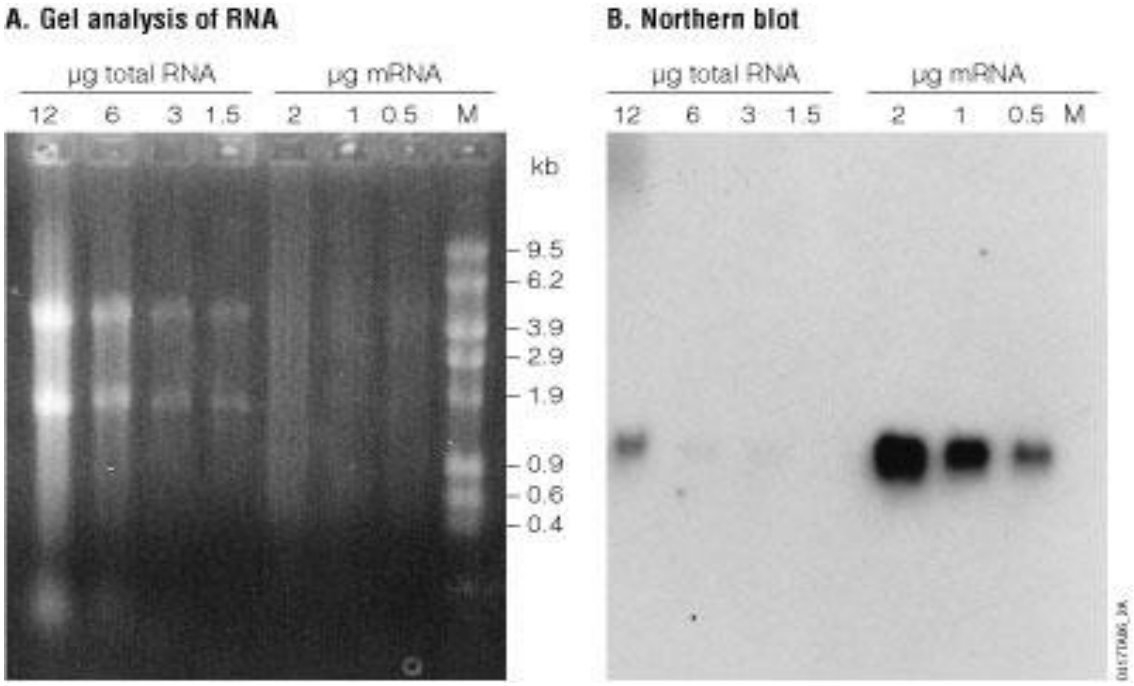

NORTHERN BLOTTING

Prepared by

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- The technique was developed by James Alwine, David Kemp, and George Stark at Stanford University in 1977.
- The Northern blot is used to detect the presence of particular mRNA in a sample.
- **The term "Northern" has no scientific significance.** It was given in connection with the name of blotting of DNA which is known as Southern Blotting after the name of the discoverer E. M. Southern.

- **Principle: The key to this method is transfer of RNA from gel followed by nucleic acid hybridization, which is the process of forming a double-stranded DNA-RNA hybrid molecule between a single-stranded DNA probe and a single-stranded target RNA.**
- The reactions are very specific – the probes will only bind to specific target with a complementary sequence. The probe can find one molecule of target in a mixture of millions of related but non-complementary molecules.

PROCEDURE

- Northern blotting procedure starts with extraction of total RNA from a homogenized tissue sample or from cells. Eukaryotic mRNA molecules can then be isolated through the use of oligo-dT cellulose chromatography to isolate only those RNAs with a polyA tail.
- RNA samples are then separated by gel electrophoresis. The RNA samples are most commonly separated on agarose gels containing formaldehyde as a denaturing agent for the RNA to limit secondary structure.
- The gels can be stained with ethidium bromide (EtBr) and viewed under UV light to observe the quality and quantity of RNA before blotting.
- Polyacrylamide gel electrophoeresis with urea can also be used in RNA separation but it is commonly used for fragmented RNA or microRNAs.
- An RNA ladder is often run alongside the samples on an electrophoresis gel to observe the size of fragments obtained.

Procedure [cont.]

