Week 12. Hybridization Methods

HYBRIDIZATION METHODS

Department of Microbiology

- Detection of specific genes in the DNA of disease agents or the host's own cells in various materials (tissue, organ, cell culture, excreta, secretions, etc.) using labeled probes
- Probe hybridization methods

Hybridization Methods

1. Southern Blot Hybridization
2. Northern Blot Hybridization
3. Dot Blot Hybridization
4. In situ Hybridization

Southern Blot Hybridization

1. Genomic DNA Isolation
2. Specific Restriction Enzyme (RE) Digestion
3. Fragment Electrophoresis
4. Transfer of Bands to a Membrane (Southern transfer)
5. Denaturation and Fixation
6. Hybridization
7. Autoradiography

1. Genomic DNA Isolation

- The process involves isolating DNA from materials.
- Cell walls and outer membranes of microorganisms are lysed using lysozyme and other enzymes.
- SDS (sodium dodecyl sulfate), proteinase K, and RNases are added to the extraction solution to remove components other than DNA.
- EDTA is added to protect the DNA from endogenous nucleases.

2. Specific Restriction Enzyme (RE) Digestion

- DNA is cut into numerous smaller and larger segments using specific restriction enzymes like EcoRI, Hind III, and BamHI.
- This process is typically performed at 37°C for 4-6 hours.

3. Fragment Electrophoresis

- The DNA fragments are separated by size on an agarose gel, typically overnight at 40-50V.
- The bands that form can be visualized under UV light after staining with ethidium bromide.

4. Transfer of Bands to a Membrane (Southern Transfer)

- The DNA bands are transferred from the agarose gel to a nylon or nitrocellulose membrane using methods such as electrotransfer or alkali capillarity.
- Nylon membranes are often preferred.

5. Denaturation and Fixation

- The DNA bands on the membrane are denatured with 0.5 N NaOH and then heated to 80°C to fix the single-stranded DNA to the membrane.

6. Hybridization

- The membrane is pre-hybridized for 2-4 hours at 60-65°C.
- A labeled DNA or RNA probe (e.g., with 32P or biotin) is then added.
- The single-stranded DNA on the membrane hybridizes with the complementary probe, forming a double-stranded DNAxDNA duplex.
- This process is typically left overnight.

7. Autoradiography

- An X-ray sensitive film is placed over the nylon membrane and left at -70°C overnight.
- After the film is developed, the regions where the labeled probes hybridized to the target DNA sequences appear as black spots.
- For biotin-labeled probes, a color change is used to locate the target DNA segment.

Northern Blot Hybridization

- This method analyzes RNA instead of DNA, specifically mRNA, viral RNA, or total RNA.
- It is used to analyze genes at the transcription level, determine mRNA levels in tissues and organs, and detect differences between viral RNAs.

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1. RNA Isolation
2. RNA Separation via Electrophoresis
3. RNA Transfer to Nylon Membrane (Northern Transfer)
4. Hybridization with Labeled DNA or RNA Probes
5. Visualization via Autoradiography

3. Dot Blot Hybridization

- In this method, nucleic acids from pathogenic materials are extracted, concentrated, and then spotted directly onto a nylon or nitrocellulose membrane in various dilutions.
- The samples are denatured and fixed onto the membrane.
- A specific, single-stranded probe labeled with 32P is added.
- The subsequent steps are similar to Southern Blot.
- Dark spots on the film indicate the hybridization regions.

4. In Situ Hybridization

- This method is used to detect microorganism DNA directly in infected tissue cultures, biopsy materials, tissue suspensions, or a drop of peripheral blood on a slide.
- After denaturation, a labeled cDNA probe (e.g., with 32P, biotin-avidin) is added to facilitate hybridization.
- The location of the microorganism's DNA can be easily determined.

IMMUNOGENIC SUBSTANCES DETECTION

- Biotechnological methods are used to detect pathogenic agents or the antigenic substances derived from them (proteins, glycoproteins, etc.).
- Immuno (Western) Blotting

1. Protein Dot Blot Assay
2. In Situ Immunoperoxidase Assay
3. Use of Monoclonal Antibodies

Immuno (Western) Blotting

- Proteins from the agent are purified, treated with SDS, and separated by their molecular weight using SDS-polyacrylamide gel electrophoresis.
- They are then transferred to nitrocellulose paper via electrotransfer.
- The paper strips are treated with polyclonal or monoclonal antibodies to allow them to bind to the proteins (antigens).
- After washing, secondary antibodies (e.g., goat anti-IgG) labeled with 32P or biotin are added.
- The results are evaluated by autoradiography for 32P-labeled antibodies or by a color change for biotin-labeled antibodies.

Protein Dot Blot Assay

- Specific monoclonal antibodies against the target agent are spotted onto nitrocellulose paper and dried.
- Supernatants from microorganism suspensions (protein antigens) from infected tissues are then dropped onto the dried antibodies.
- After incubation and washing, a biotin-labeled antiserum prepared against the agent is added.
- Avidin enzyme conjugate and a substrate are added, which leads to a color change, indicating a positive result.

In Situ Immunoperoxidase Assay

- Bacterial or viral proteins are detected in formalin-fixed infected tissue sections using polyclonal or monoclonal antibodies.
- After incubating the sections with specific antibodies and adding amino ethyl-carbazole as a substrate, the microbial proteins in the tissues are identified by a color change.

Use of Monoclonal Antibodies

- Monoclonal antibodies labeled with substances like fluorescein, peroxidase-antiperoxidase, biotin-avidin, radioisotopes, and enzymes are successfully used to detect microorganisms grown in tissues, tissue cultures, or embryonated eggs.