

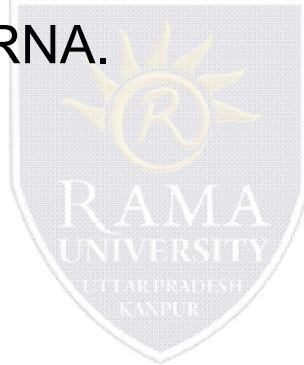


FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

Northern Blotting

- › The northern blot is a technique used in molecular biology research to study gene expression by detection of RNA
- › The northern blot technique was developed in 1977 by James Alwine, David Kemp, and George Stark at Stanford University.
- › The Northern blot, also known as the RNA blot, is one of the blotting techniques used to transfer DNA and RNA onto a carrier for sorting and identification.
- › The Northern blot is similar to the Southern blot except that RNA instead of DNA is the subject of analysis in this technique.

- › It is **mRNA** which is isolated and hybridized in northern blots
- › The **formaldehyde** was use in electrophoresis gel as a denaturant because the sodium hydroxide treatment used in the Southern blot procedure would degrade the RNA.



Applications:

- › The Northern blot is useful for the study of gene expression in two ways.
- › First, the position of bands on the blot provides a direct measure of RNA size.
- › Knowing the size of the RNA will provide an estimate for the transcript's coding capacity and thus the size of the protein it encodes.

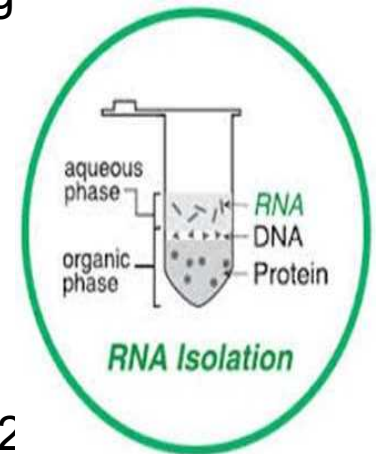
- › Second, the Northern blot analysis of RNA samples from many different tissues enables researchers to determine which specific tissue a gene is expressed in along with the relative levels of its expression in all cells where transcription is occurring.
- › The Northern blot is a valuable method used by researchers in determining gene expression patterns.
- › For example, many scientists researching Huntington disease or breast cancer are able to determine the expression patterns of the genes responsible for these diseases using blotting techniques.



Process:

1. RNA isolation:

- This part of the Northern Blot is an important step because high quality mRNA is extracted from the cells and purified.



2. Probe generation:

- The mRNA is loaded onto a gel for electrophoresis.
- Lane 1 has size standards (a mix of known RNA fragments) Lane 2
- Northern blots can be probed with radioactively or nonisotopically labeled RNA, DNA or oligodeoxynucleotide probes.



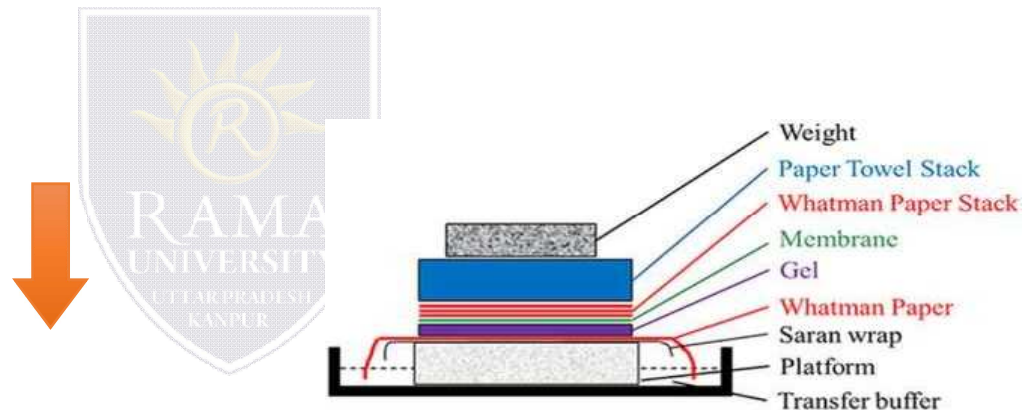


3. Denaturing agarose gel electrophoresis:

- Formaldehyde has traditionally been used as the denaturant, although the glyoxal system has several advantages over formaldehyde.
- An electric current is passed through the gel and the RNA moves away from the negative electrode.
- The distance moved depends on the size of the RNA fragment.
- Since genes are different sizes the size of the mRNAs varies also.
- This results in a smear on a gel.
- Standards are used to quantitate the size.
- The RNA can be visualized by staining first with a fluorescent dye and then lighting with UV.

4. Transfer to solid support and immobilization:

- RNA is single-stranded, so it can be transferred out of the gel and onto a membrane without any further treatment.
- The transfer can be done electrically or by capillary action with a high salt solution.



5. Prehybridization and hybridization with probe:

- A labelled probe specific for the RNA fragment in question is incubated with the blot.
- The blot is washed to remove non-specifically bound probe and then a development step allows visualization of the probe that is bound.

