Polymerase Chain Reactions

The polymerase chain reaction (PCR) is used to amplify a segment of DNA that lies between two regions of known sequence.

Components of PCR:

Oligonucleotides

Standard Buffer

Taq DNA Polymerase

Target Sequences

Deoxyribonucleoside triphosphates (dNTPs)

The standard buffer for PCR has the following components:

500 mM KCl 100 mM Tris. Cl 15 mM $MgCl_2$

Amplification Reactions:

1. In a sterile 0.5 mL eppendorf tube, mix in the following order:

Sterile Water	30 uL
10 X amplification buffer	10 uL
Mixture of 4 dNTPs (conc. 1.25 mM)	16 uL
primer 1 (in 5 uL of water)	100 pmoles
primer 2 (in 5 uL of water)	100 pmoles
template DNA (up to 2 ug)	
Water to a final volume of 100 uL	

2. Heat the reaction mixture for 5 minutes at 94°C to denature the DNA completely.
3. While the mixture is still at 94°C, add 0.5 uL of *Taq* DNA Polymerase.

Typical Conditions for the Cycles in PCR

Cycle	Denaturation	Annealing	Polymerization
1st	5 min at 94°C	2 min at 50°C	3 min at 72°C
Subsequen	1 min at 94°C	2 min at 50°C	3 min at 72°C
Final	1 min at 94°C	2 min at 50°C	10 min at 72°C

4. Withdraw a sample of the amplified DNA from the reaction mixture and analyze it by gel electrphoresis.