

NORTHERN BLOTTING

Q. Give a common illustration of southern and northern blotting technique.

1. A mixture of either single stranded RNA molecules (northern blotting) or the double- stranded DNA fragments created by restriction nuclease treatment (southern blotting) is separated according to length by electrophoresis.
2. A sheet of nitrocellulose or nylon paper is laid over the gel and the separated RNA or DNA fragments are transferred to the sheet by blotting.
3. The nitrocellulose sheet is carefully peeled off the gel.
4. The sheet containing the bound nucleic acids is placed in a sealed plastic bag together with a buffered salt solution containing a radioactively labeled DNA probe. The sheet is exposed to a labeled DNA probe for a prolonged period under conditions favouring hybridization.
5. The sheet is removed from the bag and washed thoroughly, so that only probe molecules that have hybridized to the RNA or DNA immobilized on the paper remain attached.
6. After autoradiography, the DNA that has hybridized to the labeled probe shows up as bands on the autoradiograph.
7. For southern blotting, the strands of the double-stranded DNA molecules on the paper must be separated before the hybridization process, this is done by exposing the DNA to alkaline denaturing conditions after the gel has been run.

Q. How blotting technique is effective in the field of molecular biology?

- i) Blotting is used in molecular biology for the identification of proteins and nucleic acids and is widely used for diagnostic purposes.
- ii) This technique *immobilizes* the molecule of interest on a support, which is a **nitrocellulosic** membrane or nylon. It uses hybridization techniques for the identification of the specific nucleic acids and genes.
- iii) The blotting technique is a tool used in the identification of biomolecules such as DNA, mRNA and protein during different stages of gene expression. Protein synthesis involves expression of a DNA segment which gets converted to mRNA to produce the respective protein.
- iv) Subtypes of blotting such as *northern*, *western* & *southern* depend upon the target molecule that is being sought.
- v) When a DNA sequence is the foundation or code for a protein molecule, the particular DNA molecule of interest can be blotted using Southern Blotting technique.
- vi) During gene expression, when the DNA is expressed as mRNA for a protein production, this process can be identified by *Northern blotting*.
- vii) Finally, the coded mRNA produces the concerned protein, this protein identification can be done by Western Blotting.

Q. What is the general procedure of blotting?

Key points:

- i) Homogenize the sample.
- ii) Separation of the molecule of interest by an electrophoresis membrane.
- iii) Transferring the molecules to a nitro cellulose membrane/ nylon membrane.
- iv) Hybridization or identification of the molecule

Q. Describe the northern blotting with proper labeled set up. Q. Make a brief note on application of this technique.

Northern Blotting is a technique used for the study of gene expression. It is done by detection of particular RNA (or isolated mRNA). This method reveals the identity, number, activity, and size of the particular gene. This blotting technique can also be used for the growth of a tissue or organism.