POLYMERASE CHAIN REACTION

Prof. Arjumand S. Warsy
Department of Biochemistry
College of Science, King Saud University, Riyadh

Introduction

- This method was developed by Saiki, et al in 1985 and has revolutionized molecular diagnosis and molecular analysis of genetic diseases.

- It can selectively amplify a single molecule of DNA or RNA several million fold in a few hours.

- Specific gene sequence can be determined in a patient sample without cloning or Southern blotting.

- PCR can be carried out even on a single cell obtained from:

  Mouth wash, drop of dried blood, hair root, semen, chorionic villus amniotic fluid, etc.

- PCR is based on the enzymatic amplification of a DNA fragment that is flanked by two primers (short oligonucleotides that hybridize to the opposite strands of the target sequence and prime synthesis of complementary DNA sequence by the enzyme DNA polymerase. A special heat resistant polymerase is used known as 'Taq polymerase' which is obtained from a bacterium, (Bacillus thermophilus aquaticus) which grows naturally is hot springs and is heat stable.

  The techniques involves:

  (a) obtaining two oligonucleotide primers approx. 20 bp complementary to the DNA flanking a particular DNA region of interest.
  (b) Heat denaturation: The DNA to be amplified is denatured by heating.
  (c) Hybridization: The primers are added and at a suitable temperature annealing occurs with the complementary sequence.
  (d) Sequence synthesis: DNA synthesis occurs attached to the primers in presence of the deoxyribonucleotides and DNA polymerase.
  (e) The cycles of heat denaturation, hybridization of the primers and enzymatic DNA synthesis are repeated 4-30 times and result in an exponential amplification of the target DNA sequence (10^5 - 10^6 fold).
     - DNA product can be directly visualized by UV fluorescent after ethidium bromide staining.

Advantages

Using the automated "PCR machines", a round of amplification takes approx. 10 min. Thus in a few hours millions of copies of DNA can be produced.

The DNA so amplified can be used for analysis i.e.

(a) Determination of DNA sequences
(b) Direct detection of mutation by treatment with ASO.
(c) RFLP studies
(d) DGGE, SSCP and other methods.

Disadvantages

The amplification should be done in completely sterile conditions, in order to avoid contamination with other organisms.