# Southern blotting

## Usage and Derived Methods

Gel electrophoresis is a powerful tool for separating macromolecules of different sizes and charges. DNA molecules have essentially the same charge per unit mass, so they are separated on agarose or polyacrylamide gels almost exclusively based on their size or conformation. Agarose and polyacrylamide gels act as molecular sieves that slow the passage of large molecules more than small ones. Agarose gels are better sieves for large molecules (over a few hundred nucleotides), while polyacrylamide gels are better for resolving small DNA molecules.

## DNA Analysis by Southern Blotting

In 1975, E.M. Southern published an important new procedure that made it possible to determine the location of genes and other sequences in restriction fragments separated by gel electrophoresis. The basic feature of this method is the transfer of DNA molecules separated by gel electrophoresis to a nitrocellulose or nylon membrane. Such transfer of DNA is called Southern transfer. The DNA is denatured either before or during transfer by placing the gel in an alkaline solution. After transfer, the DNA is immobilized on the membrane by drying or irradiation with UV light and hybridized with a radioactive DNA-probe containing the studied sequence. The probe will only hybridize to DNA molecules that contain a nucleotide sequence complementary to the probe sequence.

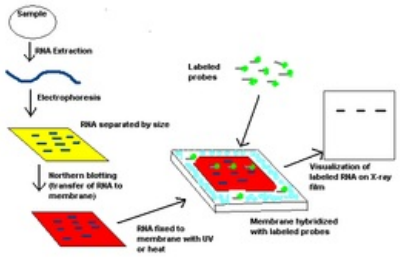
Unhybridized probes are then washed from the surface of the membrane and the membrane thus treated is exposed to X-ray film so that the presence of radioactivity can be detected. After developing the film, the dark bars show the position of the DNA sequences that hybridized with the probe. The ability to transfer DNA molecules separated by gel electrophoresis onto nylon membranes for hybridization studies and other types of analysis has proven extremely useful. For example, it can be used to detect the cystic fibrosis gene, etc.

Southern blot membrane

## RNA Analysis by Northern Blotting

The following photo describes transfer of RNA molecules after their separation by electrophoresis. This transmission is called northern transmission.

The only difference with Southern blotting is that RNA molecules are transferred to the membrane. However, RNA molecules are very sensitive to degradation by RNA-ases, and therefore contamination of the material by this extremely stable enzyme must be avoided. In addition, most RNA molecules contain secondary structure and must therefore remain denatured during electrophoresis to ensure size-based separation. Denaturation is achieved by adding formaldehyde or some denaturing agent to the buffer used in electrophoresis.




Northern Blot Scheme

After transfer to the membrane, RNA hybridizes with either DNA or RNA probes, just as in Southern blotting. Northern hybridization is extremely useful in gene expression studies. It can be used to determine when and where individual genes are expressed. However, northern hybridization only determines the degree of accumulation of RNA-transcripts and does not provide any information about why the accumulation occurred. Changes in the accumulation of transcripts can be caused by changes in the rate of transcription or, conversely, in the rate of degradation of transcripts.

## Protein Analysis by Western Blotting

Sometimes also referred to as an immunoblot. Because many functional proteins are composed of two or more subunits, the individual polypeptides are separated in the presence of the detergent sodium dodecyl sulfate (SDS), which denatures the proteins. After electrophoresis, proteins are detected by Coomassie blue staining or silver labeling. Split polypeptides can also be transferred from the gel to a nitrocellulose membrane and individual proteins can be detected using antibodies. The transmission is referred to as western transmission and is carried out using an electric current

After this transfer, the specific protein is identified by placing the membrane with the immobilized proteins in a solution containing an antibody to that protein. Subsequently, unbound antibodies are removed from the membrane by washing. The presence of the first (primary) antibody is detected by incubation with the secondary antibody. This secondary antibody generally reacts with immunoglobulins. The secondary antibody is linked (conjugated) to either a radioactive isotope or an enzyme.

## Links

## Related articles

- Methods of nucleic acid analysis

## References


- MURRAY, Robert K. *Harperova biochemie.* 2. české (v H & H 1.) edition. H & H, 1998. 872 pp. ISBN 80-85787-38-5.
- GOETZ, Pavel. *Vybrané kapitoly z lékařské biologie II.* Karolinum edition. 2002. 139 pp. ISBN 80-246-0320-9.