Aim: To study various blotting techniques- Southern, Northern and Western.

Southern blotting

It 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 method is named after the British biologist Edwin Southern, who first published it in 1975.

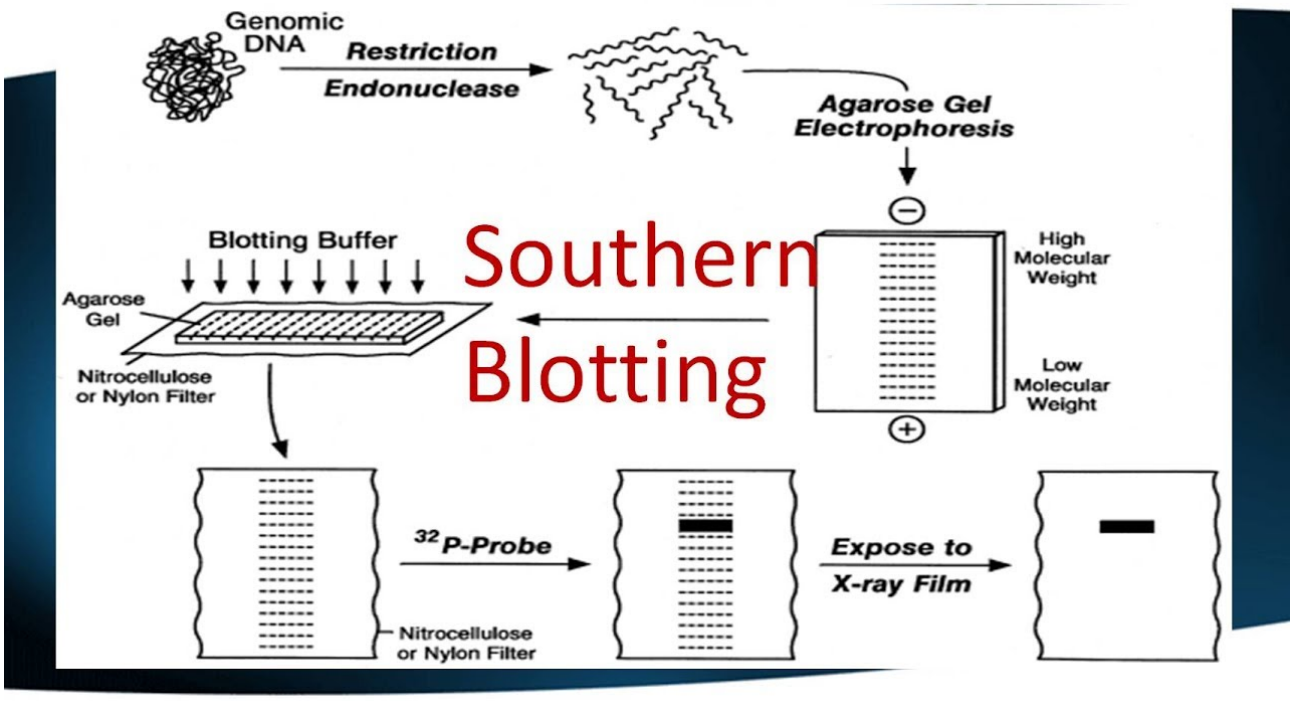
Procedure

1. Restriction endonucleases are used to cut high-molecular-weight DNA strands into smaller fragments.
2. The DNA fragments are then electrophoresed on an agarose gel to separate them by size.
3. If some of the DNA fragments are larger than 15 kb, then prior to blotting, the gel may be treated with an acid, such as dilute HCl. This depurinates the DNA fragments, breaking the DNA into smaller pieces, thereby allowing more efficient transfer from the gel to membrane.
4. If alkaline transfer methods are used, the DNA gel is placed into an alkaline solution (typically containing sodium hydroxide) to denature the double-stranded DNA. The denaturation in an alkaline environment may improve binding of the negatively charged thymine residues of DNA to a positively charged amino groups of membrane, separating it into single DNA strands for later hybridization to the probe.
5. A sheet of nitrocellulose (or, alternatively, nylon) membrane is placed on top of (or below, depending on the direction of the transfer) the gel. Pressure is applied evenly to the gel (either using suction, or by placing a stack of paper towels and a weight on top of the membrane and gel), to ensure good and even contact between gel and membrane.
6. The membrane is then baked in a vacuum or regular oven at 80 °C for 2 hours or exposed to ultraviolet radiation to permanently attach the transferred DNA to the membrane.
7. The membrane is then exposed to a hybridization probe—a single DNA fragment with a specific sequence whose presence in the target DNA is to be determined. The probe DNA is labelled so that it can be detected, usually by incorporating radioactivity or tagging the molecule with a fluorescent or chromogenic dye.
8. After hybridization, excess probe is washed from the membrane (typically using SSC buffer), and the pattern of hybridization is visualized on X-ray film by autoradiography in the case of a radioactive or fluorescent probe, or by development of colour on the membrane if a chromogenic detection method is used.

