

Polymerase Chain Reaction

Material

DNA polymerase (Ex Taq from Takara)

10x Buffer

Forward primer

Reverse primer

dNTPs

Template DNA

ddH₂O

Procedure

1. Keep everything on ice.
2. Make up a master mix of everything into PCR tubes and microcentrifuge 1 minute (25.0 μL reaction system)

19.10 μL ddH₂O
2.5 μL 10x Buffer (Mg²⁺)
2.0 μL dNTPs
0.5 μL forward primer
0.5 μL reverse primer
0.15 μL DNA polymerase
0.25 μL template DNA

----- 25.0 μL Total

3. Choose a suitable program, and adjust your annealing temperature and extension time as described below:

95 °C 5 min

95 °C 30s

— °C 30s

72 °C 1 min

72 °C 5 min

12 °C ∞

} 30x