Southern Blot

IV

Southern blotting is a laboratory technique used to detect a specific DNA sequence in a blood or tissue sample. A Restriction enzyme is used to cut a sample of DNA into fragments that are separated using gel electrophoresis. The DNA fragments are transferred out of the gel to the surface of a membrane.

Step - 1 - DNA digestion

2 - Gel electrophoresis

3. - Blotting

4 - Probe labelling

5 - Hybridization & washing

6 - Detection.

Principle of Southern blotting :-

Southern blotting is based on the principle of separation of DNA fragments by gel electrophoresis followed by the identification by Labeled probe hybridization. The DNA fragments are separated based on their size and charge during electrophoresis.

Applications :-

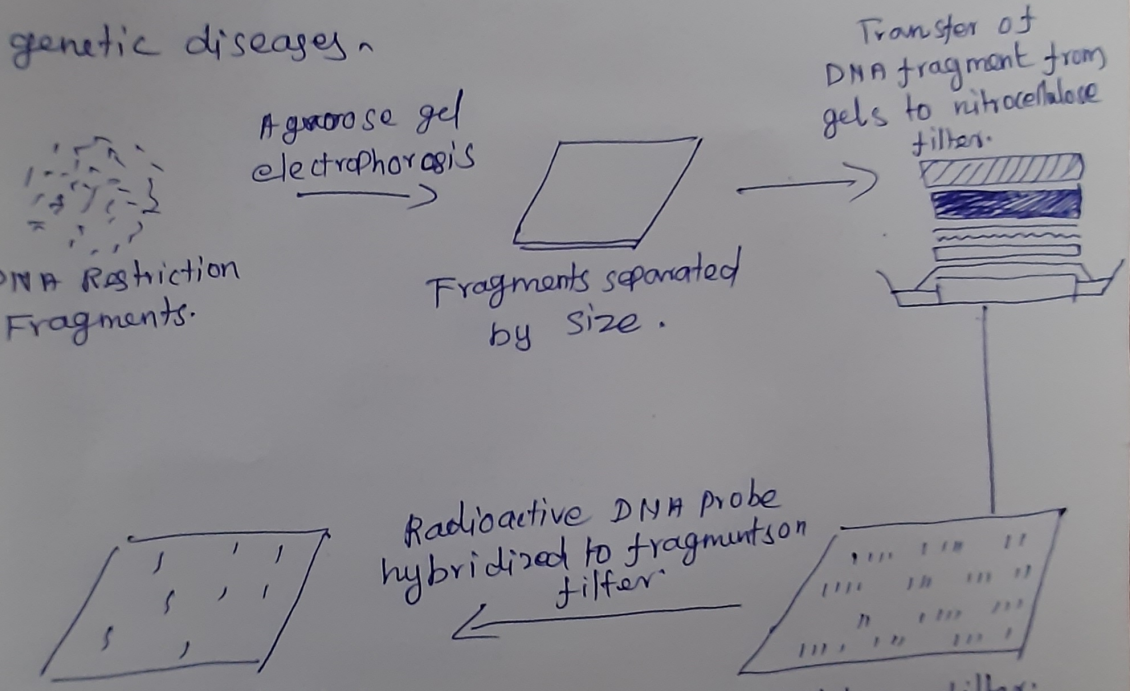
1. Identifying specific DNA in a DNA sample
2. Preparation of RFLP fragment length polymorphism maps.

3. Detection of mutations, deletions or gene rearrangements in DNA

4. For criminal identification and DNA Finger printing.

5. mapping of restriction sites.

6. prognosis of cancer and Prenatal diagnosis of genetic diseases.



Southern Blotting Techniques.

Western blotting.

unit - IV

The Western blot or western blotting is a widely used analytical technique is molecular biology and immunogenetics to detect specific protein in a sample of tissue homogenate or extract.

Principle :.

In western blotting target protein are transferred to a hydrophobic membrane after SDS - PAGE and detected using specific antibodies. After SDS - PAGE a membrane is placed on the gel, to which the separated Protein in the gel are electrophoretically transferred.

