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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.

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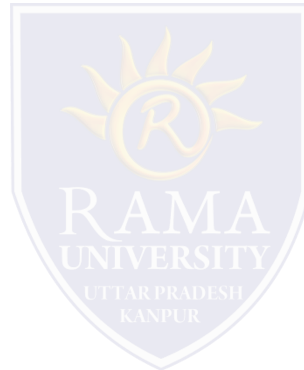
- It is mRNA which is isolated and hybridized in northern blots
- The formaldehyde was use in electrophoresis gel as a denaturant because the sodium hydroxide treatment used in the Southern blot procedure would degrade the RNA.



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.

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- 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.

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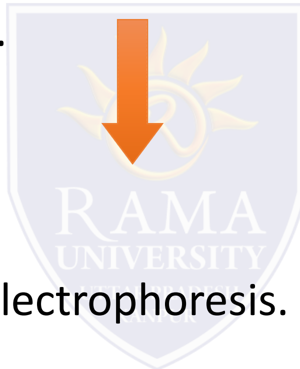
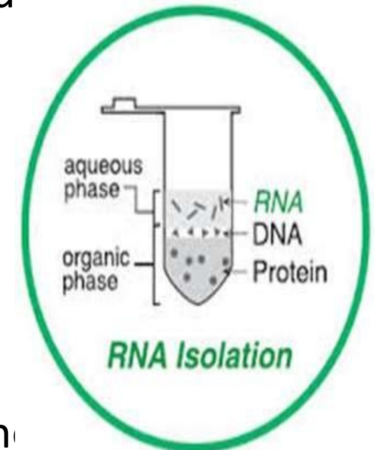
Process:

1.RNA isolation:

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





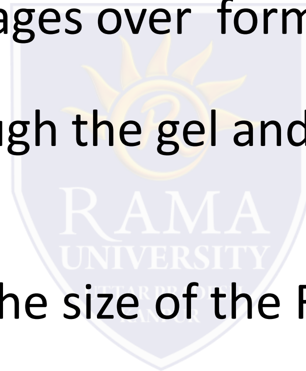
2. Probe generation:

•The mRNA is loaded onto a gel for electrophoresis.

•Lane 1 has size standards (a mix of known RNA fragments) Lane 2 has th

•Northern blots can be probed with radioactively or nonisotopically labeled RNA, DNA or oligodeoxynucleotide probes.

3.Denaturing agarose gel electrophoresis: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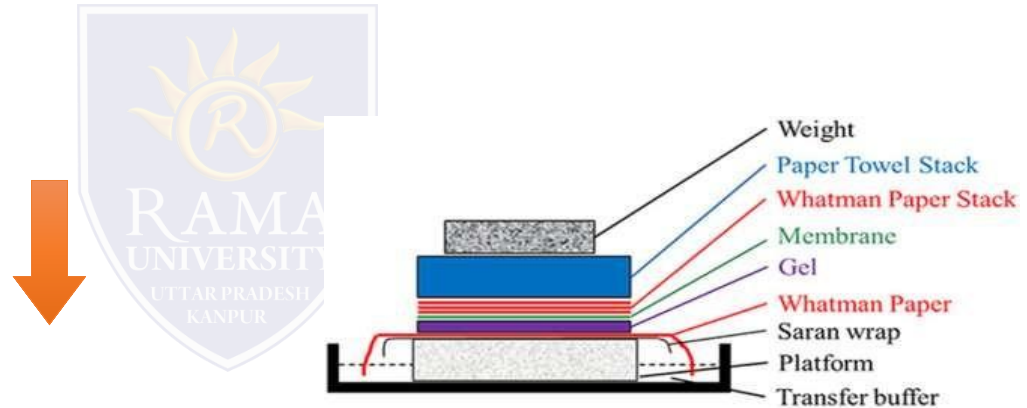
- Formaldehyde has traditionally been used as the denaturant, although the glyoxal system has several advantages over formaldehyde.
- An electric current is passed through the gel and the RNA moves away from the negative electrode.
- The distance moved depends on the size of the RNA fragment.
- Since 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:

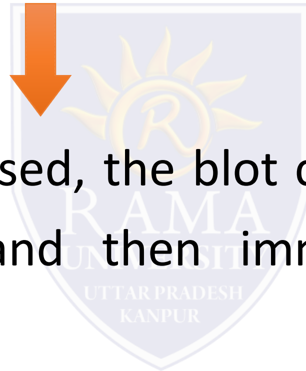
•A labelled probe specific for the RNA fragment in question is incubated with the blot.

•The blot is washed to remove non-specifically bount 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.

