

Conventional Polymerase Chain Reaction (PCR)



General Aim

To amplify DNA fragments using Polymerase Chain Reaction (PCR) technique.

Method

Taq polymerase PCR.

Learning Objectives (ILOs)

- Demonstrate proficiency with the protocol involved in PCR.
- Identify the role of specific reagents and equipment in PCR.
- Practice basic laboratory techniques.
- Conclude downstream applications of PCR.

Theoretical Background/Context

Conventional Polymerase Chain Reaction is a technique for in vitro amplification of a specific short defined segment of DNA. Most PCR methods typically amplify DNA fragments of up to ~10kb. In a test tube, A pair of oligonucleotide primers directs the polymerase enzyme and the deoxyribonucleotides to a specific gene in the DNA sample. A replication like process occurs in cycles. This repetition results in DNA amplification and as much as a billion copies can be the result of 30 cycles. Therefore, minimal amounts of DNA can be detected.

Principle of Work

A PCR setup requires several components and reagents. These components include :

- Two primers (Forward and reverse).
- Taq polymerase.
- Deoxynucleoside triphosphates (dNTPs).
- Buffer solution.
- Distilled water.
- Template DNA.

The PCR reaction follows the following steps:

1. Denaturation: High temperature (95 degree centigrade) allows the separation of the two DNA strands due to break down of hydrogen bonds linking bases together.
2. Primer annealing: Primers are short, synthetic sequences of single-stranded DNA typically consisting of 20-30 bases. Annealing usually takes place between 40 degrees Centigrade and 65 degrees centigrade, depending on the length and base sequence of the primers.
3. Extension (elongation): Taq polymerase is a recombinant thermo-stable DNA polymerase, from the organism *Thermus aquaticus*. It is active at high temperatures. Temperature is raised at this stage to 72 degree centigrade and complementary dNTPs are added starting the 3' end of the primer. Hence; Taq DNA polymerase synthesized in the 5'-3' direction.