THE POLYMERASE CHAIN REACTION (PCR)

DEFINITION:

Polymerase Chain Reaction **(PCR)** is a technique for *in vitro* amplify of specific DNA, that produces millions of exact copies of a selected DNA sequence out of a mixed population of DNA molecules or from very few DNA copies (even from single DNA molecules).

History

A 1971 paper in the Journal of Molecular Biology by Kleppe and co-workers first described a method using an enzymatic assay to replicate a short DNA template with primers *in vitro*. However, this early manifestation of the basic PCR principle did not receive much attention, and the invention of the polymerase chain reaction in 1983 is generally credited to Kary Mullis in 1983. Mullis summarized the procedure: "Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon". He was awarded the Nobel Prize in Chemistry in 1993 for his invention.

The discovery in 1976 of Taq polymerase , a DNA polymerase purified from the thermophilic bacterium, Thermus aquaticus, which naturally lives in hot (50 to 80 °C) environments such as hot springs

PRINCIPLE:

PCR is based on the ability of single-stranded DNA to pair up with a complementary single-stranded molecule to form a double-stranded molecule. This annealing or

hybridization of single-stranded DNA into duplexes occurs spontaneously under certain well-understood conditions of temperature, pH, and salt.

With the proper choice of sequence, one can readily anneal a short oligonucleotide (DNA Primer) to a much longer molecule and expect to have exact base pairing so that the oligomer attaches to the long DNA at only the exact complementary sequence. The resulting DNA molecule will have at least one duplex region flanked by single-stranded regions. If one then adds DNA polymerase and sufficient amounts of all four deoxy- ribonucleotide triphosphates to a solution containing the hybridized DNA, the DNA polymerase will bind to the duplex region and start to synthesize new DNA that is complementary in sequence to the single-stranded region.

With PCR, one can amplify the desired DNA sequence from a mixture of DNA to the point where the rest of the DNA can be ignored, and so the viral gene's presence can be easily detected, for example, by using a hybridization assay.

REQUIREMENTS

A basic PCR set up requires several components and reagents. These components include:

- DNA template that contains the DNA region (target) to be amplified.
- Two primers (foreword primer and reverse primer) that are complementary to the 3' ends of each of the sense and anti-sense strand of the DNA target.
- Taq polymerase or another DNA polymerase with a temperature optimum at around 70 °C.
- Deoxynucleoside triphosphates (dNTPs),

- Buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
- Bivalent cations, magnesium or manganese ions; generally Mg^{2+} is used, but Mn^{2+} can be utilized for PCR-mediated DNA mutagenesis, as higher Mn^{2+} concentration increases the error rate during DNA synthesis.
- Monovalent cation potassium ions.

The PCR is commonly carried out in a reaction volume of 10–200 µl in small reaction tubes (0.2–0.5 ml volumes) in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction.