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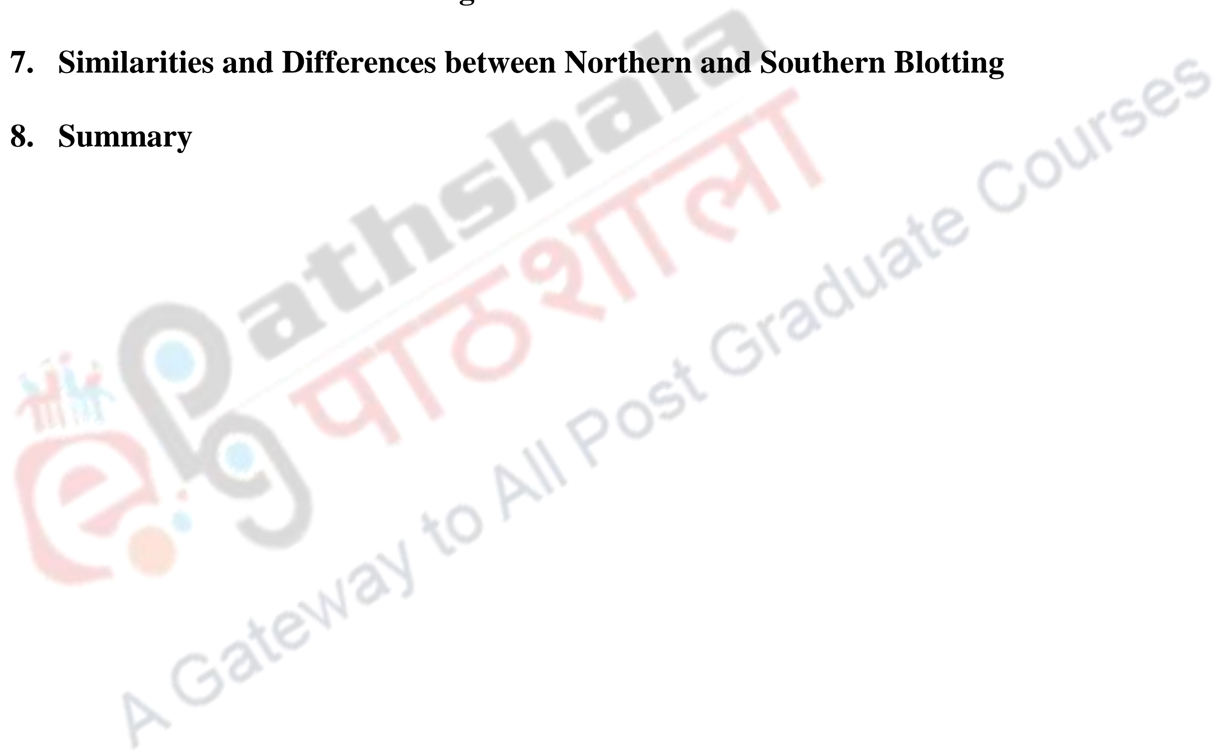


1. Learning Outcomes

After studying this module, you shall be able to-

- Know about the principle and methodology followed for the northern and southern blotting procedure
- Learn about the applications for northern and southern blotting and lastly,
- Learn the similarities and differences between northern and southern blotting

2. Introduction

A Blot or the procedure of Blotting is a technique that allows the transfer proteins, DNA or RNA, onto a carrier membrane, such as a nitrocellulose or a PVDF membrane. This transfer is generally done after gel electrophoresis, by transferring the molecules from the gel onto the desired blotting membrane or can be done by directly adding the samples onto the membrane. Further, blotting is of different types depending upon the type of molecule transferred. These are –

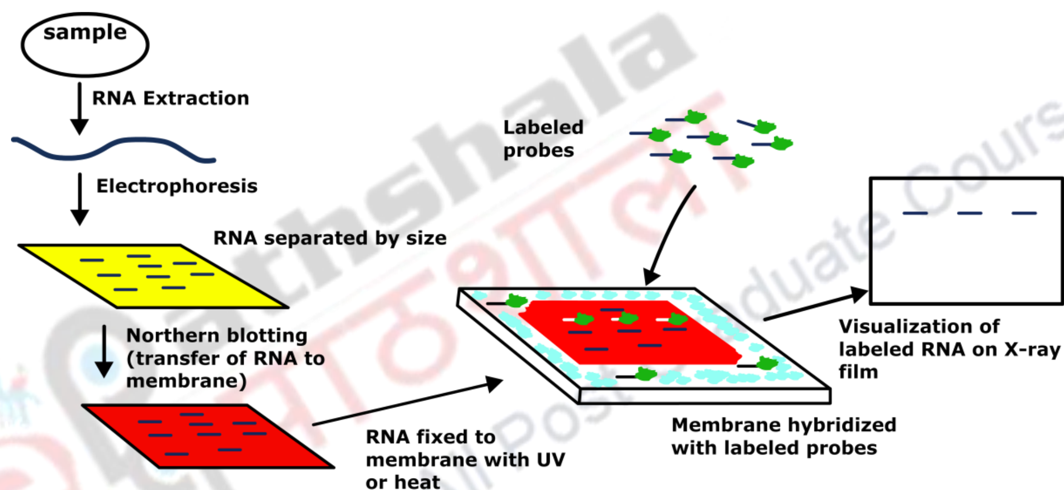
S. No	Name	Molecule transferred
1.	Northern Blotting	RNA
2.	Southern Blotting	DNA
3.	Western Blotting	Proteins