Western Blot procedures :.

1. WB buffers preparation
2. Samples preparation.
3. Gel electrophoresis
4. protein transfer
5. membrane blocking
6. antibody incubation
7. WB detection and imaging
8. Data analysis.

western Blot Applications :.

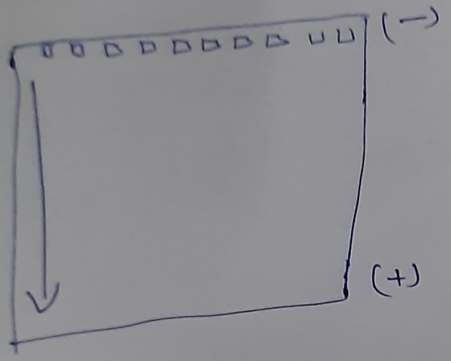
1. Detecting phosphorylation states of protein.
2. Detecting changes in protein levels across treatment groups.

3. Detecting changes in protein levels across time points.

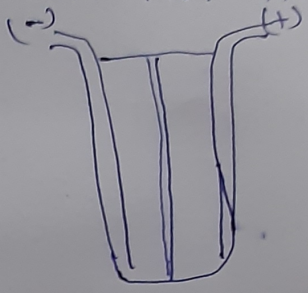
4. Detecting Truncated Isoforms of proteins.

5. Detecting tagged proteins.

① Load and separate protein samples on SDS-PAGE

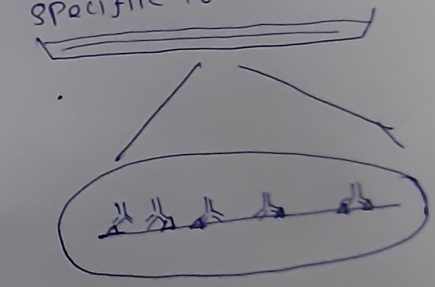


② Electrophoretically Transfer fractionated proteins onto PVDF Membrane.



③ Block the membrane with neutral protein (BSA (or) milk casein)

④ . incubate the membrane with primary antibody specific to target protein.



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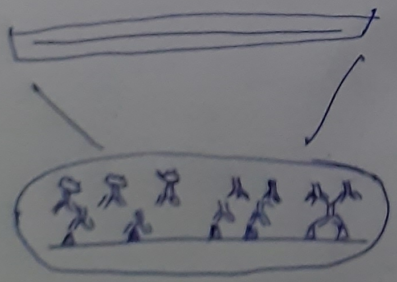


Incubate the membrane with HRP-labeled secondary antibody specific to primary antibody

⑥

incubate the blot with chemiluminescent HRP substrate and expose to film.

ELISA

An enzyme-linked immunosorbent assay, also called Elisa or EIA is a test that detects and measures antibodies in your blood. This test can be used to determine if you have antibodies related to certain infectious conditions.

procedure:-

1. Antibody coating:-
   specific capture antibody is immobilized on high protein binding plates by overnight incubation. plates are blocked with irrelevant protein (eg) albumin.

2. Protein capture:-
   samples and standard dilutions are added to the walls and will be captured by the bound antibodies.

3. Detection antibody:
   specific biotinylated detection antibody is added to the wells to enable detection of the captured protein.

4. streptavidin enzyme conjugate:
   streptavidin conjugated with alkaline phosphatase or horseradish peroxidase and will bind to the biotinylated antibody.

5. Addition of substrate:-
   colorimetric substrate is added to the wells and will form a colored solution when catalyzed by the enzyme.

6. Analysis:-

Absorbance is measured in a ELISA reader and the amount of protein in the samples is determined.

