HYBRIDIZATION METHODS

Week 12

- Detection of disease agents in materials (tissue, organ, cell culture, excretion, secretion, etc.) or specific genes in cell DNA of these tissues with labeled probes
- Probe hybridization methods

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

Southern Blot Hybridization

1. Isolation of genomic DNA
2. Cutting with specific restriction endonuclease (RE) enzyme
3. Electrophoresis of fragments
4. Transfer of the bands on the membrane (Southern transfer)
5. Denaturation and fixation
6. Hybridization
7. Autoradiography

- Developed by Southern
- Specific sequences in isolated and then purified DNA of m.o. found in diagnostic materials are revealed with the help of labeled probes (DNA or RNA probe).

1. Isolation of genomic DNA

- Cell walls and outer membranes of m.o. are lysed using lysozyme and other enzymes.
- Components other than DNA are removed by adding SDS (sodium dodecyl sulfate), proteinase K and Rnase to the extraction solution.
- EDTA is added to protect DNA from endogenous nuclease.
- Phenol chloroform extraction in eukaryotes
- Cesium chloride density gradient centrifugation to remove polysaccharides

- The concentration of DNA; spectrometric or electrophoretic methods
- Tissue or organ cell DNA suitable extraction method
- Genetic materials belonging to plasmids, phages and viruses, with appropriate methods ...

2. Cutting with specific RE

- Enzymes like EcoRI, Hind III, BamHI
- One or two or more enzymes in the experiment
- 4-6 hours at 37C
- A large number of DNA segments, small and large, by cutting DNA from certain bases

3. Electrophoresis of Fragments

- Electrophoresis in agarose gel at 40-50 V overnight
- Fragments are ordered in descending order
- Bands formed on the gel can be stained with ethidium bromide and visible under UV-rays.

- Nearly 100 bands larger than 20 kb in a good sectionIn revealing the differences in DNAs
- RFLP, RAPD techniques in making restriction analysis
- PAGE is used alongside agarose gel

4. Transfer of the bands on the membrane (Southern transfer)

- Transfer of bands on agarose gel on nylon or nitrous cellulose membrane
- Electrotransfer, alkaline capillarity
- Nylon membranes are preferred.

5. Denaturation and Fixation

- DNA bands transferred onto nylon are dried
- Denatured with 0.5 N NaOH
- It is heated up to 80C, single strands are fixed on the membrane.
- Fixation process is done in vacuum to secure fixation

6. Hybridization

- 2-4 hours prehybridization at 60-65C
- Washing
- Labeled DNA probe (32P or biotin) or RNA probe addition
- Single-stranded and labeled probe on the membrane are mutually joined by non-covalent bonds with the single-stranded DNA segment that is self-complementary and the double-stranded DNAxDNA duplex is formed (hybridization).
- One night
- Washing

7. Autoradiography

- A film sensitive to X-rays is covered on the nylon membrane.
- It is kept at 70C for 1 night.Bath of the film
- Regions with target DNA sequences combined with labeled probes appear in black. These regions refer to the places between which the gene is localized.
- In biotin-labeled probes, the color change caused by avidin, peroxidase and chromogen substance indicates the location of the target DNA segment to be sought

Northern Blot Hybridization

- Instead of DNA, mRNA, viral RNA or total RNA
- Separation of RNA on gel, transfer to filter, hybridization with specific probe (RNA or DNA) and autoradiography Northern Blot Hybridization
- It is used to analyze genes at the transcription level, to determine the level of mRNA in tissues and organs, and to detect the difference between viral RNAs.

Northern Blot Hybridization

1. Isolation of RNA
2. Separation of RNA by electrophoresis
3. Transfer of RNA onto nylon membrane (Northern transfer)
4. Hybridization with labeled DNA or RNA probesImaging by autoradiography

- RNAs are separated from the DNA by selectively precipitation with lithium chloride or sodium acetate by precipitation method.
- No RE cutting required before separation of RNA molecules in agarose gelIf desired,
- Southern blotting can be performed by converting mRNA or viral RNA into cDNA with reverse transcriptase enzyme.

3. Dot Blot Hybridization

- Nucleic acids belonging to the causative agent are extracted from pathological materials and concentrated.
- Samples are diluted x2 or x5One drop of each dilution is applied to the nylon or nitrocellulose membrane.
- Fixed on the membrane by denaturationSpecific single strand probe marked with 32P is added
- Next transactions are as in Southern Blot
- Dark spots on film indicate hybridization zone

4. In Situ Hybridization

- Infected tissue cultures, biopsy materials, tissue suspensions are taken on a drop of peripheral blood on a slide.
- After denaturation, a labeled (32P, biotin-avidin)
- cDNA probe is added and hybridization is achieved.
- The location of M.o.'s DNA is easily determined.

DETERMINATION OF IMMUNOGENIC SUBSTANCES

- Biotechnological methods are used to reveal disease factors and antigenic substances (protein, glycoprotein, etc.) obtained from them.

1. Immuno (Western) Blotting
2. Protein Dot Blotting Experiment
3. In Situ Immunoperoxidase Assay
4. Using Monoclonal Antibodies

Immuno (Western) Blotting

- Proteins belonging to the agents are obtained in pure form and treated with SDS and then separated according to their molecular weights by SDS-polyacrylamide gel electrophoresis.
- Transferred to nitrouscellulose papers by electrotransfer and air dried
- The paper strips are treated with polyclonal or monoclonal antibodies to allow the antibodies to bind with proteins (antigen).

- Washing
- Goat anti-IgG specific antibodies prepared against the first antibodies and labeled (32P, biotin) are placed on the washed paper strips.
- If 32P marked abs are used, the result is autoradiography, if biotiny ab is used, avidin-peroxidase conjugate 4-chloro-1-naphtol substrate is added to evaluate according to the color change.

Protein Dot Blot Assay

- Specific monoclonal abs against the target agent are put on nitrocellulose papers and 10-15 min. Dried
- Supernatants of microorganism suspensions (protein antigen) prepared from infected tissues are dripped onto the antibodies dried on filter paper.
- 2 hours standby (ab-ag conjugate)
- The filter is washed, antiserum prepared against the agent and labeled with biotin is dropped onto the conjugate and incubated for 2 hours and washed.
- Avidin enzyme conjugate and substrate are added.

In Situ Immunoperoxidase Assay

- By using polyclonal or monoclonal antibodies, bacterial or viral proteins are detected in formalin-fixed infected tissue sections. Sections are incubated with specific ab, amino ethyl-carbazole is added as substrate. Microbial proteins in tissues are detected according to color change.

Use of Monoclonal Antibodies

- Fluorescein, peroxidase-antiperoxidase, biotin-avidin, radioisotopes, enzymes, etc. Monoclonal antibodies marked with are used successfully.