Applications

- Southern blotting transfer may be used for homology-based cloning on the basis of amino acid sequence of the protein product of the target gene.

- Oligonucleotides are designed so that they are similar to the target sequence. The oligonucleotides are chemically synthesized, radiolabeled, and used to screen a DNA library.
- Sequences that hybridize with the hybridization probe are further analysed, for example, to obtain the full length sequence of the targeted gene.
- Southern blotting can also be used to identify methylated sites in particular genes.



Northern blotting

The **northern blot**, or RNA blot is a technique used in molecular biology research to study gene expression by detection of RNA. The northern blot technique was developed in 1977 by James Alwine, David Kemp, and G Stark at Stanford University. Northern blotting takes its name from its similarity to the first blotting technique, the Southern blot. The major difference is that RNA, rather than DNA, is analyzed in the northern blot.

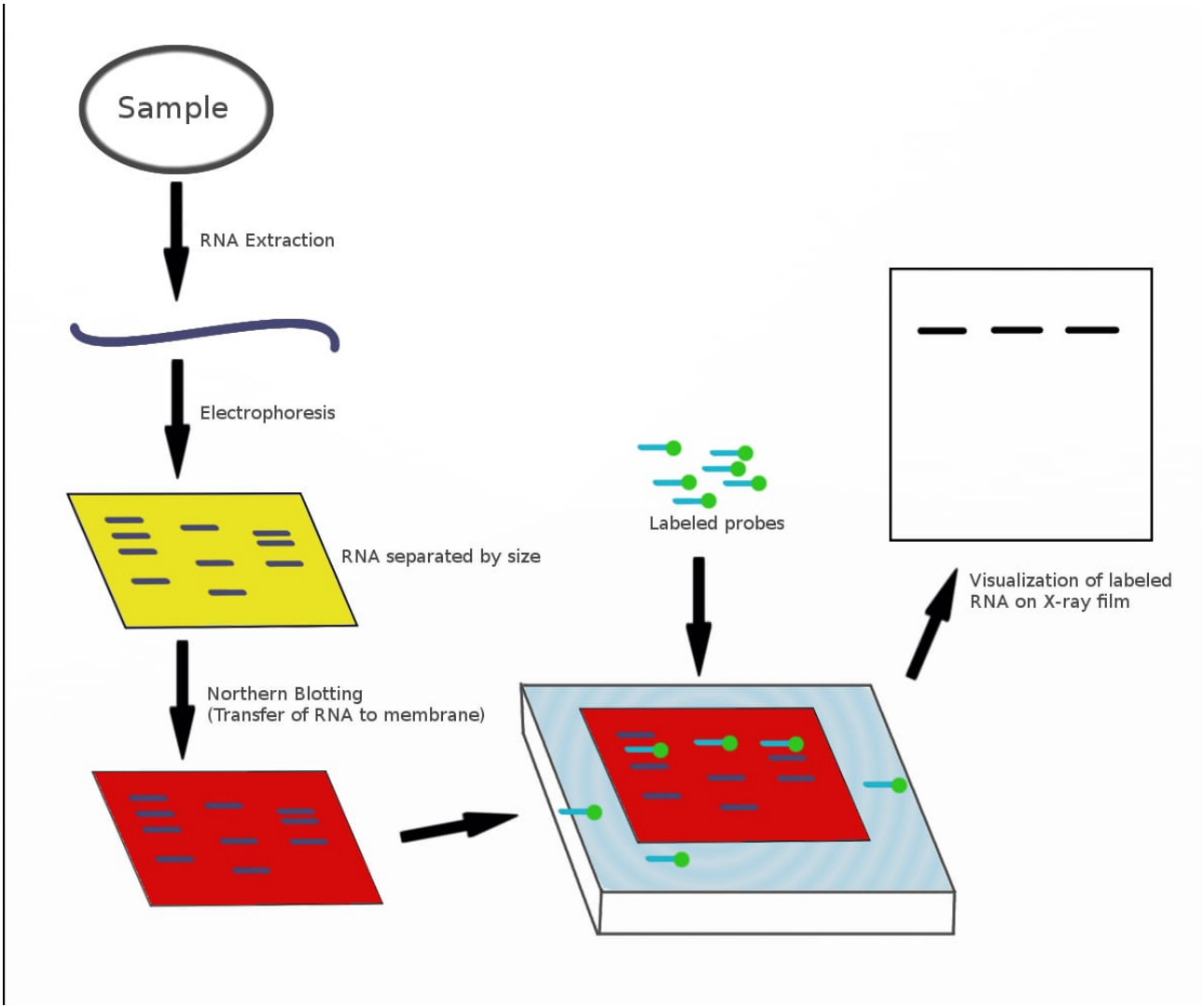
Procedure

- starts with extraction of total RNA from a homogenized tissue sample or from cells.
- Eukaryotic mRNA can then be isolated through the use of oligo (dT) cellulose chromatography to isolate only those RNAs with a poly(A) tail.
- RNA samples are then separated by gel electrophoresis. Since the gels are fragile and the probes are unable to enter the matrix, the RNA samples, now separated by size, are transferred to a nylon membrane through a capillary or vacuum blotting system.

- A nylon membrane with a positive charge is the most effective for use in northern blotting since the negatively charged nucleic acids have a high affinity for them.
