Northern Blotting: RNA Detection Using DNA Hybridization

Northern blotting is a laboratory technique used to detect **specific RNA sequences** in a sample. It is similar to **Southern blotting (for DNA)** but is used for **RNA analysis**. This technique relies on **DNA-RNA hybridization**, where a labeled **DNA or RNA probe** binds to its complementary **RNA sequence**.

Steps of Northern Blotting

1. RNA Extraction

- Extract **RNA** from cells or tissues using methods like **phenol-chloroform extraction**.
- Treat with **DNase** to remove any contaminating DNA.

2. Gel Electrophoresis

- RNA is loaded onto an **agarose gel with formaldehyde** to keep it denatured.
- Electrophoresis is run to **separate RNA molecules by size**.

3. Transfer to a Membrane (Blotting)

- The separated RNA is transferred onto a **nylon or nitrocellulose membrane** using **capillary action or electrophoretic transfer**.

4. Hybridization with a Probe

- A **radioactive or fluorescently labeled DNA/RNA probe** (complementary to the target RNA sequence) is added.
- The probe binds (hybridizes) to its specific RNA sequence.

5. Detection

- Excess probe is washed away.
- The labeled probe is detected using **X-ray film (autoradiography) or fluorescence imaging**, revealing the **target RNA bands**.

Applications of Northern Blotting

✅ **Gene Expression Analysis** – Determines **when and where** a gene is active.
✅ **Viral RNA Detection** – Identifies **viral infections** like HIV or influenza.
✅ **Cancer Research** – Studies **changes in gene expression in tumors**.