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PCR Protocol for Taq DNA Polymerase with ThermoPol® Buffer (M0267) V.2

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

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 PCR 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 ThermoPol Reaction Buffer is 2 mM. This supports satisfactory amplification of most amplicons. However, Mg^{++} can be further optimized in 0.5 or 1.0 mM increments using MgSO4 (NEB# B1003).

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.

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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 T_m Calculator is recommended for calculation of 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 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.
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3. Sun, Y., Hegamyer, G. and Colburn, N. (1993). Biotechniques. 15, 372-374.
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MATERIALS

Taq DNA Polymerase with ThermoPol Buffer - 400 units **New England Biolabs Catalog #M0267S**

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

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

1 

Assemble the following reaction **On ice** :

A	B	C	D
Component	**25 µl reaction**	**50 µl reaction**	**Final Concentration**
10X ThermoPol 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	

For amounts of DNA needed, see below:

A	B
DNA	**Amount**
genomic	1 ng–1 µg
plasmid or viral	1 pg–10 ng

2 

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.

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4 Quickly 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

6 

Perform PCR using the general routine below or using your own optimized routine:

A	B	C
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	

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