- RNA samples, now separated by size, are transferred to a membrane through a capillary or vacuum blotting system.
- A nylon membrane with a positive charge is the most effective for use in northern blotting since the negatively charged nucleic acids have a high affinity for them.
- **The transfer buffer used for the blotting usually contains formamide because it lowers the annealing temperature of the probe-RNA interaction, thus eliminating the need for high temperatures, which could cause RNA degradation.**
- Once the RNA has been transferred to the membrane, it is immobilized through **covalent linkage to the membrane by UV light or heat**.

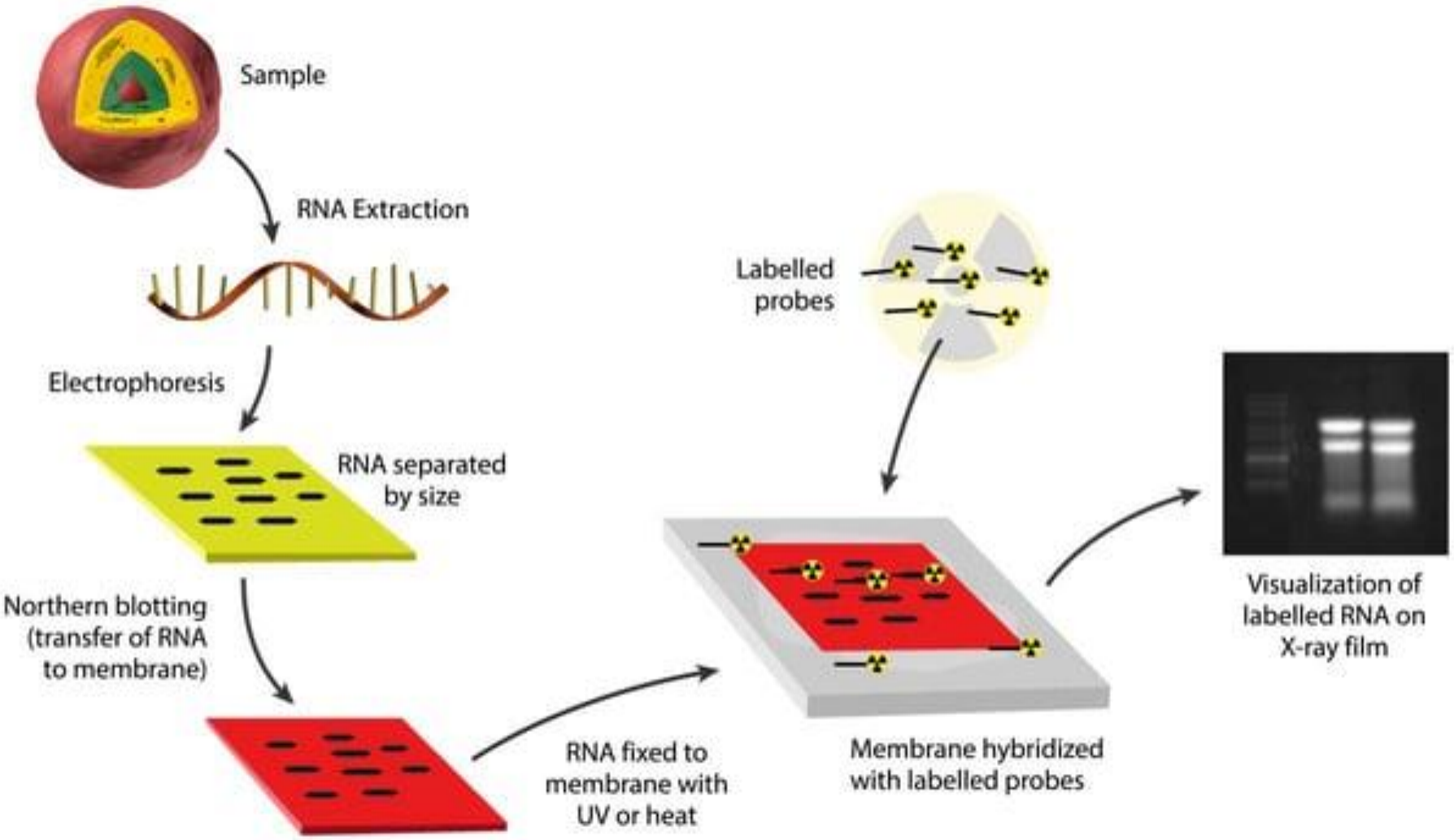
Procedure [cont.]