- The transfer buffer used for the blotting usually contains formamide because it lowers the annealing temperature of the probe-RNA interaction, thus eliminating the need for high temperatures, which could cause RNA degradation.
- Once the RNA has been transferred to the membrane, it is immobilized through covalent linkage to the membrane by UV light or heat.
- After a probe has been labeled, it is hybridized to the RNA on the membrane. Experimental conditions that can affect the efficiency and specificity of hybridization include ionic strength, viscosity, duplex length, mismatched base pairs, and base composition.
- The membrane is washed to ensure that the probe has bound specifically and to prevent background signals from arising.
- The hybrid signals are then detected by X-ray film and can be quantified by densitometry.

Applications

- With northern blotting it is possible to observe cellular control over structure and function by determining the particular gene expression rates during differentiation and morphogenesis, as well as in abnormal or diseased conditions.



- Northern blotting allows one to observe a particular gene's expression pattern between tissues, organs, developmental stages, environmental stress levels, pathogen infection, and over the course of treatment.
- The technique has been used to show overexpression of oncogenes and downregulation of tumor-suppressor genes in cancerous cells when compared to 'normal' tissue,as well as the gene expression in the rejection of transplanted organs.

WESTERN BLOTTING

W**estern blotting**, is a widely used technique to detect specific proteins in a sample of tissue homogenate or extract.

Principle

Western blotting is a rapid and sensitive assay for detection and characterization of proteins. It is based on the principle of immunochromatography where proteins are separated into polyacrylamide gel according to their molecular weight.

