

## **Principle of Northern hybridization**

The principle of Northern hybridization, also known as RNA hybridization or RNA blotting, is a technique used to detect and analyze specific RNA molecules in a sample. Northern hybridization is based on the hybridization of complementary RNA probes with target RNA sequences of interest. It allows researchers to study gene expression patterns, identify RNA transcripts, and analyze RNA processing events. Here is an outline of the principle:

**RNA sample preparation:** The RNA sample of interest is isolated from cells or tissues using appropriate methods, such as phenol-chloroform extraction or column-based purification kits. The RNA is then treated to remove any contaminating DNA and proteins.

**RNA separation by gel electrophoresis:** The RNA sample is subjected to gel electrophoresis, typically denaturing formaldehyde-agarose gel electrophoresis. This separates the RNA molecules based on their size, with larger RNA molecules migrating more slowly through the gel.

**Transfer to a solid support:** After gel electrophoresis, the separated RNA molecules are transferred from the gel to a solid support, such as a nitrocellulose or nylon membrane, in a process called blotting. This is achieved by capillary action or electroblotting, preserving the relative positions and sizes of the RNA molecules on the membrane.

**RNA fixation and cross-linking:** The transferred RNA on the membrane is usually fixed and cross-linked to the membrane by UV irradiation or chemical cross-linking agents, such as formaldehyde, to immobilize the RNA and prevent its diffusion or degradation during subsequent steps.

**Hybridization:** The membrane with the transferred RNA is incubated with a specific RNA probe that is complementary to the target RNA sequence of interest. The RNA probe is typically labeled with a detectable marker, such as a radioactive isotope, fluorescent dye, or enzyme, to enable visualization or detection. The probe binds specifically to its complementary target RNA sequence on the membrane through base pairing, forming an RNA duplex or hybrid.

**Washing:** After hybridization, the membrane is washed to remove any unbound or non-specifically bound probes. This step helps to reduce background noise and enhance the specificity of the hybridization signal.

**Detection:** The presence of the hybridized RNA-probe complexes is detected by imaging or visualizing the labeled probe using appropriate detection methods. This can include autoradiography for radioactive probes, fluorescence detection for fluorescent probes, or enzymatic detection for enzyme-labeled probes.

**Data analysis:** The obtained hybridization signals are analyzed to determine the presence or abundance of the target RNA sequence in the original sample. The signal intensity or presence can be compared to known standards or controls to provide qualitative or quantitative information about the target RNA.

Northern hybridization allows for the detection and analysis of specific RNA molecules in a complex mixture. It provides valuable insights into gene expression patterns, alternative splicing events, RNA processing, and RNA abundance in different tissues or under different experimental conditions. The technique has been widely used in molecular biology and genetic research.