- After a probe has been labelled, it is hybridized to the RNA on the membrane.
- Experimental conditions that can affect the efficiency and specificity of hybridization include ionic strength, viscosity, duplex length, mismatched base pairs, and base composition.
- Probes for northern blotting are composed of DNA or RNA oligonucleotides with a minimum of 25 complementary bases to the target sequence. Commonly cDNA is created with labelled primers for the RNA sequence of interest to act as the probe in the northern blot.
- RNA probes (riboprobes) transcribed *in vitro* are able to withstand more rigorous washing steps preventing some of the background noise.
- The membrane is washed to ensure that the probe has bound specifically and to prevent background signals from arising. The hybrid signals are then detected.

Membrane used in Northern Blotting

- RNA does not bind to nitrocellulose membrane.
- Therefore, in Northern blotting, mRNAs are blot-transferred from the gel into a chemically reactive paper, instead of nitrocellulose membrane, where they are bound covalently.
- The **aminobenzyloxymethyl** paper used as membrane can be prepared from Whatman 540 paper by a series of uncomplicated reactions.
- Once covalently bound to this aminobenzyloxymethyl membrane, the mRNA is available for hybridization.
- Later, it was found that mRNA bands can be blotted directly into nylon membrane under appropriate conditions.

Procedure of Northern Blotting

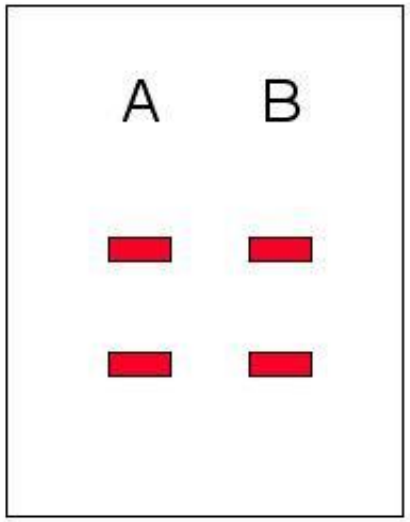


Dr. Subhadeep Sarker

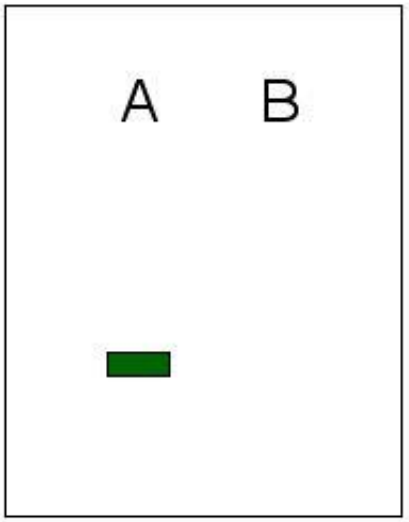
SOUTHERN BLOTTING	NORTHERN BLOTTING
1. Transfer of DNA.	1. Transfer of RNA.
1. Named after the discoverer E.M. Southern.	2. Since transfer of DNA is called Southern blotting, the name of Northern blotting is given to denote related but distinct procedure.
1. Requires denaturation of DNA to produce single stranded poly-nucleotide chain to facilitate hybridization.	3. Does not require denaturation as RNA is single stranded but requires destruction of any secondary structure formed by RNA.
1. Originally nitrocellulose membrane was used for transfer.	4. Originally aminobenzyloxymethyl membrane was used for transfer since RNA has very poor binding capacity towards nitrocellulose.

Northern Blotting for Study of Gene Expression

DNA from two tissues (A & B) probed with a particular gene fragment on a Southern blot



RNA from two tissues (A & B) probed with a particular gene fragment on a Northern blot



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Some Applications of Northern Blotting

- Observe a particular gene's expression pattern between tissues, organs, developmental stages, environmental stress levels, pathogen infection, and over the course of treatment.
- Used to show overexpression of oncogenes and down regulation of tumor-suppressor genes in cancerous cells.
- Detecting a specific mRNA in sample, used for screening recombinants which are successfully transformed with transgene.
- mRNA splicing studies