ELISA test may be used to diagnose:-

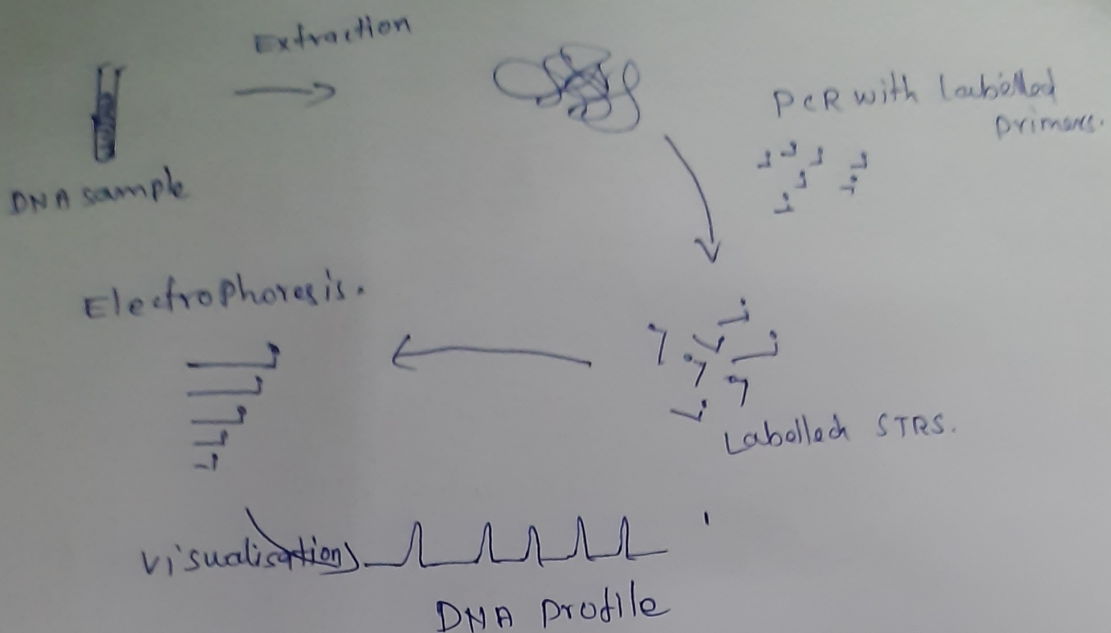
- HIV
- Lyme disease
- Pernicious anemia
- Rocky mountain spotted fever
- rotavirus
- squamous cell carcinoma
- syphillis
- toxoplasmosis.
- zika virus.

DNA Finger printing.

DNA Finger printing is a laboratory techniques used to establish a link between biological evidence and a suspect in a criminal investigation. A DNA sample taken from a crime scene is compared with a DNA sample from a suspect. If the two DNA profiles are a match, then the evidence came from the suspect.

PROCESS OF DNA Finger printing:-

1. The process begins with a blood or cell sample from which the DNA is extracted.
2. The DNA is cut into fragments using a restriction enzyme. The fragments are then separated into bands by electrophoresis through an agarose gel.
3. The DNA band pattern is transferred to a nylon membrane.
4. A Radio active DNA probe is introduced. The DNA probe binds to specific DNA sequences on the nylon membrane
5. The excess probe material is washed away leaving unique DNA band pattern.
6. The Radioactive DNA pattern is transferred to x ray film by direct exposure. When developed, the resultant visible pattern is the DNA Finger print.



Application:

1. It is used in forensic Science to identify potential crime suspects.
2. establish paternity and family relationship
3. used to identify and protect the commercial varieties of crops and livestock
4. used to find out the evolutionary history of an organism and trace out the linkage between groups of various organisms.

Bio sensors :-

Biosensors are devices used to detect the presence or concentration of a biological analyte, such as a Biomolecule, a biological structure or a micro organism. Biosensors consist of three parts: a component that recognizes the analyte and produces a signal a signal transducer, and a reader device.

Principle :-

Biosensors are operated based on the principle of signal transduction. These components include a bio recognition element, a biotransducer and an electronic system composed of a display, processor and amplifier. The bio-recognition element, essentially a bio receptor, is allowed to interact with a specific analyte.

Applications of Biosensors :-

1. Food Analysis
2. Study of biomolecules and their interaction
3. Drug Development.
4. crime detection
5. medical diagnosis
6. environmental field monitoring
7. Quality control
8. Industrial Process control
9. Detection systems for biological warfare agents.
10. manufacturing of Pharmaceuticals and replacement organs.