PCR Protocol for Taq DNA Polymerase with Standard Taq Buffer

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

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	Temperature	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 (http://frodo.wi.mit.edu/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 1.5–2.0mM is optimal for most PCR products generated with *Taq* DNA Polymerase. The final Mg^{++} concentration in 1X Standard *Taq* Reaction Buffer is 1.5mM. This supports satisfactory amplification of most amplicons. However, Mg^{++} can be further optimized in 0.5 or 1.0mM 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 200μM 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/50ul reaction). However, the optimal concentration of *Taq* DNA Polymerase may range from 5–50 units/ml (0.25–2.5 units/50ul 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 T_m. 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	Temperature	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.

Product: Taq *DNA Polymerase with Standard* Taq *Buffer (M0273)*

Protocol from New England Biosciences
https://international.neb.com/protocols/0001/01/01/taq-dna-polymerase-with-standard-taq-buffer-m0273