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Blotting Techniques

8.1 INTRODUCTION

Blotting techniques are widely used analytical tools for the specific identification of desired DNA or RNA or protein fragments on to a blotting membrane.

This technique is accomplished by separation of macro molecules on the basis of electrophoresis and detection of desired fragment with a labeled probe.

There are four types of blotting techniques

1. Southern blotting: It was developed by a scientist named as Edward Southern. It is used for the analysis of DNA sequences.

2. Western blotting: It was developed by George Stark and is used for the detection and analysis of protein.

3. Northern blotting: It was developed by James Alwine and is used for the detection and analysis of RNA.

4. Eastern blotting: Eastern blotting is a technique used to detect specific post-translational modifications (PTMs) of proteins, such as glycosylation or lipidation, after they have been separated by electrophoresis and transferred to a membrane. Instead of using antibodies like in Western blotting, Eastern blotting typically uses lectins or chemical probes that specifically bind to functional groups added to proteins.

It is mainly applied in the identification and analysis of glycoproteins, lipid-modified proteins, and other protein modifications, especially in studies related to cell signaling, cancer biomarkers, and immune responses.

Let us discuss these techniques in detail:

8.2 SOUTHERN BLOTTING

It is the technique used for identification of DNA sequences named after the discoverer Edward M. Southern in 1975.

Principle: The southern blotting technique is based on nucleic acid hybridization in which DNA fragments are separated by gel-electrophoresis and is identified by labeled probe hybridization.

Procedure: There are various steps for Southern blotting

Step 1: DNA digestion: The chromosomal DNA of an organism is digested by restriction enzyme and targeted DNA sequence would be present within a specific restriction site.

Step 2: DNA Gel Electrophoresis: The DNA fragment get separated according molecular weights but the separated DNA fragments are double stranded the hybridization there is requirement of single stranded DNA fragments before moving to the next step, these DNA fragments are denatured in th mild alkali i.e. NaOH. This denatures the DNA by disrupting hydroge between the two complementary strands.

Step 3: Blotting: The third step is the blotting in which the DNA fragments are tra from the gel to a suitable membrane like nitrocellulose or nylon membrane days nylon membrane is preferred over nitro cellulose membrane because high tensile strength and a better binding capacity for nucleic acid.

Step 4: Baking and blocking: After the DNA of interest is bound on the memb transferred into autoclave to fix the membrane.

The membrane is further treated with Casein or Bovine serum albumin which saturates all the binding site of the membrane.

Step 5: Hybridization and washing: In this step many copies of nucleic acid p added. The labeled probe is bind with complementary sequences to the interest and form double stranded DNA hybrid.

So the probe hybridization with the DNA fragment that is complementary t unbounded probes are removed by washing.

Step 6: Visualization by Autoradiogram: In this step detection of the boun with DNA fragment is carried out by locating double stranded hybrid for hybridization this probe can be visualized by autoradiography, fluoresc colored pattern on the membrane.

Application of Southern blotting

1. It is used to detect DNA in given sample.
2. It is used to identify desired gene of interest through gene mapping.
3. It is used in diagnosis of disease caused by genetic defects.
4. It is used in DNA fingerprinting which has