

DNA Electrophoresis

- DNA electrophoresis is a laboratory technique used to separate DNA fragments based on their size.
- It is commonly performed using agarose gel.
- DNA molecules move in an electric field because they carry a negative charge.

Principle of DNA Electrophoresis

- DNA fragments move from the negative electrode toward the positive electrode.
- Smaller DNA fragments move faster through the gel pores than larger fragments.
- This results in separation of DNA fragments according to size.

Components Required



Agarose Gel



Buffer
Solution



DNA Sample



Loading Dye



DNA Marker



Power Supply

1. Agarose Gel

- It acts as a molecular sieve for DNA separation.

2. Buffer Solution

- It maintains pH and conducts electricity.

3. DNA Sample

- The DNA fragments to be analyzed.

4. Loading Dye

- It helps visualize sample loading and movement.

5. DNA Marker or Ladder

- It contains DNA fragments of known sizes for comparison.

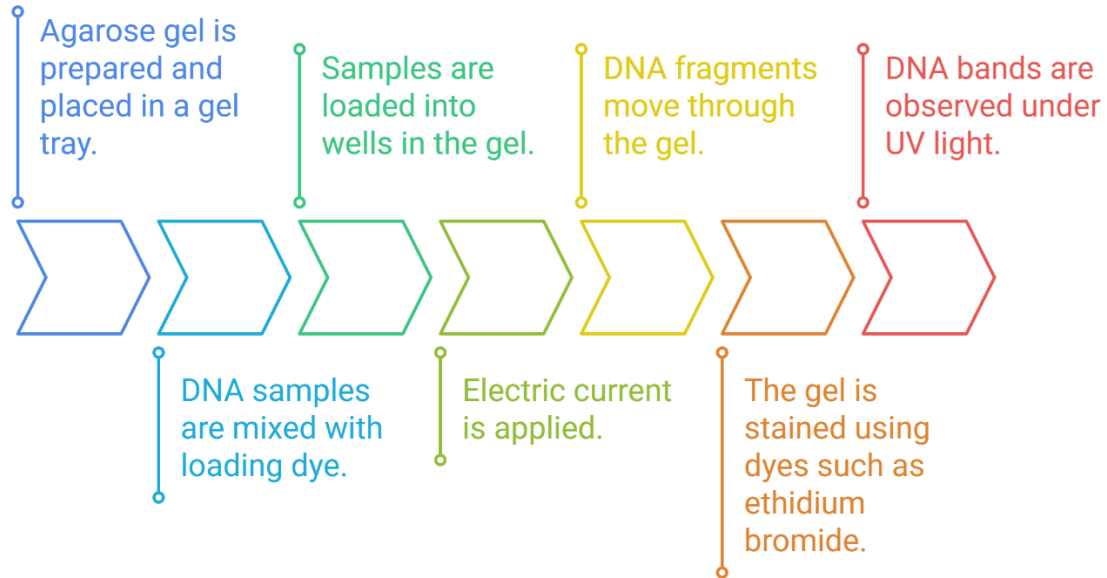
6. Power Supply

- It provides electric current.

Steps of DNA Electrophoresis

1. Agarose gel is prepared and placed in a gel tray.
2. DNA samples are mixed with loading dye.
3. Samples are loaded into wells in the gel.
4. Electric current is applied.
5. DNA fragments move through the gel.

6. The gel is stained using dyes such as ethidium bromide.
7. DNA bands are observed under UV light.



Types of Electrophoresis

1. Agarose Gel Electrophoresis

- Used mainly for DNA and RNA separation.

2. Polyacrylamide Gel Electrophoresis (PAGE)

- Used for smaller DNA fragments and proteins.

Applications of DNA Electrophoresis

- It is used in DNA fingerprinting.
- It helps analyze PCR products.
- It is used in gene cloning and sequencing.
- It helps detect genetic mutations.

PCR (Polymerase Chain Reaction)

- PCR is a technique used to amplify a specific DNA sequence.
- It can produce millions of copies of a DNA fragment in a short time.

Principle of PCR

- PCR works by repeated cycles of DNA denaturation, primer binding, and DNA synthesis.
- A heat-stable DNA polymerase enzyme is used.

Components Required for PCR

1. Template DNA

- The DNA containing the target sequence.

2. Primers

- Short DNA sequences that bind to target DNA.

3. DNA Polymerase

- Usually Taq polymerase is used.

4. Nucleotides (dNTPs)

- Building blocks for new DNA synthesis.

5. Buffer Solution

- Maintains suitable conditions for the reaction.

Steps of PCR

1. Denaturation

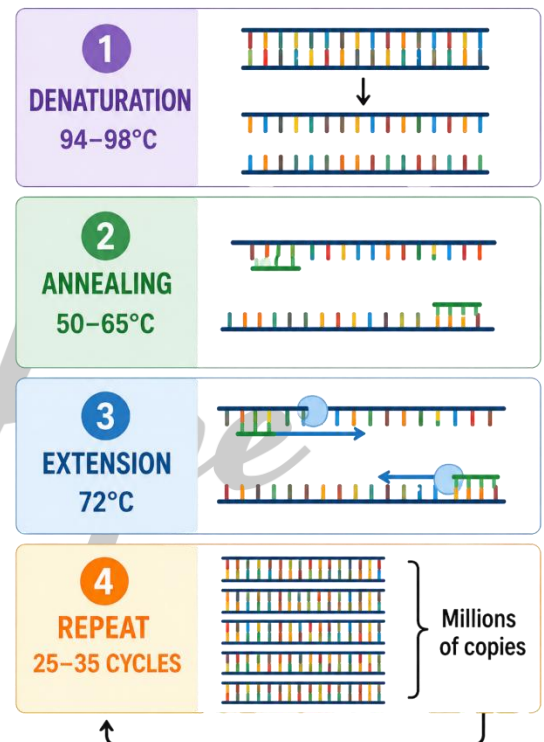
- DNA strands separate at high temperature.

