ can be further optimized in 0.5 or 1.0 mM increments using $MgCl_2$.

   Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO (3) or formamide (4).

4. **Deoxynucleotides**:
   The final concentration of dNTPs is typically 200 µM of each deoxynucleotide.

5. ***Taq* DNA Polymerase Concentration**:
   We generally recommend using *Taq* DNA Polymerase at a concentration of 25 units/ml (1.25 units/50 µl reaction). However, the optimal concentration of *Taq* DNA Polymerase may range from 5–50 units/ml (0.25–2.5 units/50 µl reaction) in specialized applications.

6. **Denaturation**:
   An initial denaturation of 30 seconds at 95°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer initial denaturation of 2–4 minutes at 95°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5-minute denaturation at 95°C is recommended.

   During thermocycling a 15–30 second denaturation at 95°C is recommended.

7. **Annealing**:
   The annealing step is typically 15–60 seconds. Annealing temperature is based on the Tm of the primer pair and is typically 45–68°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated Tm. The NEB Tm Calculator is recommended to calculate an appropriate annealing temperature.

When primers with annealing temperatures above 65°C are used, a 2-step PCR protocol is possible (see #10).

8. **Extension**:
   The recommended extension temperature is 68°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68°C is recommended.

9. **Cycle number**:
   Generally, 25–35 cycles yield sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

10. **2-step PCR**:
    When primers with annealing temperatures above 65°C are used, a 2-step thermocycling protocol is possible.

11. **PCR product**:
    The PCR products generated using *Taq* DNA Polymerase contain dA overhangs at the 3´–end; therefore, the PCR products can be ligated to dT/dU-overhang vectors. The Monarch® Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1130) is recommended as an efficient method for purification and concentration up to 5 µg of high-quality, double-stranded and single-stranded DNA.

Thermocycling conditions for a routine 2-step PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 65-68°C	15-30 seconds 1 minute/kb
Final Extension	65-68°C	5 minutes
Hold	4-10°C	

References:
1. Saiki R.K. et al. (1985). *Science*. 230, 1350-1354.
2. Powell, L.M. et al. (1987). *Cell*. 50, 831-840.
3. Sun, Y., Hegamyer, G. and Colburn, N. (1993). *Biotechniques*. 15, 372-374.
4. Sarkar, G., Kapelner, S. and Sommer, S.S. (1990). *Nucleic Acids Res.*. 18, 7465.

Related Resources

- Tm Calculator
- Guidelines for PCR Optimization with *Taq* DNA Polymerase
- *Taq* PCR Kit Troubleshooting Guide