Polymerase Chain Reaction (PCR) Principles and Applications

The **polymerase chain reaction (PCR)** is a rapid, highly sensitive, and specific **in vitro** enzymatic technique used to amplify targeted DNA sequences exponentially. It was developed by Kary Mullis in 1983 and revolutionized molecular biology by enabling the detection and analysis of minute amounts of DNA.

PCR is essential in clinical diagnostics, research, forensic science, and infectious disease detection (e.g., SARS-CoV-2 causing COVID-19).

Key Concepts and Definitions

- **PCR:** Amplifies specific DNA sequences using repeated thermal cycles that enable DNA denaturation, primer annealing, and extension by DNA polymerase.
- **Reverse Transcription (RT):** The enzymatic synthesis of complementary DNA (cDNA) from an RNA template using **reverse transcriptase** .
- **Reverse Transcription PCR (RT-PCR):** A technique that converts RNA into cDNA (via reverse transcription) before amplifying the DNA target by PCR. Used for detecting RNA viruses (e.g., SARS-CoV-2) and measuring gene expression.
- **Reverse Genetics:** Creation of viral RNA genomes from cloned DNA to study viral functions.
- **Taq DNA Polymerase:** A thermostable enzyme from *Thermus aquaticus* bacteria that synthesizes new DNA strands during PCR and withstands repeated heating cycles.

DNA Structure Refresher

- DNA is a **double helix** formed by two complementary strands.
- Each strand consists of nucleotides made of:
    - A **nitrogenous base** (adenine [A], thymine [T], cytosine [C], guanine [G])
    - A **deoxyribose sugar**
    - A **phosphate group**
- Base pairing occurs via hydrogen bonds: A pairs with T, C pairs with G.

PCR Mechanism: Step-by-Step

PCR relies on **thermal cycling** , involving repeated cycles of three main steps to exponentially amplify DNA:

1. Denaturation (90–98°C)

- Double-stranded DNA melts into single strands by breaking hydrogen bonds.

2. Annealing (37–65°C)

- Synthetic **oligonucleotide primers** bind (anneal) to complementary sequences flanking

the target DNA on opposite strands.
- Primers are short, specific DNA sequences that define the region to be amplified.

3. Extension (72°C)

- **Taq DNA polymerase** extends primers by adding nucleotides to the 3'-end, synthesizing new DNA strands complementary to the template.

Cycle repetition:

- Typically 20–30 cycles are run.
- Each cycle doubles the amount of target DNA, resulting in exponential amplification (up to billions of copies).

Detection of PCR Products

- Amplified DNA (amplicons) can be visualized by:
  - **Gel electrophoresis:** Separation by size in agarose gel.
  - **Southern blotting:** Transfer to membrane and hybridization with labeled probes.
  - **Real-time PCR (qPCR):** Monitors DNA amplification in real-time using fluorescent dyes or probes.

Real-Time PCR (qPCR)

- Measures DNA concentration **during** amplification.
- Fluorescent signal increases as the product accumulates.
- The **Cycle threshold (Ct)** is the PCR cycle number when fluorescence exceeds background; lower Ct = higher initial DNA quantity.
- Enables **quantitative** analysis of nucleic acid concentration.

Reverse Transcription PCR (RT-PCR)

RT-PCR is used for RNA targets, such as viral RNA or gene expression analysis.

Steps:

1. **Reverse Transcription:**
   - RNA is converted to complementary DNA (cDNA) using **reverse transcriptase** enzymes (e.g., from avian myeloblastosis virus or Moloney murine leukemia virus).
2. **PCR Amplification:**
   - The cDNA is amplified by conventional PCR cycles.

RT-PCR Variants

- **One-step RT-PCR:**
  - Both reverse transcription and PCR amplification occur in a single tube.
  - Minimizes contamination and variability but may be less flexible.

- **Two-step RT-PCR:**
  - Reverse transcription and PCR amplification performed separately.
  - Allows storage of cDNA and optimization of each step independently.
  - Preferred for higher sensitivity and specificity.

Quantification in RT-PCR

- Quantitative RT-PCR (qRT-PCR) assesses gene expression by measuring cDNA amplification in real-time.
- **Ct values** are compared to standard curves or reference genes.
- Amplification efficiency must be validated to ensure accurate quantification.

Clinical and Research Applications

- Detection of pathogens (e.g., SARS-CoV-2, HIV, Hepatitis viruses).
- Genetic mutation analysis.
- Gene expression profiling.
- Forensic DNA fingerprinting.
- Prenatal genetic diagnosis.

High-Yield Notes:

- PCR requires:
  - Two primers flanking the target DNA sequence.
  - Thermostable DNA polymerase (e.g., Taq polymerase).
  - Thermal cycling for denaturation, annealing, extension.
- RT-PCR first converts RNA to cDNA, then amplifies DNA; crucial for RNA virus detection.
- Real-time PCR (qPCR) allows quantification by fluorescence, with Ct value inversely proportional to initial nucleic acid amount.
- Taq polymerase tolerates repeated heating, making automated thermal cycling possible.
- Annealing temperature depends on primer melting temperature (Tm) and affects specificity.
- PCR can detect as low as a single DNA molecule.
- PCR product detection methods: gel electrophoresis, Southern blot, or fluorescence in qPCR.
- PCR efficiency affects quantification; proper controls and standard curves are essential.
- RT-PCR is gold standard for COVID-19 diagnostic testing.