Review of Nucleic Acid Labeling, Northern Blot, Southern Blot, and DNA Sequencing (Sanger Method)

1. Labeling of Nucleic Acids (DNA and RNA)

Nucleic acid labeling is a technique used to trace or detect DNA and RNA molecules in various biological applications. These methods are essential for:

- Investigating nucleic acid–protein interactions
- Identifying specific nucleotide sequences
- Performing molecular hybridization experiments (e.g., Northern blot and Southern blot)
- Real-time PCR assays and diagnostic tests

➢ Types of Labels

A. Radioactive (Hot) Labels

These labels incorporate radioactive isotopes into nucleic acids. Commonly used isotopes include:

- **[^{32}P] Phosphorus-32** – incorporated into phosphate groups of DNA/RNA
- **[^{3}H] Tritium** – incorporated into nucleobases
- **[^{125}I] Iodine-125** – attached to modified nucleotides

Radioactive labeling provides high sensitivity but requires specialized handling.

B. Non-Radioactive (Cold) Labels

Chemical, enzymatic, or fluorescent tags that enable safer detection.

a. Fluorescent Labels

- *Cy3, Cy5*: produce detectable fluorescent signals
- *Alexa Fluor dyes*: highly sensitive and suitable for imaging

b. Enzymatic Labels

- *Biotin*: detected using streptavidin conjugated to enzymes (e.g., peroxidase)
- *Digoxigenin (DIG)*: detected with specific anti-DIG antibodies

c. Chemical Labels

- Intercalating or binding dyes such as acridine orange

d. Modified Oligonucleotide Probes

- Chemically modified with amino, thiol, or other reactive groups.

2. Southern Blot

The Southern blot, developed by Edwin Southern in 1975, is a method used to detect specific DNA sequences within a complex genomic sample. Applications include:

- Identification of mutations
- Gene mapping
- Verification of gene presence in a genome

➢ Principle and Steps

1. **DNA Extraction and Restriction Digestion**
   - Genomic DNA is isolated and digested using restriction enzymes that cut at specific sequences.
2. **Agarose Gel Electrophoresis**
   - Digested fragments are separated by size.
   - The gel is then treated with an alkaline solution to denature double-stranded DNA into single-stranded DNA.
3. **Transfer to a Membrane**
   - DNA fragments are transferred onto a nylon or nitrocellulose membrane by capillary action, electrotransfer, or pressure blotting.
4. **Fixation**
   - Single-stranded DNA is immobilized on the membrane.
5. **Hybridization with a Labeled Probe**
   - A labeled probe complementary to the target sequence is incubated with the membrane.

6. **Detection**
    - Non-specifically bound probes are washed away.
    - Visualization:
        - **Autoradiography** for radioactive probes
        - **Chemiluminescence** for enzyme-linked probes
        - **Fluorescence** for fluorophore-labeled probes

A detected signal confirms the presence of the target DNA sequence.

3. Northern Blot

The Northern blot is a molecular biology technique used to detect and analyze specific RNA molecules within a complex mixture. It provides information about RNA **size**, **abundance**, and **expression patterns** under various biological conditions.

Steps of the Northern Blot Procedure

1. **Extraction of Total RNA**
   Includes rRNA, tRNA, mRNA, and other RNA species.
2. **RNA Denaturation**
   Achieved by heat (95°C) or chemical denaturants (e.g., glyoxal, formaldehyde) to prevent secondary structures.
3. **Agarose Gel Electrophoresis**
   Denaturing gels allow separation of RNA molecules based on size.
4. **Transfer to a Membrane**
   RNA is transferred to a nylon or nitrocellulose membrane (see schematic).
5. **Hybridization with a Labeled Probe**
   A probe is a DNA or RNA fragment labeled for detection.
6. **Stringent Washing**
   Removes probes bound non-specifically.
7. **Signal Detection**
    - For radioactive probes: autoradiography using X-ray film
    - For fluorescent or enzymatic probes: fluorescence imaging or chemiluminescence.

4. DNA Sequencing: Sanger Method

The Sanger sequencing method, also known as the **chain-termination method**, is a classical and widely used approach for determining the nucleotide sequence (A, T, C, G) of DNA.

A. Background

Developed by Frederick Sanger in 1977, this method earned him the 1980 Nobel Prize in Chemistry. It enabled precise determination of DNA sequences and laid the foundation for modern genetics.

B. Principle

Sanger sequencing relies on **dideoxynucleotides (ddNTPs)**, modified nucleotides that lack a 3'-OH group. Their incorporation terminates DNA chain elongation.

C. Key Steps:

- **DNA synthesis**
  The target DNA is amplified and a primer anneals to initiate DNA polymerization.
- **Chain termination reactions**
  The reaction mixture contains:
  - Standard dNTPs
  - Fluorescently or radioactively labeled ddNTPs
    Incorporation of a ddNTP terminates the chain, generating fragments of varying lengths.
- **Separation and detection**
  DNA fragments are separated by capillary or gel electrophoresis.
  Each ddNTP emits a specific signal allowing sequence determination based on fragment length and label.

D. Required Reagents and Equipment

- Template DNA
- Primer complementary to the target region

- DNA polymerase
- Labeled ddNTPs (ddA, ddT, ddC, ddG)
- Electrophoresis equipment
- Detection system for fluorescence or radioactivity

E. Sanger Sequencing Workflow

1. **Sample preparation**
   Isolation and optional amplification of the target DNA.
2. **Sequencing reaction**
   Incorporation of dNTPs and ddNTPs results in terminated fragments.
3. **Fragment separation**
   Electrophoresis sorts fragments by size.
4. **Data analysis**
   Detection of labeled fragments produces a chromatogram. Each peak corresponds to a specific base at a defined position.

F. Evolution Toward Modern Technologies

Although largely replaced by **Next-Generation Sequencing (NGS)** for high-throughput applications, Sanger sequencing remains widely used for:

- Verification of cloned DNA
- Sequencing small genomic regions
- Clinical confirmation of mutations

It remains the gold standard for accuracy in DNA sequence determination.