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PCR with *Taq* DNA Polymerase with Standard *Taq* Buffer(M0273) V.2

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dx.doi.org/10.17504/protocols.io.bdd5i286**New England Biolabs (NEB)**Tech. support phone: **+1(800)632-7799** email: **info@neb.com****New England Biolabs**

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This Protocol explains methods for *Taq* DNA Polymerase with Standard *Taq* Buffer (M0273).

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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).

A	B	C	D
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:

A	B	C
STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C	15-30 seconds
	45-68°C	15-60 seconds
	68°C	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:

A	B
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](#) 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 MgCl₂.

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 T_m. The NEB [T_m 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:

A	B	C
STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C	15-30 seconds
	65-68°C	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.

MATERIALS

 [Taq DNA Polymerase with Standard Taq Buffer - 400 units New England](#)


Biolabs Catalog #M0273S

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

Annealing temperatures should be determined using the [NEB Annealing Temp Calculator](#).

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Set up the following reaction  **On ice** .

A	B	C	D
Component	25 µl reaction	50 µl reaction	Final Concentration
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10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
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Template DNA	variable	variable	<1,000 ng
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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:

A	B
DNA	Amount
genomic	1 ng–1 µg
plasmid or viral	1 pg–1 ng

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Gently mix the reaction.

3 Collect all liquid to the bottom of the tube by a quick spin if necessary and overlay the sample with mineral oil if using a PCR machine without a heated lid.

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

5 Select between thermocycling conditions for a routine PCR and for a routine 2-step PCR:

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

Step 5 includes a Step case.

Routine PCR

Routine 2-step PCR

step case

Routine PCR

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Thermocycling conditions for a routine PCR:

A	B	C
STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C	15-30 seconds
	45-68°C	15-60 seconds
	68°C	1 minute/kb
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