B. Sc (P) Life Science III year Semester VI

DSE-1 Analytical Techniques in Plant Sciences

Practical- Study of Blotting Techniques

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- The blot analysis technique is a powerful method to detect specific biomolecules in samples of complex composition.
- Blotting methods are the adjunct to gel electrophoresis, a method for separating DNA, RNA and proteins with exceptional resolving power.
- It can be applied to biomolecules that will adhere stably to a support material such as nitrocellulose, nylon or paper membrane and are still able to bind their cognate ligand. Usually the biomolecules of interest are first separated according to size and/or charge before transfer to the membrane.
- The method was originally developed for DNA by **Edwin M. Southern** in 1975. He demonstrated that the DNA restriction fragments that had been electrophoretically fractionated in agarose gel could be transferred to a solid support (nitrocellulose) and detected as discrete bands following hybridization to a complimentary DNA probe.
- When the Southern blotting method was applied to RNA, it was termed as **Northern Blotting**. The **Western blot** analysis refers to the transfer of proteins to membrane and their detection with antibody probe.
- All methods have in common a step, in which molecules are transferred from the gel to a porous membrane, most often achieved by soaking solution through the gel and the membrane using absorbent paper.
- For DNA and RNA, specific sequences are detected in the membrane by molecular hybridization with labelled nucleic acid probes. The proteins are detected using labelled antibodies.
- The original protocol was adapted for radioactive probes labelled with, for example, ^{32}**P**, ^{3}**H**, ^{35}**S** or ^{125}**I**. Since then, other labelling systems have been developed, including fluorescent and chemiluminescent reagents.

General principle

All the three blotting methods are fairly simple and usually consist of four separate steps:

1. Electrophoretic separation of protein or of nucleic acid fragments in the sample,
2. Transfer to and immobilization on paper support,
3. Binding of analytical probe to target molecule on paper, and
4. Visualization of bound probe.

Molecules in a sample are first separated by electrophoresis and then transferred on to an easily handled support medium or membrane. This immobilizes the protein or DNA fragments, provides a faithful replica of the original separation, and facilitates subsequent biochemical analysis.

After being transferred to the support medium the immobilized protein or nucleic acid fragment is localized by the use of probes, such as antibodies or DNA, that specifically bind to the molecule of interest.

Finally, the position of the probe that is bound to the immobilized target molecule is visualized usually by autoradiography.

Procedure

1. Run the DNA/RNA/Protein gel to desired distance.
2. Visualize gel on UV box.

3. Soak gel in 0.2 N HCl until the bromophenol blue band begins to change color. (About 10 min usually). Alternatively, expose to UV transilluminator for 90 sec.
4. Rinse once with H2O.
5. Soak in 0.4N NaOH/0.6M NaCl for 30 min.
6. set up a transfer pyramid: put a glass plate on a support in a tray containing 1 litre of 0.5 M NaOH, and cover it with two sheets of filter paper (Whatman 3MM or other suitable paper) such that on all sides the filter paper contacts the solution and functions as a wick.
7. Wet the filter paper with 0.5 M NaOH and remove air bubbles by gently rolling a glass pipette back and forth on the paper.
8. Put the gel on the filter paper. Place a piece of nylon membrane slightly larger than the gel (prewet in 0.5 M NaOH) on the gel. Put 4pieces of prewet filter paper cut to size. Make sure to remove air bubbles between each layer.
9. Put a 10 cm stack of dry filter paper or paper towels, cut to size, on top of the pyramid. Place a glass plate and a weight of about 0.5 Kg on top.
10. The orientation of the gel and the nylon filter should be marked, for instance by cutting off a corner. Place strips of parafilm or plastic wrap around the gel to prevent direct contact between the stack of filter paper and the wick causing a short-circuit in the flow of the transfer solution. Allow the transfer to proceed for 12 h or longer.
11. Carefully disassembles the pyramid.
12. Remove membrane from blotter. Discard gel, whatman, and paper towels.
13. Rinse membrane in 2X SSC. Blot dry on a piece of whatman.
14. Sandwich blot between two pieces of dry whatman and bake in vacuum oven for 1 hour.

