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Lab 8 **Nucleic acid hybridization**

Southern Blot

Introduction:

Nucleic acid hybridization is a basic technique in molecular biology which takes advantage of the ability of individual single-stranded nucleic acid molecules to form double-stranded molecules. According to Watson-Crick base pairing, adenine binds with thymine and guanine binds with cytosine by hydrogen bonding.

The analytical technique that involves the transfer of a specific DNA, RNA or a protein separated on gel to a carrier membrane, for their detection or identification is termed as blotting. The process of transfer of the denatured fragments out of the gel and onto a carrier membrane makes it accessible for analysis using a probe or antibody. Depending upon the substance to be separated, blotting techniques may be – Southern blot, Northern blot or Western blot which separates DNA, RNA and proteins respectively.

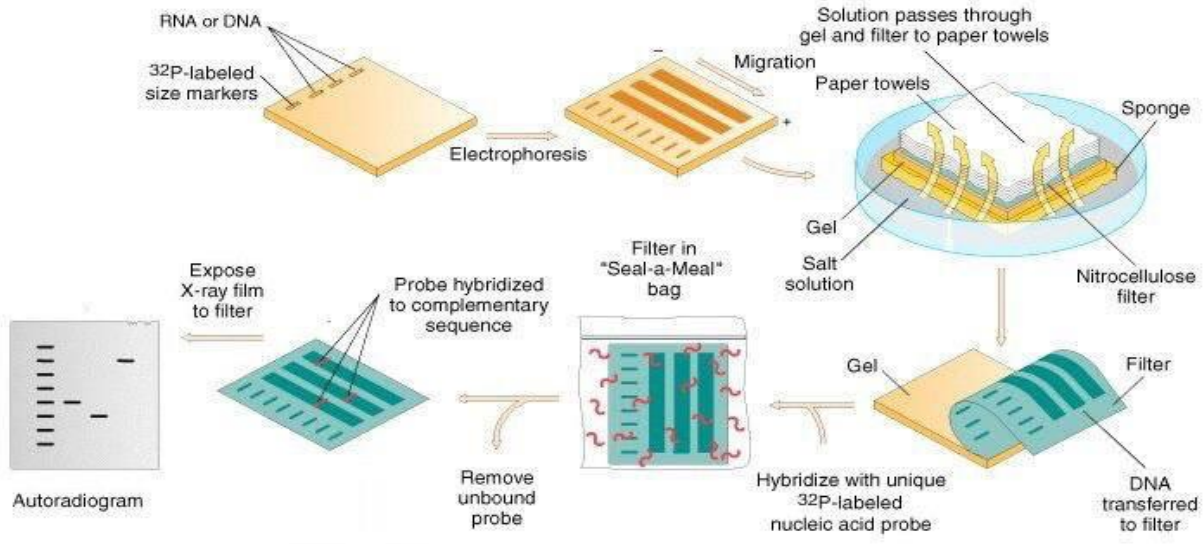
Southern Blot is the analytical technique used in molecular biology, immunogenetics and other molecular methods to detect or identify DNA of interest from a mixture of DNA sample or a specific base sequence

within a strand of DNA. The technique was developed by a molecular biologist E.M. Southern in 1975 for analyzing the related genes in a DNA restriction fragment and thus named as Southern blotting in his honour.

Principle of Southern Blot

The process involves the transfer of electrophoresis-separated DNA fragments to a carrier membrane which is usually nitrocellulose and the subsequent detection of the target DNA fragment by probe hybridization. Hybridization refers to the process of forming a double-stranded DNA molecule between a single-stranded DNA probe and a single-stranded target DNA. Since the probe and target DNA are complementary to each other, the reaction is specific which aids in the detection of the specific DNA fragment.

Steps Involved in Southern Blot



1. Extraction and purification of DNA from cells

DNA is first separated from target cells following standard methods of genomic DNA extraction and then purified.

2. Restriction Digestion or DNA Fragmentation

Restriction endonucleases are used to cut high-molecular-weight DNA strands into smaller fragments. One or more restriction enzymes can be used to achieve such fragments.

3. Separation by Electrophoresis

The separation may be done by agarose gel electrophoresis in which the negatively charged DNA fragments move towards the positively charged anode, the distance moved depending upon its size.

4. Depurination

Partial depurination is done by the use of dilute HCl which promotes higher efficiency transfer of DNA fragments by it breaking down into smaller pieces.

5. Denaturation

DNA is then denatured with a mild alkali such as an alkaline solution of NaOH. This causes the double stranded DNA to become single-stranded, making them suitable for hybridization. DNA is then neutralized with

NaCl to prevent re-hybridization before addition of the probe.

6. Blotting

The denatured fragments are then transferred onto a nylon or nitrocellulose filter membrane which is done by placing the gel on top of a buffer saturated filter paper, then laying nitrocellulose filter membrane on the top of gel. Finally some dry filter papers are placed on top of the membrane. Fragments are pulled towards the nitrocellulose filter membrane by capillary action and result in the contact print of the gel.

7. Baking

The nitrocellulose membrane is removed from the blotting stack, and the membrane with single stranded DNA bands attached on to it is baked in a vacuum or regular oven at 80 °C for 2-3 hours or exposed to ultraviolet radiation to permanently attach the transferred DNA onto the membrane.

8. Hybridization

The membrane is then exposed to a hybridization probe which is a single DNA fragment with a specific sequence whose presence in the target DNA is to be determined. The probe DNA is labeled so that it can be detected, usually by incorporating radioactivity or tagging the molecule with a fluorescent or chromogenic dye. 9. Washing of unbound probes

After hybridization, the membrane is thoroughly washed with a buffer to remove the probe that is bound nonspecifically or any unbound probes present.

10. Autoradiograph

The hybridized regions are detected autoradiographically by placing the nitrocellulose membrane in contact with a photographic film which shows the hybridized DNA molecules. The pattern of hybridization is visualized on X-ray film by autoradiography in case of a radioactive or fluorescent probe is used or by the development of color on the membrane if a chromogenic detection method is used.

Applications of Southern Blot

1- Identifying specific DNA in a DNA sample.

2- Preparation of RFLP (Restriction Fragment Length Polymorphism) maps

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

4- For criminal identification and DNA fingerprinting (VNTR)

5-Detection and identification of trans gene in transgenic individual

6- Mapping of restriction sites

7- For diagnosis of infectious diseases

8- Prognosis of cancer and prenatal diagnosis of genetic diseases

9-Determination of the molecular weight of a restriction fragment and to measure relative amounts in different samples.