ELECTROPHORASIS UNIT III

Principle, procedure and applications of native polyacrylamide gel electrophoresis

Native polyacrylamide gel electrophoresis (PAGE) is a widely used technique for the separation and analysis of proteins and nucleic acids based on their size and charge. It is called "native" because the molecules are not denatured, which means they retain their native shape and structure.

Principle: The principle of native PAGE is based on the fact that different biomolecules have different charge-to-mass ratios. When an electric field is applied to a gel matrix containing the sample, the molecules migrate through the gel based on their charge-to-mass ratio, with smaller molecules migrating faster than larger molecules.

Procedure: The procedure for native PAGE involves the following steps:

Preparation of the gel: A gel matrix is prepared by polymerizing acrylamide and bisacrylamide monomers with a crosslinker to form a porous gel matrix. The percentage of acrylamide and bisacrylamide used in the gel determines the pore size of the gel, which affects the separation of the molecules.

Preparation of the sample: The sample is prepared by mixing the biomolecules with a loading buffer containing a tracking dye, which allows for visualization of the sample during electrophoresis.

Loading the sample: The sample is loaded onto the gel using a sample loading apparatus. The gel is then submerged in a buffer solution that conducts electricity.

Electrophoresis: An electric field is applied to the gel using an electrophoresis apparatus. The biomolecules migrate through the gel matrix based on their charge-to-mass ratio.

Staining and visualization: After electrophoresis, the gel is stained with a protein stain or a nucleic acid stain, and the bands are visualized using imaging techniques such as UV transillumination or chemiluminescence.

Applications: Native PAGE is used for a variety of applications, including:

- Separation and analysis of proteins and nucleic acids based on their size and charge.
- Detection of protein-protein interactions, protein-DNA interactions, and other biomolecular interactions.

- Quantification of the amount of protein or nucleic acid in a sample.
- Purification of proteins or nucleic acids by cutting out the band of interest from the gel.
- Characterization of protein isoforms or post-translational modifications.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a commonly used technique for the separation and analysis of proteins based on their molecular weight. Unlike native PAGE, SDS-PAGE denatures proteins, meaning that they lose their native shape and structure.

Principle: The principle of SDS-PAGE is based on the fact that SDS, a detergent, binds to proteins and denatures them, giving them a uniform negative charge per unit of mass. When an electric field is applied to a gel matrix containing the sample, the proteins migrate through the gel based solely on their size.

Procedure: The procedure for SDS-PAGE involves the following steps:

Preparation of the gel: A gel matrix is prepared by polymerizing acrylamide and bisacrylamide monomers with a crosslinker to form a porous gel matrix. The percentage of acrylamide and bisacrylamide used in the gel determines the pore size of the gel, which affects the separation of the proteins.

Preparation of the sample: The sample is prepared by mixing the protein sample with a loading buffer containing SDS, which denatures the proteins and gives them a uniform negative charge per unit of mass. The sample is also typically treated with a reducing agent such as beta-mercaptoethanol, which breaks disulfide bonds and unfolds any protein domains that may interfere with the migration through the gel.

Loading the sample: The sample is loaded onto the gel using a sample loading apparatus. The gel is then submerged in a buffer solution that conducts electricity.

Electrophoresis: An electric field is applied to the gel using an electrophoresis apparatus. The proteins migrate through the gel matrix based solely on their size.

Staining and visualization: After electrophoresis, the gel is typically stained with a protein stain such as Coomassie blue or silver stain, and the protein bands are visualized using imaging techniques such as UV transillumination or chemiluminescence.

Applications: SDS-PAGE is used for a variety of applications, including:

- Separation and analysis of proteins based on their molecular weight.
- Purification of proteins by cutting out the band of interest from the gel.
- Quantification of the amount of protein in a sample.
- Characterization of protein subunits and oligomeric complexes.
- Western blotting, which involves transferring the separated proteins onto a membrane and probing with specific antibodies to detect the presence of a particular protein.

Isoelectric focusing

Isoelectric focusing (IEF) is a technique used to separate and analyze proteins based on their isoelectric point (pI), which is the pH at which a protein has a net charge of zero. In IEF, a pH gradient is established in a gel matrix, and proteins are then separated based on their pI.

Principle: The principle of IEF is based on the fact that proteins have different charges at different pH values. In an electric field, proteins will migrate towards the electrode of opposite charge. If the pH of the environment is such that the protein has a net charge of zero, it will stop migrating and will remain in that position. By creating a pH gradient in the gel, each protein will migrate until it reaches the pH at which it has a net charge of zero, resulting in the separation of the proteins based on their pI.

Procedure: The procedure for IEF involves the following steps:

Preparation of the gel: A gel matrix is prepared with a pH gradient. This can be achieved by mixing ampholytes, which are small molecules that have acidic and basic groups, with the gel matrix. Alternatively, a precast gel with a pH gradient may be used.

Preparation of the sample: The protein sample is prepared by mixing it with a solution containing a detergent and a reducing agent. The detergent solubilizes the proteins and the reducing agent breaks disulfide bonds, which can interfere with the separation.

Loading the sample: The sample is loaded onto the gel using a sample loading apparatus.

Electrophoresis: An electric field is applied to the gel using an electrophoresis apparatus. Proteins migrate through the pH gradient until they reach the pH at which they have a net charge of zero.