3. Northern Blotting

Northern blotting is a procedure used to identify specific RNA molecules from a mixture of RNA molecules. It is generally used for the analysis of the RNA sample from a particular tissue or cell which is used to quantify the RNA expression of particular genes. This process was invented by Alwine and is known for its resemblance to the technique of Southern blot, which operates over DNA.

Common protocol followed for Northern Blotting:

1. The initial step in any northern blotting experiment is to denature or separate the RNA within the sample into single strands. This step ensures that the strands are not bonded together through hydrogen bonds and are unfolded.
2. The denatured RNA molecules are separated according to their molecular weight by using agarose gel electrophoresis.
3. Following the electrophoretic separation, the resolved RNAs are transferred from the gel onto a blotting membrane.
4. After the completion of transfer, blotting membrane carries RNA molecules in the form of bands which were present originally on the gel.



5. In the subsequent step the membrane is blocked and incubated with a DNA or RNA probe, which has a sequence that is complementary to a particular RNA sequence in the sample and a molecular tag for detection of its location.
6. This tag can be radioactive labelled molecule or any other tag, i.e., non-radiolabelled.
7. After the probe has been hybridized (or bound) to a specific RNA fragment on the membrane it can be detected among the different RNA molecules on the membrane.

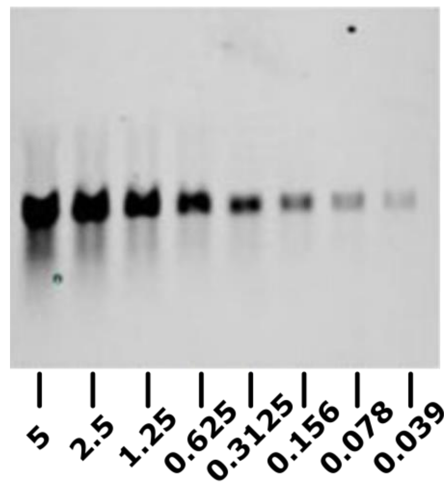


Figure 1: A typical Northern blotting result as observed on the X ray film

4. Applications of Northern Blotting

1. Northern blotting has multiple applications in modern biology, biotechnology and molecular biology laboratories. The technique allows the scientists to observe the expression pattern of genes between multiple tissues, organs, developmental stages, environmental stress levels, pathogenic state of an infection, and also to monitor responsiveness to therapy.
2. The technique has been applied to demonstrate overexpression of several oncogenes and downregulation of tumour-suppressor genes in case of cancer as compared to the normal cells.
3. It has also been applied to study the gene expression in case of rejection of a transplanted organ.
4. Northern blotting can also be used to determine if the expression profile belongs to a previously known gene or when a novel gene sequence has been identified.
5. Furthermore, the expression patterns derived on a Northern blot can provide insights into the gene functions if specific set of conditions have been used.
6. Northern blotting can also provide information if a particular mRNA molecule undergoes alternative splicing. This can be observed if same probe is hybridized at multiple molecular weight levels.
7. Alternately if the product has lower molecular weight than expected it may represent deletion mutations in the gene.

5. Southern Blotting

Southern blotting is a laboratory technique used in the field of cell and molecular biology to identify specific DNA molecules from a combination of DNA molecules. Southern blotting is the transference of DNA fragments from an electrophoresis gel on to a membrane, subsequent in the immobilization of DNA fragments, so that the membrane transports a semi-permanent reproduction of the banding arrangement of the gel. After the immobilization, the DNA can be subjected to hybridization analysis, enabling bands with sequence similarity to be attached to the labelled probe and thus, is identified. Southern blotting merges transmission of electrophoresis-separated DNA fragments to a sieve membrane and the further fragment detection is done by probe hybridization. The technique was discovered by Edwin Southern, and hence the name Southern blotting.