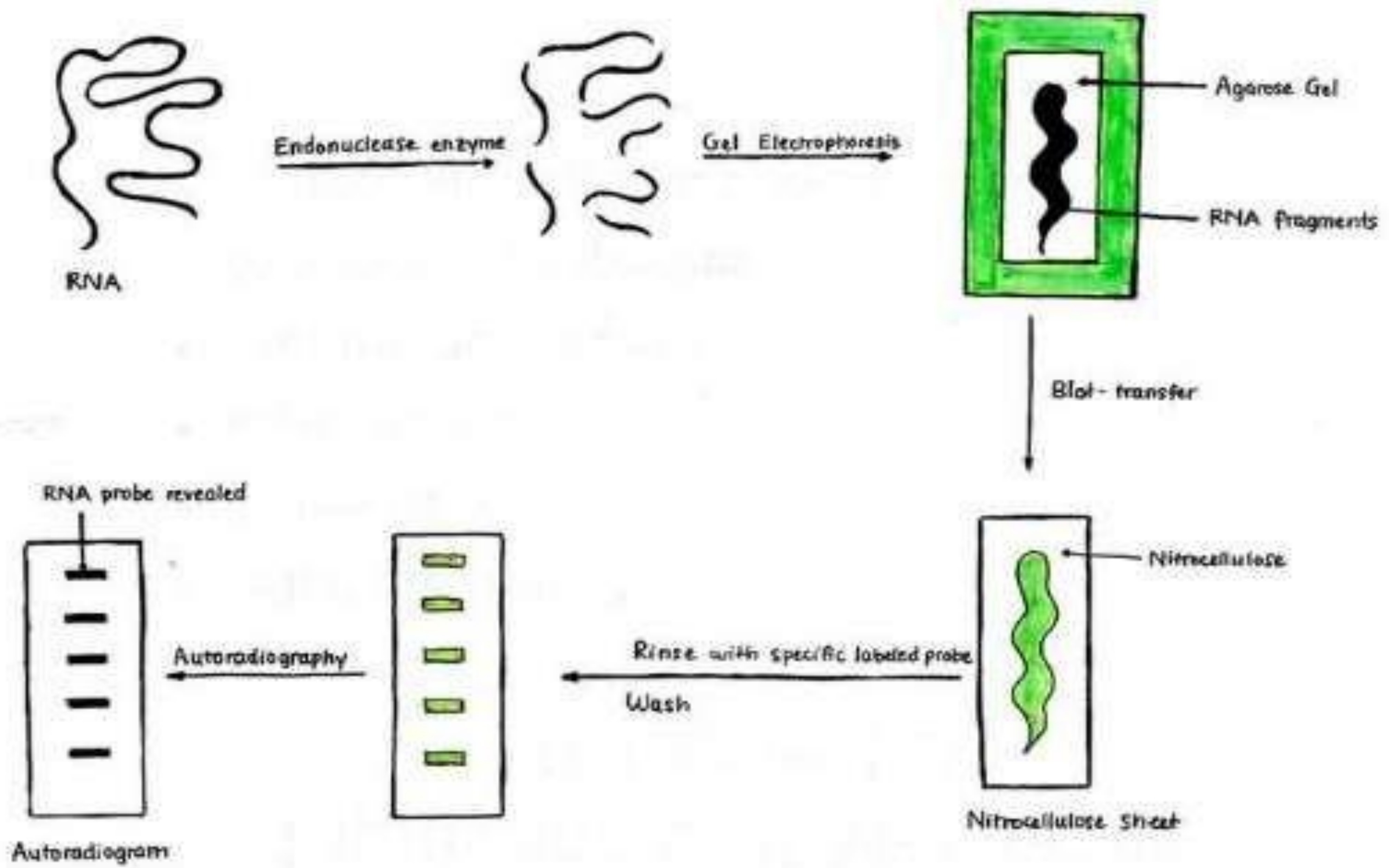
7.Detection:



- 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.


RNA
Endonuclease enzyme
Gel Electrophoresis
Agarose Gel
RNA fragments
Blot- transfer
Nitrocellulose
Nitrocellulose sheet
Rinse with specific labeled probe
Wash
Autoradiography
RNA probe revealed
Autoradiogram

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 adges.
- 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.

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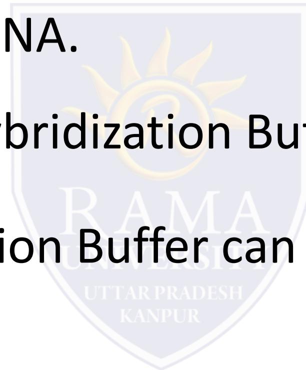
Ways to Increase the Sensitivity of Northern

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