# PCR Protocol for Taq DNA Polymerase with Standard Taq Buffer (M0273)

## 1. Reaction setup:

It is recommended to assemble all reaction components on ice and transfer the reactions to a thermocycler preheated to the denaturation temperature (95°C).

| Component | 25 µL Reaction | 50 µL Reaction | Final Reaction |
|---|---|---|---|
| 10X Standard Taq Reaction Buffer | 2.5 µL | 5 µL | 1X |
| 10 mM dNTPs | 0.5 µL | 1 µL | 200 µM |
| 10 µM Primer Forward Primer | 0.5µL | 1 µL | 0.2 µM (0.05-1 µM) |
| 10 µM Reverse Primer | 0.5µL | 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 | |

Gently mix the reaction in a PCR tube and transfer the tubes to a PCR machine and begin thermocycling.

## 2. Thermocycling conditions:

| Step | Temperature | 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 | |

*Adjust accordingly to the annealing temperatures of the used primer.

From: NEB