

# Molecular techniques nucleic acid based, PCR and hybridisation

Gulshan Kumar

# What is PCR?

- **Polymerase chain reaction (PCR)** is a common laboratory technique used to make many copies (millions or billions!) of a particular region of DNA. This DNA region can be anything the experimenter is interested in. For example, it might be a gene whose function a researcher wants to understand, or a genetic marker used by forensic scientists to match crime scene DNA with suspects.
- Typically, the goal of PCR is to make enough of the target DNA region that it can be analyzed or used in some other way. For instance, DNA amplified by PCR may be sent for sequencing, visualized by gel electrophoresis, or cloned into a plasmid for further experiments.

- **Taq polymerase**
- Like DNA replication in an organism, PCR requires a DNA polymerase enzyme that makes new strands of DNA, using existing strands as templates. The DNA polymerase typically used in PCR is called **Taq polymerase**, after the heat-tolerant bacterium from which it was isolated (*Thermus aquaticus*).

- PCR primers are short pieces of single-stranded DNA, usually around 20-200 nucleotides in length. Two primers are used in each PCR reaction, and they are designed so that they flank the target region (region that should be copied).

- **The steps of PCR**
- The key ingredients of a PCR reaction are *Taq* polymerase, primers, template DNA, and nucleotides (DNA building blocks). The ingredients are assembled in a tube, along with cofactors needed by the enzyme, and are put through repeated cycles of heating and cooling that allow DNA to be synthesized.
- The basic steps are:
- **Initial denaturation (94 - 96°C)** – All the double stranded DNA template is melted to form single stranded at the beginning of reaction.
- **Denaturation (94 - 96 °C)**: Heat the reaction strongly to separate, or denature, the DNA strands. This provides single-stranded template for the next step.

# Blotting

- **Introduction**
- Blotting is technique in which nucleic acids i.e., RNA and DNA or proteins are transferred onto a specific membrane. This membrane may be nitrocellulose PVDF or nylon membrane. This process can be done just after the gel electrophoresis, by transferring the molecules from the gel onto the surface of blotting membrane. But sometimes it can be done by directly transferring the molecules onto the membrane. And then we can visualize these transferring molecules by using staining. Examples: Ethidium bromide, Crystal violet, Safranin and Osmium tetroxide etc

- *Types of Blotting*
- There are basically 4 types of blotting:
  - 1) Southern blotting
  - 2) Western blotting
  - 3) Northern blotting
  - 4) Eastern blotting

- **Southern blotting**
- Southern blotting is named after Edward M. Southern. This method is used for analysis of DNA sequences. It involves the following steps:
- **Western blotting**
- Western blotting is named after W. Neal Burnette. This method is used for detection and analysis of protein in a given sample . It involves the following steps:
- **Northern blotting**
- Northern blotting is given by Alwine. This method is used to analyse and detection of RNA in a sample