6. Protocol for Southern Blotting

1. The DNA to be analysed is isolated from the source.
2. Restriction enzymes are used to hydrolyse the larger DNA molecules into smaller fragments.
3. The DNA fragments are then separated under the influence of electric field using agarose gel electrophoresis. This results in separation according to their molecular weight or size.
4. In case DNA fragments above 10kb are present, then the agarose gel may be treated with an acid, such as dilute HCl prior to the blotting step. This acid treatment results in the depurination of DNA fragments, breaking the DNA into smaller pieces, thereby increasing efficiency of transfer from gel to nitrocellulose or nylon membrane.
5. A sheet of nylon (6, 6) or nitrocellulose membrane is placed on top in the direction of transfer from the gel. Pressure is distributed uniformly onto the gel to confirm a smooth contact among gel and membrane. Buffer transfer by capillary action also move the DNA from the gel onto the membrane where ionic interactions bind the DNA to the membrane due to the negative charge of the DNA and positive charge of the membrane.
6. The membrane is overheated in an oven at 80°C for two hours (usual conditions; nitrocellulose or nylon membrane) or uncovered to ultraviolet radiation (nylon membrane) to permanently join the transported DNA to the membrane.
7. The membrane is then unprotected to a hybridization probe—a single DNA fragment with a precise arrangement whose occurrence in the target DNA is to be known. The probe DNA is labelled with radioactivity or a fluorescent tag for its detection. The probe can be either DNA or RNA molecule.

In order to minimize the non-specific binding *Salmon* or calf thymus DNA is used for blocking of the membrane surface and target DNA along with deionized formamide, and detergents like SDS are used to reduce non-specific binding of the probe.

8. Following the hybridization, the extra probe is carried away from the membrane and the arrangement of hybridization is visible on X-ray film by autoradiography or through development of coloured bands on the membrane, in case a chromogen has been used.

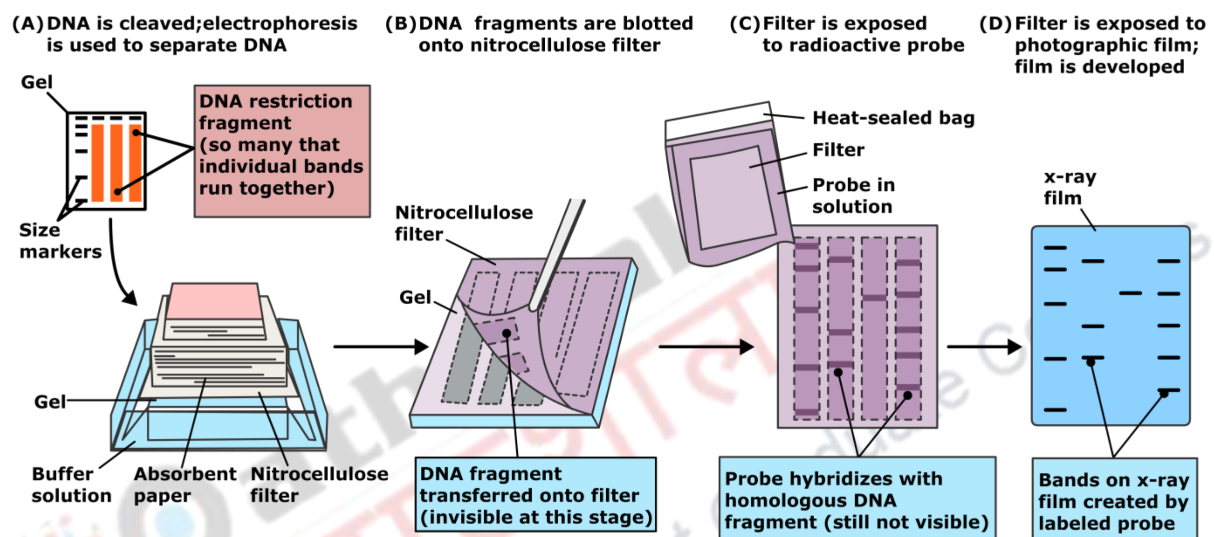


Figure 2: Southern blotting Procedure. DNA from the source is cleaved into small fragments and is electrophoresed on agarose gel. This is followed with capillary blotting and probing for the known DNA sequences

Blots can be probed using a radioactive or a non-radioactive (DIG) labelled probe. This probe binds to the target DNA fragment and helps in amplification and detection of such sequences.

The hybridization reaction involves a buffer, a detergent and a non-specific DNA to maximize the stringency. The membrane is washed with the buffer to minimize any non-specific binding.

Detection

The blots are detected by autoradiography using the X-ray films placed along the membrane. Consequently, only those places in the membrane where the probe is hybridized with the DNA, yields a band and thus, can be easily detected.

Hybridization of the probe to a specific DNA fragment on the filter membrane indicates that this fragment contains DNA sequence that is complementary to the probe. The transfer step of the DNA from the electrophoresis gel to a membrane permits easy binding of the labelled hybridization probe to the size-fractionated DNA.

