NORTHERN BLOTTING

- Northern blotting is a technique in molecular biology used to study gene expression by detecting RNA (or specifically mRNA) in a sample.
- It is similar in procedure to southern blotting and Western blotting. The key difference being that RNA is the subject rather than DNA or protein respectively
- In northern blotting, the hybridization probes are oligonucleotides (which may be either DNA or RNA, with a minimum of 25 nucleotides).
- The technique was developed by James Alwine, David Kemp and George Stark in 1977.

PROCEDURE :-

1. Extraction / Isolation of RNA - from tissue sample.
2. Electrophoresis of RNA - In agarose gel
3. Transferred to nylon paper - By capillary action.
4. Stabilization - by Heat or UV rays.
5. Hybridization - with radiolabelled probe.
6. Detection - By X-rays.

STEP I :- Isolation of RNA

RNA is isolated from several biological samples (Eg: various tissues). RNA is more susceptible to degradation than DNA.

STEP II :- Agarose Gel electrophoresis

The sample's are loaded on gel and RNA samples are separated according to their size on an agarose gel, using an electric field.

STEP III :- Blotting / transfer to nylon paper

The gel in then blotted on a nylon membrane or a nitrocellulose filter paper so it may be accessible to a probe for hybridization and detection.

The separated mRNA bands are then blotted on chemically reactive filter paper.

STEP IV :- Hybridization

The membrane is placed in a dish containing hybridization buffer with a labelled probe.

Thus, it will hybridize to the RNA on the blot that corresponds to the sequence of interest.

Later, the membrane is washed to remove unbound probe.

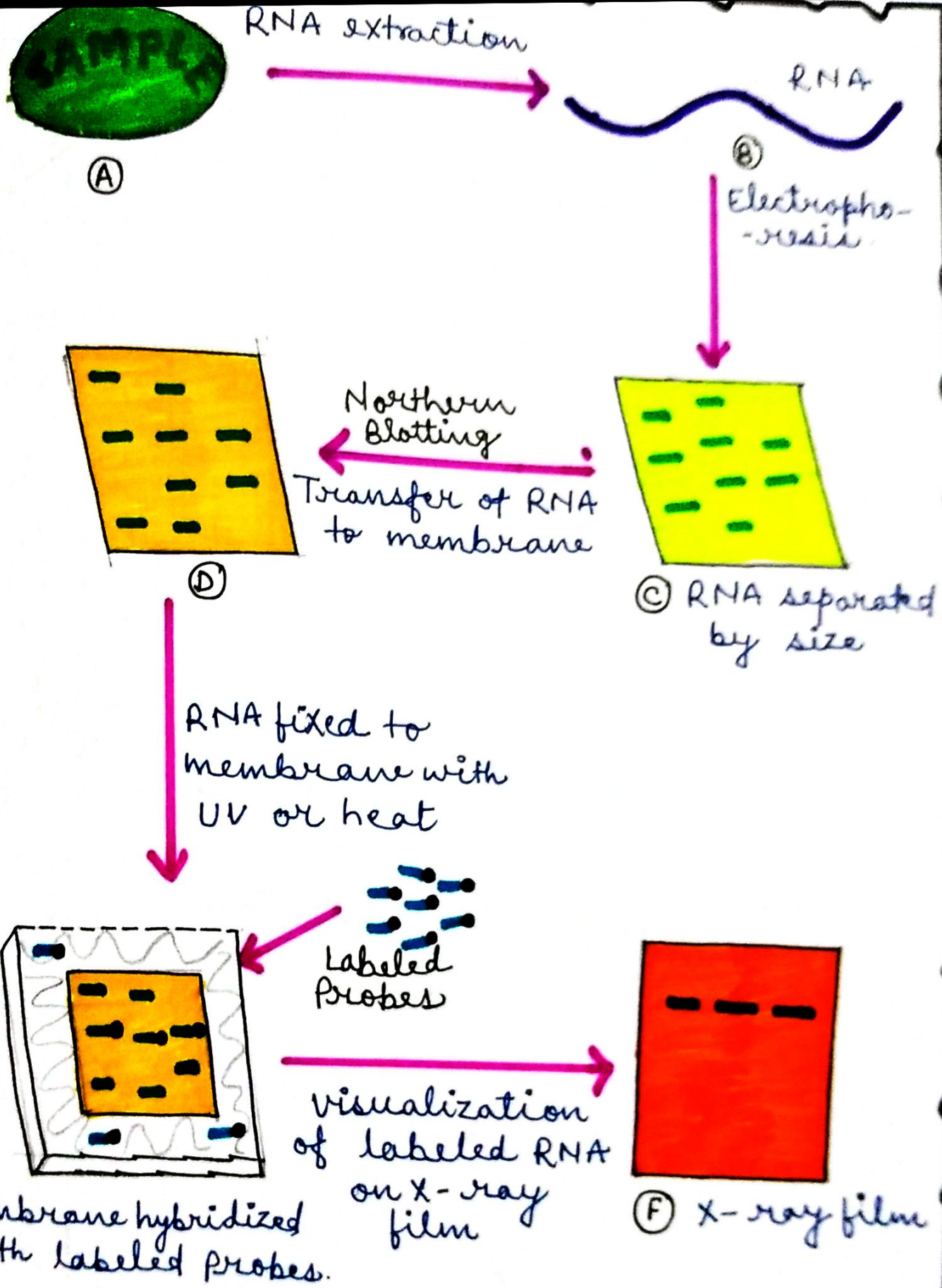

Fig: Procedure of Northern Blotting Techniques.

STEP V :- Washing and detection

The unbound probe on the membrane is washed. The labeled probe is detected via autoradiography, which results in the formation of a dark band on an X-ray film. Now, the expression patterns of the seq. of interest in the different sample can be compared.

APPLICATIONS

- A standard for the study of gene expression at the level of mRNA (messenger RNA transcripts).
- Detection of mRNA transcript size.
- Study RNA degradation.
- observe particular gene expression pattern between tissue, organ, and development stages.
- study RNA half-life.
- It is to seq. analysis, genome determination and protein structure.
- study RNA spliting.