2. Annealing

- Primers bind to complementary DNA sequences.

3. Extension

- DNA polymerase synthesizes new DNA strands.



Types of PCR

1. Conventional PCR

- Used for general DNA amplification.

2. Reverse Transcription PCR (RT-PCR)

- Used to convert RNA into complementary DNA (cDNA) before amplification.

3. Real Time PCR (qPCR)

- Used to measure DNA amplification in real time.

Reverse Transcription PCR (RT-PCR)

- RT-PCR is used for studying gene expression.
- RNA is first converted into cDNA using reverse transcriptase enzyme.
- The cDNA is then amplified by PCR.

Applications of RT-PCR

- Detection of RNA viruses.

- Analysis of gene expression.
- Research in molecular biology.

Real Time PCR (qPCR)

- Real time PCR measures amplified DNA during each PCR cycle.
- Fluorescent dyes or probes are used for detection.

Types of Real Time PCR Detection

1. SYBR Green Method

- Uses fluorescent dye that binds double-stranded DNA.

2. TaqMan Probe Method

- Uses fluorescent probes specific to target DNA.

Applications of Real Time PCR

- Quantification of gene expression.
- Detection of infectious diseases.
- Cancer diagnostics.

Applications of PCR

- Diagnosis of genetic diseases.
- Detection of pathogens.
- Forensic analysis.
- DNA cloning.

Gene Sequencing

- Gene sequencing is the process of determining the exact order of nucleotides in DNA.
- It helps identify genetic information and mutations.

Principle of Gene Sequencing

- The nucleotide sequence of DNA is determined by analyzing DNA fragments generated during the sequencing reaction.

Types of Gene Sequencing

1. Sanger Sequencing

- It is based on chain termination method.
- It is suitable for sequencing small DNA fragments.

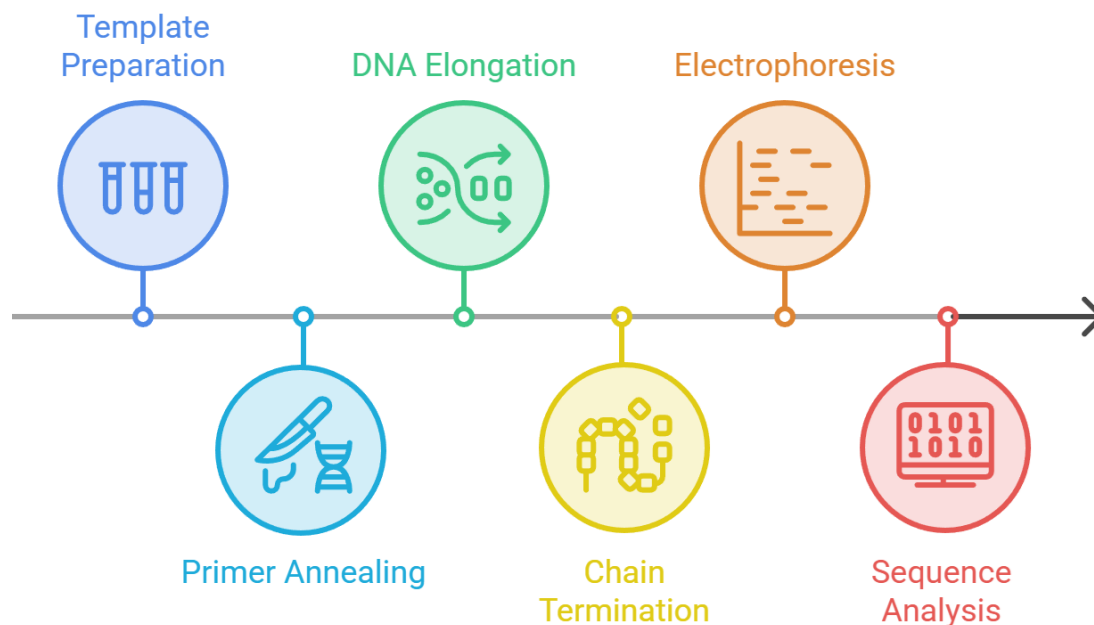
2. Next Generation Sequencing (NGS)

- It allows sequencing of millions of DNA fragments simultaneously.
- It is faster and high throughput.

Sanger Sequencing

- This method uses dideoxynucleotides to stop DNA synthesis.
- DNA fragments of different lengths are produced.
- The sequence is determined by analyzing fragment sizes.

Steps of Sanger Sequencing



1. DNA template is prepared.
2. Primer binds to the template.
3. DNA synthesis occurs using DNA polymerase.
4. Dideoxynucleotides terminate DNA elongation.
5. DNA fragments are separated by electrophoresis.
6. The nucleotide sequence is analyzed.

Next Generation Sequencing (NGS)

- NGS sequences many DNA fragments at the same time.
- It generates massive amounts of genetic data.

Steps of NGS

1. DNA is fragmented.
2. Adapters are added to fragments.
3. DNA fragments are amplified.

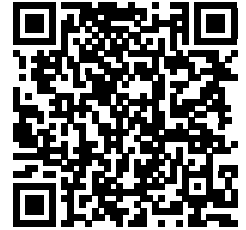


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