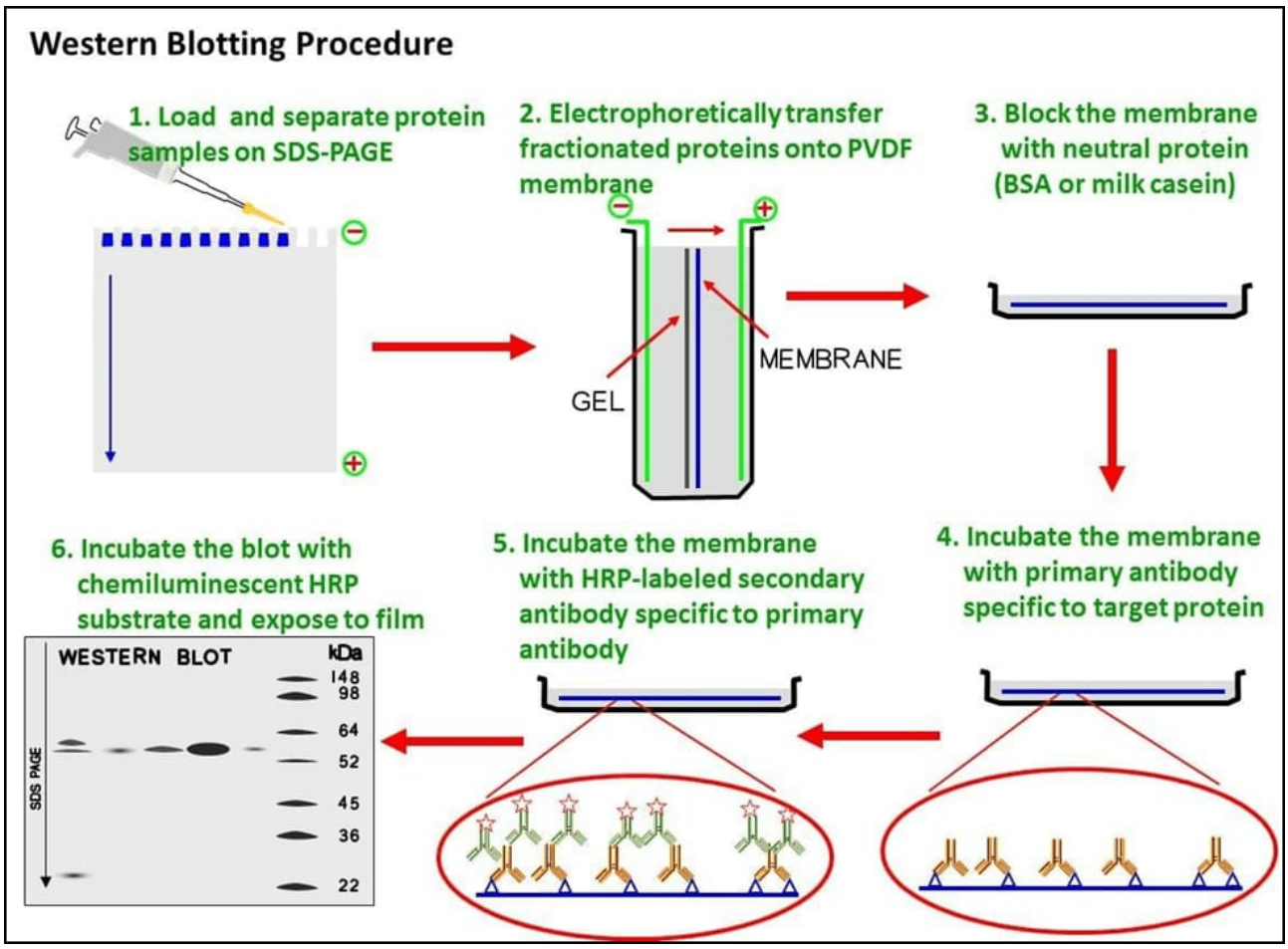
In brief, the sample undergoes protein denaturation, followed by gel electrophoresis. A synthetic or animal-derived antibody (known as the primary antibody) is created that recognises and binds to a specific target protein. The electrophoresis membrane is washed in a solution containing the primary antibody, before excess antibody is washed off. A secondary antibody is added which recognises and binds to the primary antibody. The secondary antibody is visualised through various methods such as staining, immunofluorescence, and radioactivity, allowing indirect detection of the specific target protein.

Procedure

- The western blot method is composed of a gel electrophoresis to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide, followed by an electrophoretic transfer onto a membrane (mostly PVDF or Nitrocellulose)
- SDS-PAGE is generally used for the denaturing electrophoretic separation of proteins.
- Prior to electrophoresis, protein samples are often boiled to denature the proteins present. This ensures that proteins are separated based on size and prevents proteases from degrading samples.
- Following electrophoretic separation, the proteins are transferred to a membrane (typically nitrocellulose or PVDF), where they are blocked with milk (or other blocking agents) to prevent non-specific antibody binding, and then stained with primary antibodies specific to the target protein.
- Lastly, the membrane will be stained with a secondary antibody that recognizes the first antibody staining, which can then be used for detection by a variety of methods. The gel electrophoresis step is included in western blot analysis to resolve the issue of the cross-reactivity of antibodies.

Applications

- It is most sensitive and specific test for determining size and amount of protein present in any material.
- The confirmatory HIV test employs a western blot to detect anti-HIV antibody in a human serum sample.
- A western blot is also used as the definitive test for Creutzfeldt-Jakob Disease, Lyme disease, Hepatitis B infection and Herpes Type 2 infection.



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