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

Protocols.io also provides an interactive version of this protocol where you can discover and share optimizations with the research community.

Overview

PCR

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, low template concentrations, or amplicons greater than 5 kb may require further optimization.

Protocol

Reaction setup:

We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (95°C).

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	

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Transfer PCR tubes from ice to a PCR machine with the block preheated to 95°C and begin thermocycling.

Thermocycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
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	

General Guidelines:

1. Template:

   Use of high quality, purified DNA templates greatly enhances the success of 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–1 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 (https://bioinfo.ut.ee/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 T_m 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 yields 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.

    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	

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.

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.

Links to this resource

Product Categories: *Taq* DNA Polymerase Products, One*Taq*® DNA Polymerases Products

Applications: Colony PCR, A-tailing, Polymerases for DNA Manipulation, | More +

Related Products: *Taq* DNA Polymerase with Standard *Taq* Buffer