

## Northern Blotting:

Northern blotting is used to check the expression levels of a gene by detecting the RNA of that gene in a sample. In this, we perform hybridization of a labelled complimentary oligo-ribonucleotide probe to the mRNA extracted from a sample. The probe is complimentary to the sequence of interest.

The binding is detected using an autoradiogram or fluorescence, which helps to identify the presence and concentration the RNA sequence of interest. This in turn tells us if the gene is being expressed or not and in how much quantity it is being expressed.

### Procedure

- The RNA is extracted from the sample cells
- mRNAs are extracted using oligo-dT chromatography to separate RNA with a poly(A) tail.
- mRNAs are then separated by gel electrophoresis
- Various RNA bands obtained on the agarose gel are transferred to a positively charged nylon or nitrocellulose membrane.
- Since, the nucleotides are negatively charged, they bind to the membrane.
- The nitrocellulose membrane is baked at 80°C to immobilize the RNA.
- The immobilized RNA is exposed to the hybridization probe or sequence complementary to the RNA of interest, which has been labelled using fluorescent or radioactive markers.
- The hybridization probe will bind to any complementary sequence present in the test RNA
- The membrane is washed with appropriate buffer to remove any unbound probe.
- Radioactivity is detected by autoradiography or fluorescence scanners.

The site of radioactivity or fluorescence represents the complementary RNA sequences present in the test RNA extract. The levels of fluorescence or radioactivity represent the concentration of the RNA of interest and the expression levels of gene of interest.

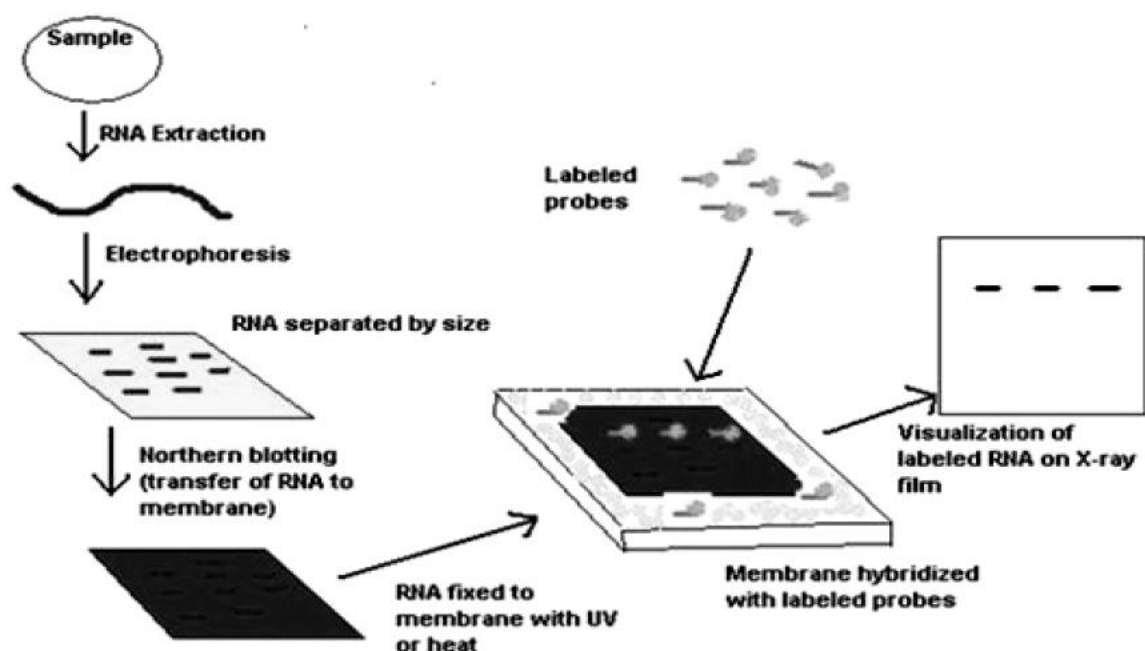


Figure: Workflow of Northern Blotting

**Applications**

1. Detect the presence of a particular RNA in a sample
2. It is used to check the expression levels of a particular gene during various developmental stages or under different environmental conditions.
3. Diagnosis of a disease
4. Check overexpression or under-expression of certain genes in a particular disease
5. Identification of viruses