NEW ENGLAND Biolabs®

Protocol for *Taq* 2X Master Mix (NEB #M0270)

Materials Required but not Supplied

Taq 2X Master Mix

- 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* 2X Master Mix. These guidelines cover routine PCR. Amplification of templates with high GC content, high secondary structure, or low template concentrations may require further optimization.

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. The entire reaction should be mixed again to ensure homogeneity. Collect all liquid to the bottom of the tube with a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

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

Component	25 µl reaction	50 µl reaction	Final Concentration
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
One*Taq* 2X Master Mix with Standard Buffer	12.5 µl	25 µl	1X
Nuclease-free water	to 25 µl	to 50 µl	

Thermocycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
25-35 Cycles	95°C 45-68°C* 68°C	15-30 seconds 15-60 seconds 1 minute/kb

STEP	TEMP	TIME
Final Extension	68°C	5 minutes
Hold	4-10°C	

**Use of the NEB TmCalculator is highly recommended.*

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–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 is typically 0.1–0.5 µM.

3. **Mg^{++} and Additives:**
   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 ^{Taq} Master Mix 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. **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.

5. **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's 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 #8).

6. **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

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

8. **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 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	

9. **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.

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