It also allows for the fixation of the target-probe hybrids, required for analysis by autoradiography or other detection methods. Southern blots performed with restriction enzyme-digested genomic DNA may be used to determine the number of sequences (e.g., gene copies) in a genome.

A probe that hybridizes only to a single DNA segment that has not been cut by the restriction enzyme will produce a single band on a Southern blot, whereas multiple bands will likely be observed when the probe hybridizes to several highly similar sequences (e.g., those that may be the result of sequence duplication). Modification of the hybridization conditions, such as increasing the hybridization temperature or decreasing salt concentration, increases the specificity and decreases the hybridization of the probe to sequences that are less than 100% similar.

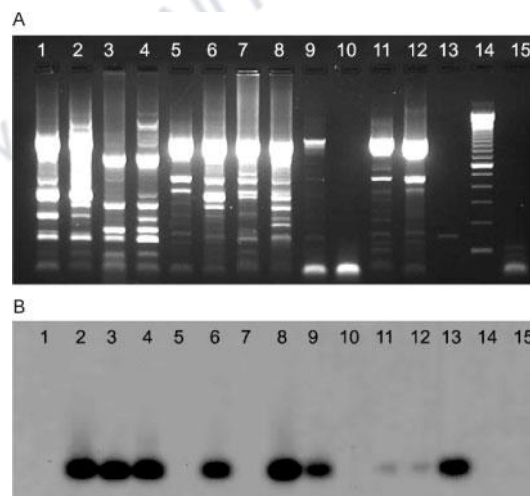


Figure 3: Southern Blot Result. Note that the gel above has multiple bands but in the Southern Blot below only those bands are visible that have been hybridized by the radioactive probe and hence could be detected

7. Applications of Southern Blotting

1. Southern blotting is used to locate specific arrangements in restriction fragments that have been alienated by gel electrophoresis. This most noticeable use of Southern blotting is to detect DNA sequences that have analogous sequences to the one which are existing, i.e., how many functionally associated genes have been known and considered. In this case, one strand of the gene being studied is applied as a probe.
2. This information can also be applied to simplify a cloning experiment. For example, if the desired gene is exposed to be encrypted on a restriction fragment 6,000 base pairs in length, then fragments of that size can be separated from the rest and cloned.
3. Further, it is also used to distinguish different strains of a given species by detecting subtle variations in their nucleotide sequences. Certain mutations create, whereas others put an end to restriction enzymes recognition sequences at particular sites in the genome. Therefore, when genomic DNA of diverse strains is processed with the similar restriction enzyme, the individual DNA will offer to some extent a dissimilar variety of restriction fragment sizes. Each variation is called a restriction fragment length polymorphism (RFLP). Southern blot hybridization is applied to selectively visualize restriction fragments that often vary in size.

8. Similarities and Differences between Northern and Southern Blotting

While there are a number of similarities between the two techniques there are significant differences as well. The most notable ones are:

Basis	Northern Blotting	Southern Blotting
Molecule detected	mRNA(ss)	DNA(ds)
Gel electrophoresis	Formaldehyde agarose gel	Agarose gel
Gel treatment	Since RNA molecules are single stranded denaturation step is not required.	Depurination, denaturation and neutralization

Membrane used	Amino-benzyloxymethyl membrane is used	Nitrocellulose or Nylon membrane can be used
Blotting method	Capillary transfer	Capillary transfer
Probes	cDNA– radioactive or non-radioactive	DNA- radioactive or non-radioactive
Detection system	Autoradiography, chemiluminescent, calorimetric	Autoradiography, chemiluminescent, calorimetric
Use	Used to study expression of a particular gene	Used to identify a particular gene

9. Summary

- Northern blotting is a method applied to identify specific RNA molecules from a mixture of several RNA molecules. It is generally used for the analysis of the RNA sample from a particular tissue or cell and is used to quantify the RNA expression of particular genes.
- This technique was invented by Alwine and known for its resemblance to the technique of Southern blot.
- It has multiple applications in modern biology, biotechnology and molecular biology laboratories. The technique allows the scientists to observe expression pattern of genes between multiple tissues, organs, developmental stages, environmental stress levels, pathogenic state of an infection, and also to monitor responsiveness to therapy.
- The technique has been applied to demonstrate overexpression of several oncogenes and downregulation of tumour-suppressor genes in case of cancer as compared to the normal cells.
- It has also been applied to study the gene expression in case of rejection of a transplanted organ.
- Southern Blotting is used to detect the sequence of interest (DNA) from a variety of /crude DNA sequences. It was invented by Edwin Southern.

- Southern Blotting involves the transmission of DNA from agarose gels onto a nylon or nitrocellulose membrane.
- The membrane bound DNA is then stabilized and probed for a sequence of interest using radiolabelled or DIG labelled probe.
- Most common applications of Southern Blotting are in field of identification of new genes, similar genes in different species and in Forensic Science, where it is used to support RFLP.