Quadrant II-Transcript and related materials

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Blotting technique is a process of transfer of DNA, RNA and proteins onto a solid membrane, so they can be separated and it often follows the use of gel electrophoresis. It is a central technique for hybridization studies.

Gel electrophoresis- method to separate a mixed population of DNA, RNA and proteins in a matrix of agarose. Agarose is a natural linear polymer extracted from sea weed. Ethidium bromide is an intercalating agent commonly used as a florescent tag. It is commonly abbreviated as 'EtBr'. When the molecules on gel electrophoresis are exposed to UV light it will fluoresce with an orange color.

There are different types of blotting techniques:

The types of blotting technique depend upon the substance to be separated, blotting techniques may be – Southern blot, Northern blot or Western blot which separates DNA, RNA and proteins respectively.

1. Southern blotting technique- used to study DNA.
2. Northern blotting technique- used to study RNA.
3. Western blotting technique- used to study proteins.

NORTHERN BLOTTING TECHNIQUE

This procedure was developed by James Alwine and George Stark in the year 1979. This technique is a modified version of southern blotting technique. This technique provides information about the length of the RNA sequences and the presence of variations in the sequence. Northern blotting was employed as the primary technique for the analysis of RNA fragments. Northern blot utilizes size-dependent separation of RNA segments and

thus can be used to determine the sizes of the transcripts.

PRINCIPLE OF SOUTHERN BLOTTING TECHNIQUE:

The principle of the northern blot involves the transfer of biomolecules from one membrane to another. The RNA samples are separated on gels according to their size by gel electrophoresis. The separated RNA fragments are then transferred to a nylon membrane. Nitrocellulose membrane cannot be used as RNA does not bind effectively to the membrane. The RNA fragments on the membrane are detected by the addition of a labeled probe complementary to the RNA sequences present on the membrane. The hybridization forms the basis of the detection of RNA as the specificity of hybridization between the probe, and the RNA allows the accurate identification of the segments.

PROCEDURE

I. **Extraction and purification of RNA from the cells:** RNA is first isolated and separated from target cells.

II. **Separation and denaturation on gel electrophoresis:** The gel is prepared by adding formaldehyde which helps in denaturation of RNA. Smaller fragments move faster. Based upon their size, RNA fragments are separated on agarose gel.

III. **Blotting:**

- After gel electrophoresis RNA gel is removed from the tank and rinsed with water.
- RNA is transferred from gel to a chemically reactive paper.
- This paper used is amino-benzyl-oxy-methyl cellulose paper prepared from Whatmann filter paper No. 540 after a series of reactions.
- The membrane is overlaid with filter papers. The fry filter paper draws the buffer through the gel.
- This allows the transfer of RNA to membrane.
- Once the transfer is complete, the gel is removed.
- Membrane is baked at 80°C for 2 hours and the RNA are permanently fixed to the membrane.
- A probe containing sequence of interest is then hybridized or annealed with the immobilized RNA by placing the filter in a solution containing either radiolabeled RNA or DNA probes
- After hybridization, the membrane is thoroughly washed with buffer to remove the nonannealed probe is washed off.
- The hybridized regions are detected by autoradiography.

APPLICATIONS

- Direct study of gene expression at the level of mRNA for studied related. overexpression of cancer-causing genes.
- Used as a molecular tool for the diagnosis of specific diseases.
- Detection and quantification of specific mRNA transcript size.
- It is also used to study RNA degradation.
- Northern blotting technique is used to study RNA splicing.
- Also used to study RNA half-life.