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PHARMACEUTICAL BIOTECHNOLOGY

UNIT 2

TOPIC :

- d) Brief introduction to PCR

Polymerase Chain Reaction (PCR)

- Polymerase Chain Reaction (PCR) is a laboratory technique used to produce millions of copies of a specific DNA segment in a short period of time.
- PCR is also called DNA amplification technology because it increases the number of copies of target DNA.
- PCR was developed by Kary Mullis in 1983. He received the Nobel Prize in Chemistry in 1993 for this invention.

Principle of PCR

PCR works on the principle of DNA replication carried out artificially in the laboratory.

The process involves:

1. Separation of DNA strands by heating.
2. Binding of primers to target DNA sequence.
3. Synthesis of new DNA strands by DNA polymerase enzyme.

Repeated cycles lead to exponential amplification of DNA.

Components of PCR

1. Template DNA

- DNA molecule to be amplified.
- Contains the target sequence.

2. Primers

- Short single-stranded DNA sequences.
- Bind to complementary DNA regions.
- Provide starting point for DNA synthesis.

Two primers are generally used:

- Forward primer
- Reverse primer

3. DNA Polymerase

Heat-stable enzyme that synthesizes DNA.

Common example:

- Taq Polymerase

Source:

- *Thermus aquaticus* bacteria

Function:

- Adds nucleotides to form new DNA strands.

4. dNTPs (Deoxynucleotide Triphosphates)

Building blocks of DNA:

- dATP
- dTTP
- dGTP
- dCTP

Function:

Provide nucleotides required for DNA synthesis.

5. Buffer Solution

Maintains suitable conditions for enzyme activity.

Functions:

- Maintains pH
- Provides proper ionic environment

6. Magnesium Ions (Mg^{2+})

Acts as cofactor for DNA polymerase enzyme.

Steps of PCR

PCR involves three major steps:

1. Denaturation

Temperature:

94–98°C

Process:

- Double-stranded DNA separates into two single strands.
- Hydrogen bonds between DNA strands break.

Purpose:

Prepare DNA template for primer attachment.

2. Annealing

Temperature:

50–65°C

Process:

- Primers bind to complementary DNA sequences.

Purpose:

Provides starting point for DNA synthesis.

3. Extension (Elongation/Synthesis)

Temperature:

72°C

Process:

- DNA polymerase adds nucleotides.
- New complementary DNA strand is synthesized.

DNA synthesis occurs in:

5′ → 3′ direction

PCR Cycle

One PCR cycle includes:

Denaturation → Annealing → Extension

These steps are repeated **25–35 cycles**.

Result:

Exponential amplification of target DNA.

Example:
1 DNA molecule → Millions of DNA copies

Diagrammatic Flow of PCR

DNA Template
↓
Denaturation (94–98°C)
↓
Annealing (50–65°C)
↓
Extension (72°C)
↓
Repeated Cycles (25–35)
↓
Millions of DNA Copies

Advantages of PCR

1. Very rapid process
2. Highly sensitive
3. Highly specific
4. Requires very small DNA sample
5. Produces large amount of DNA
6. Accurate detection method

Applications of PCR

1. Medical Applications

PCR is used for diagnosis of diseases.
Examples:

- COVID-19
- Tuberculosis (TB)
- HIV infection
- Hepatitis
- Genetic disorders

Uses:

- Early disease diagnosis

- Detection of mutations
- Prenatal diagnosis

2. Forensic Science

Applications:

- DNA fingerprinting
- Crime investigation
- Identification of criminals
- Paternity testing

3. Agriculture

Applications:

- Detection of genetically modified organisms (GMOs)
- Plant disease identification
- Crop improvement programs

4. Environmental and Microbiology Studies

Applications:

- Detection of microorganisms in water
- Soil microbial analysis
- Environmental pollution monitoring

5. Research Applications

Used in:

- Molecular biology research
- Genetic engineering
- Recombinant DNA technology
- DNA sequencing studies

Limitations of PCR

1. Risk of contamination
2. Requires specialized equipment
3. Expensive technique
4. Requires trained personnel