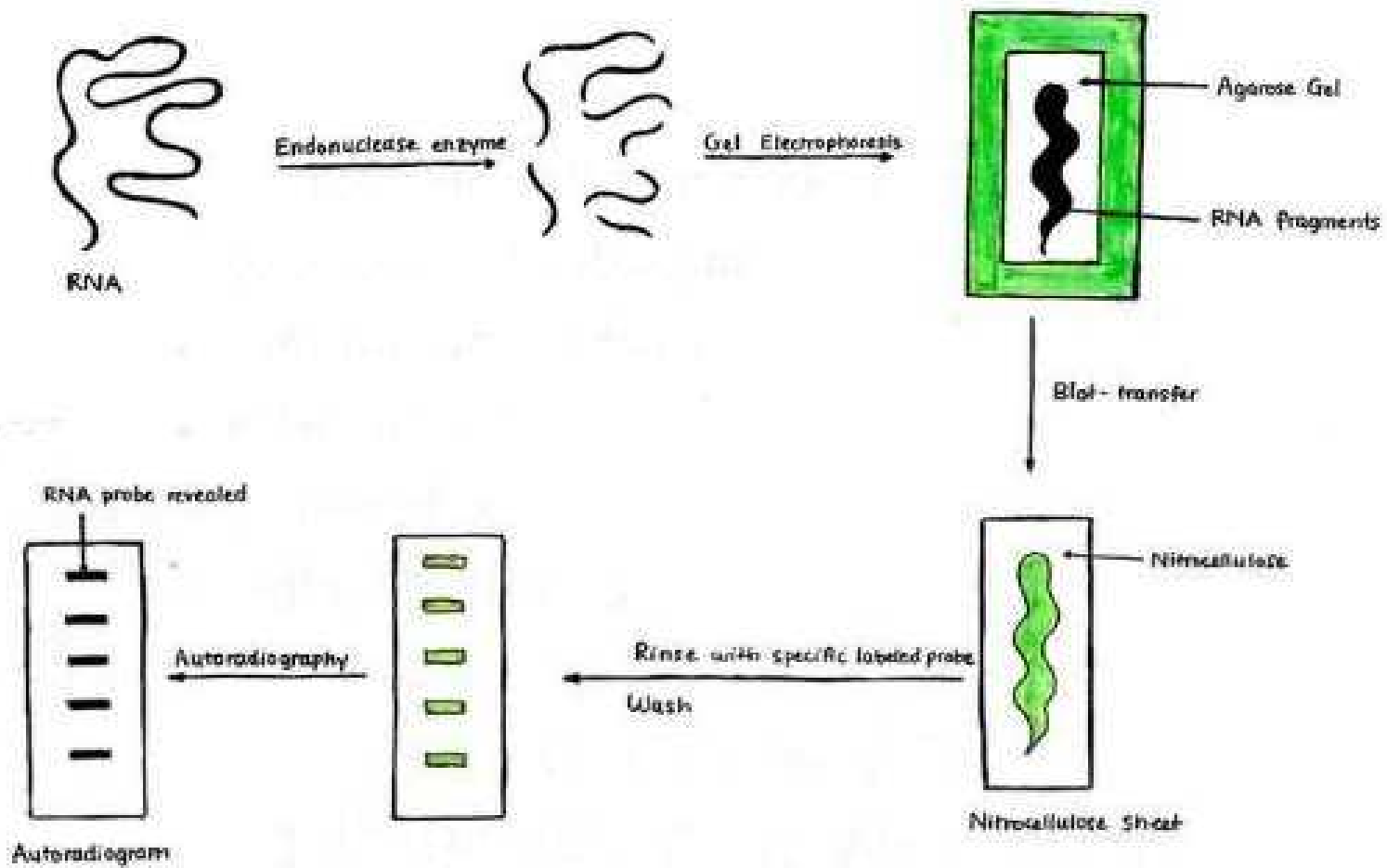
6. Washing:

- The probe is bound specifically to the target mRNA and that there is negligible non-specific binding to other mRNA or the nylon membrane itself.

7. Detection:

- If a radiolabeled probe was used, the blot can be wrapped in plastic wrap to keep it from drying out and then immediately exposed to film for autoradiography.
- If a nonisotopic probe was used, the blot must be treated with nonisotopic detection reagents prior to film exposure.
- › Hybridization signals are then detected.





Precautions for blotting

- › Remove air bubbles trapped between the gel & the membrane.
- › Ensure that all buffer components are fully dissolved before using.
- › Check whether the membrane has damaged at cut edges.
- › Ensure that the electrophoresis tanks are rinsed with distilled water after used.
- › Control the temperature during hybridization
- › Always check for the incorporation of the radioactive label before using the probe.
- › Don't reuse electrophoresis buffer & radioactive probes.

Ways to Increase the Sensitivity of Northern

- 1) Increase the Amount of RNA Loaded in Each Lane.
- 2) Use Poly(A) RNA Instead of Total RNA.
 - 10 μg of mRNA is equivalent to 300–350 μg of total RNA. (mRNA comprises only about 0.5–3% of total RNA.)
- 3) Switch From a Traditional Hybridization Buffer to ULTRAhyb.
 - Ultrasensitive Hybridization Buffer can increase the sensitivity of a blot hybridization up to 100-fold.
- 4) Use High Specific Activity Probes. The specific activity of the probe should be at least 10⁸ cpm/ μg