Taq DNA Polymerase Guidelines for PCR Optimization

Introduction

Taq DNA Polymerase is an enzyme widely used in PCR (7). The following guidelines are provided to ensure successful PCR using NEB's *Taq* DNA Polymerase. These guidelines cover routine PCR reactions. PCR of templates with high GC content, high secondary structure, low template concentrations, or amplicons greater than 5 kb may require further optimization.

Protocol

DNA Template: Use of high quality, purified DNA templates greatly enhances the success of PCR reactions. It is also critical that contamination from outside sources, especially previous PCR reactions, be avoided.

Approximately 10^4 copies of the target DNA are required to detect a product in 25–30 cycles of PCR. Typically, this means a 0.1–1 ng/ml final concentration of plasmid or viral templates, and 1–10 µg/ml of genomic templates. In general, the higher the DNA concentration and number of cycles, the lower the primer specificity. High DNA concentrations can be advantageous when fewer cycles are desired to increase the fidelity of the reaction.

Primers: Oligonucleotide primers are generally 20–30 nucleotides in length and ideally have a GC content of 40–60%, with GC residues spaced evenly within the primer. Calculated melting temperatures T_m for the two primers should be above 45°C and the Tm for the two primers should be within 5°C of each other.

The final concentration of each primer in a PCR reaction may be 0.05–1.0 µM (typically 0.1–0.5 µM). Higher concentrations increase the possibility of secondary priming, which potentially creates spurious amplification products.

Magnesium Concentration: A magnesium concentration of 1.5–2.0 mM is optimal for most PCR products generated with *Taq* DNA Polymerase. Optimization normally involves supplementing the magnesium concentration in 0.5 or 1.0 mM increments.

Deoxynucleotides: The final concentration of dNTPs is typically 200 µM of each deoxynucleotide. Concentrations > 500 µM should be avoided.

***Taq* DNA Polymerase Concentration:** *Taq* DNA Polymerase is normally present at a final concentration of 25 units/ml (1.25 units/50 µl reaction), but can range from 5–50 units/ml (0.25–2.5 units/50 µl reaction) in specialized applications. Enzyme dilutions can be made in 1X reaction buffer if used immediately. If dilutions must be stored, use Diluent F (NEB

#B8006).

Hot Start: Non-specific primed synthesis during the assembly of the reaction prior to PCR cycling has been identified as a source of nonspecific products in some PCR reactions. These undesired products can often be avoided by assembling all components on ice, adding the polymerase last and immediately transferring the reactions to a thermocycler preheated to the denaturation temperature (95°C). If this approach continues to yield non-specific products, use of a hot start polymerase is recommended. One*Taq*™ Hot Start DNA Polymerase (NEB #M0481) is available from NEB to accommodate these situations.

DNA Contamination: To minimize DNA contamination during reaction setup, it is recommended that positive-displacement pipets or aerosol barrier tips be used to aliquot and mix reagents. In order to further minimize contamination, as well as decrease pipetting errors, users are encouraged to make master mixes of reagents.

Master Mix: When setting up multiple reactions it is faster and more accurate to create a master mix of the components that are common to all reactions. In general, this involves creating a stock solution of polymerase, nucleotides, reaction buffer, water, and occasionally primers. The master mix is then aliquotted and mixed with the DNA template and any required primers.

Denaturation Temperature and Duration: 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 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, although this can depend on the thermocycler and tubes used. Consult the product literature accompanying the thermocycler being used for more specific recommendations.

. **Annealing Temperature and Duration:** Annealing temperatures should be chosen to match the T_m values of the primer pair and are typically 45–68°C. If extra bands are observed, higher annealing temperatures should be considered. The absence of product can indicate the need for a lower annealing temperature. Annealing times of 15–60 seconds are usually adequate.

. **Extension Time:** Extensions are normally done at 68°C. As a general rule, extension times of one minute per kb should be used. For products less than one kb, an extension time of 45–60 seconds should be used. A final extension of 5 minutes at 68°C is recommended.