Applications of Blotting Techniques

Southern Blotting

Southern blotting has many applications in molecular biology, including the identification of one or more restriction fragments that contain a gene or other DNA sequence of interest and in the detection of RFLPs used in construction of genomic maps.

Northern blotting

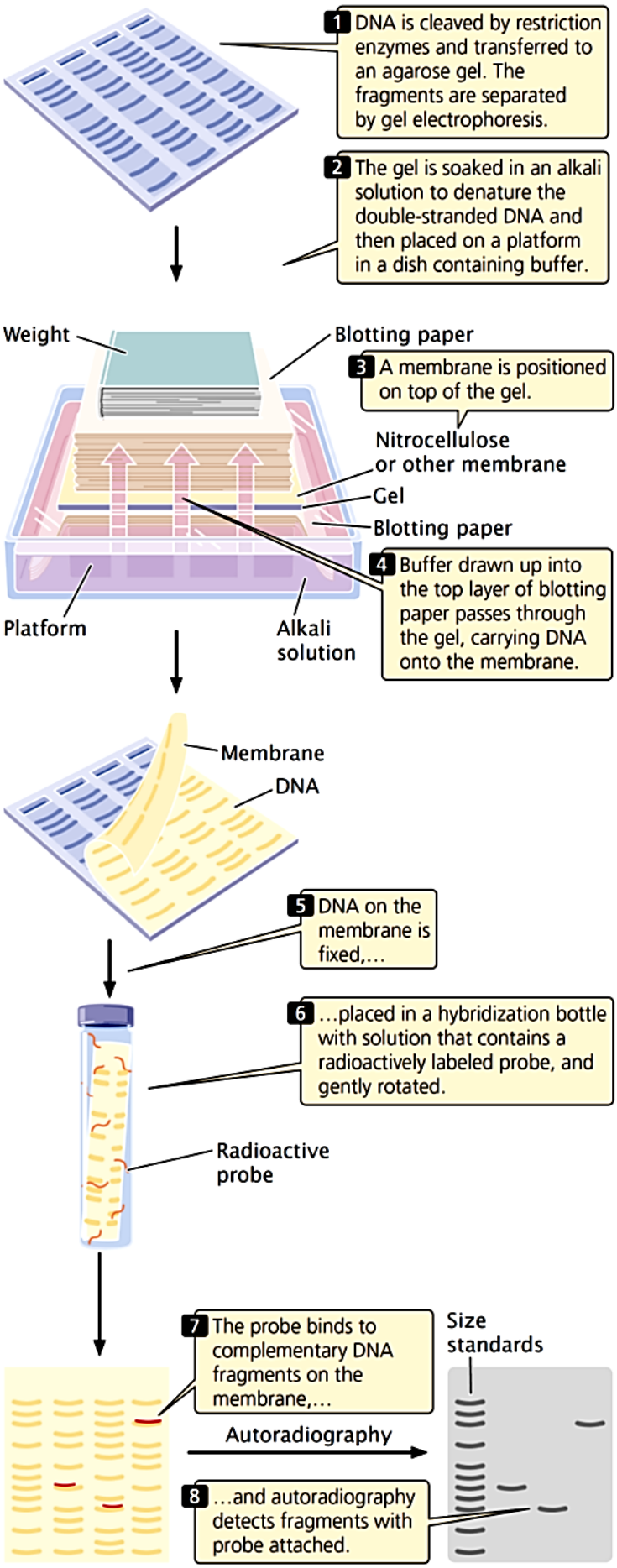
Northern blotting allows in observing a particular gene's expression pattern between tissues, organs, developmental stages, environmental stress levels, pathogen infection. The technique has been used to show over expression of oncogenes and down regulation of tumor-suppressor genes in cancerous cells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

Western blotting

The western blot (alternatively, immunoblot) is used to detect specific proteins in a given sample of tissue homogenate or extract. The method originated from the laboratory of George Stark at Stanford. The name western blot was given to the technique by W. Neal Burnette.

Source:

- Pierce, Benjamin A. Genetics: A conceptual approach. 4th edn.
- Primrose S B, R M Twyman and R W Old. Principles of Gene Manipulation. 6th edn.



Blotting Technique