PCR Amplification

Materials

DNA template
Forward and reverse primers
TaKaRa Taq DNA polymerase (5U/μL)
10× PCR Buffer (Mg^{2+} plus)
dNTP Mixture (2.5 mM each)
Sterile distilled water

Procedure

Preparation of the PCR Reaction Mixture

1. Place all PCR reagents on ice before use.
2. Prepare a 50 μL PCR reaction mixture on ice according to the following table.

Component	Amount
TaKaRa Taq (5 U/μL)	0.25 μL
10× PCR Buffer (Mg^{2+} plus)	5 μL
dNTP Mixture (2.5 mM each)	4 μL
Forward primer	0.2–1.0 μM (final)
Reverse primer	0.2–1.0 μM (final)
Template DNA	<500 ng
Sterile distilled water	Up to 50 μL

3. Gently mix the reaction mixture and keep it on ice until amplification.

PCR Amplification

1. Place the PCR tube into the thermocycler.
2. Run the following PCR program (example for a 1 kb DNA fragment):

Step	Temperature	Time
Denaturation	98 °C	10 s
Annealing	55 °C	30 s
Extension	72 °C	1 min

Repeat for 30 cycles.

Analysis of PCR Products

1. Analyze the PCR products by agarose gel electrophoresis.
2. Verify the amplified DNA fragment by comparing its size with the expected product size.