

### 8.3 NORTHERN BLOTTING

The procedure for Northern blotting is similar to that of Southern blotting with few differences. However RNA sequences are separated on denaturing agarose gel incorporated with formaldehyde. The northern blotting is completed in the following steps:

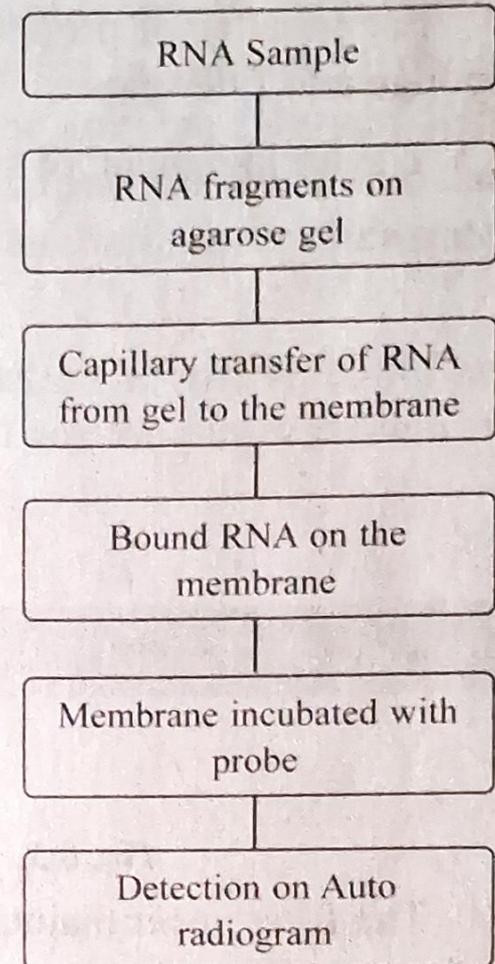
- Step 1: RNA Gel Electrophoresis:** The RNA molecules isolated from cells are separated according to size by gel electrophoresis. The RNA molecules are negatively charged so they move from negative to positive electrode during gel electrophoresis.
- Step 2: Denaturation:** Although RNA is a single stranded molecule through it need to be denatured because RNA molecules fold onto themselves and because of intramolecular base pairing they form secondary structures so there is need to denature RNA by formaldehyde which is the denaturing agent.
- Step 3: Blotting and blocking:** The third step is blotting and blocking in which the separated RNA molecules are transferred from the gel to the suitable solid support such as nylon membrane. The method of transfer is similar to that was used in Southern blotting.

**Step 4: Hybridization with Probe Washing:** In this step many copies of probe are added. The labeled probe is built to the target RNA molecules with complementary sequences. When nylon membrane are incubated with probe molecules, then these probe will specifically binds with target RNA molecules. The unbound probes are removed by washing.

**Step 5: Detection:** The detection and visualization method depends upon the type of labeled molecule used for hybridization. The probe can be visualized by autoradiography, fluorescence or color intensity on the membrane.

### Application of Northern Blotting

1. Northern blotting can be used to study the expression of specific genes in different tissue or under different conditions.
2. It is used to study the expression and processing of small RNA's, such as micro RNA's and si RNA's.
3. It is used to study RNA-splicing and degradation.
4. It is used in diagnosis of several diseases including a viral infection.



**Fig. 8.1.** Flowchart of northern blotting.