Staining and visualization: After electrophoresis, the gel is typically stained with a protein stain such as Coomassie blue or silver stain, and the protein bands are visualized using imaging techniques such as UV transillumination or chemiluminescence.

Applications: IEF is used for a variety of applications, including:

- Separation and analysis of proteins based on their pI.
- Purification of proteins by cutting out the band of interest from the gel.
- Identification of post-translational modifications such as phosphorylation, which can change the pI of a protein.
- Characterization of protein subunits and oligomeric complexes based on their pI.

Two dimensional gel electrophoresis and agarose gel electrophoresis

Two-dimensional gel electrophoresis (2D gel electrophoresis) and agarose gel electrophoresis are two additional types of gel electrophoresis used to separate and analyze proteins and nucleic acids, respectively.

Two-dimensional gel electrophoresis: Two-dimensional gel electrophoresis is a technique used to separate complex mixtures of proteins based on both their isoelectric point (pI) and their molecular weight. The procedure involves two separate electrophoresis steps: the first separates proteins based on their pI using isoelectric focusing, and the second separates the proteins based on their molecular weight using SDS-PAGE. The resulting 2D gel image provides a two-dimensional map of the separated proteins.

Agarose gel electrophoresis: Agarose gel electrophoresis is a technique used to separate and analyze nucleic acids, such as DNA and RNA, based on their size. The procedure involves the separation of nucleic acid fragments in a gel matrix made from agarose, a polysaccharide derived from seaweed. The gel matrix creates a molecular sieve that separates the nucleic acid fragments based on their size, with smaller fragments migrating further through the gel than larger fragments. The separated fragments can then be visualized using a stain that intercalates with the DNA, such as ethidium bromide.

Applications:

- Two-dimensional gel electrophoresis and agarose gel electrophoresis have a wide range of applications in molecular biology and biochemistry. For example:
- Two-dimensional gel electrophoresis can be used to analyze changes in protein expression levels and modifications in response to various stimuli, such as changes in growth conditions or disease states.
- Agarose gel electrophoresis can be used to analyze the size of nucleic acid fragments, such as PCR products, restriction fragments, and genomic DNA.
- Both techniques can be used in conjunction with other techniques, such as western blotting or hybridization, to identify specific proteins or nucleic acids.

Southern blotting;

Blotting is a technique used in molecular biology to detect the presence of specific DNA sequences in a sample. The technique is named after its inventor, Edwin Southern.

The procedure involves the separation of DNA fragments by gel electrophoresis, followed by transfer of the separated fragments onto a membrane. The membrane is then hybridized with a labeled DNA probe that is complementary to the target sequence of interest. The probe binds specifically to the target sequence, allowing the location of the sequence to be identified.

The main applications of Southern blotting include:

DNA fingerprinting: Southern blotting can be used to analyze DNA from different individuals and identify unique DNA profiles, which can be used for forensic purposes.

Genetic diagnosis: Southern blotting can be used to identify the presence or absence of specific genes in individuals, which can be useful in diagnosing genetic disorders.

Genetic engineering: Southern blotting can be used to confirm the presence or absence of a specific DNA sequence in a genetically modified organism (GMO).

Epigenetics: Southern blotting can be used to detect DNA methylation patterns, which play a role in gene expression regulation.

Southern blotting is a sensitive technique, but it requires a relatively large amount of starting material and is time-consuming. It has been largely replaced by more sensitive and efficient methods, such as polymerase chain reaction (PCR) and next-generation sequencing (NGS), but it remains a valuable tool in molecular biology research.

Northern blotting

Northern blotting is a technique used in molecular biology to detect the presence of specific RNA sequences in a sample. The technique is similar to Southern blotting, which is used to detect specific DNA sequences, but uses RNA instead of DNA.

The procedure involves the separation of RNA fragments by gel electrophoresis, followed by transfer of the separated fragments onto a membrane. The membrane is then hybridized with a labeled RNA probe that is complementary to the target sequence of interest. The probe binds specifically to the target sequence, allowing the location of the sequence to be identified.

The main applications of Northern blotting include:

- Gene expression analysis: Northern blotting can be used to analyze the expression of specific genes in different tissues or under different conditions.
- mRNA quantification: Northern blotting can be used to quantify the amount of specific mRNA transcripts in a sample.
- Alternative splicing analysis: Northern blotting can be used to analyze the splicing patterns of specific genes, which can provide insight into gene function and regulation.
- Like Southern blotting, Northern blotting is a sensitive technique but requires a relatively large amount of starting material and is time-consuming. It has been largely replaced by more sensitive and efficient methods, such as reverse transcription-quantitative PCR (RT-qPCR) and RNA sequencing (RNA-seq), but it remains a valuable tool in molecular biology research.

western blotting;

Western blotting, also known as immunoblotting, is a technique used in molecular biology to detect and analyze specific proteins in a sample. The technique is named after its inventor, Dr. George Stark.

The procedure involves the separation of proteins by gel electrophoresis, followed by transfer of the separated proteins onto a membrane. The membrane is then incubated with a primary antibody that is specific to the protein of interest. The primary antibody binds to the protein and is detected by a secondary antibody that is labeled with an enzyme or a fluorescent tag. The labeled secondary antibody allows the visualization of the protein of interest.

The main applications of Western blotting include:

- Protein expression analysis: Western blotting can be used to analyze the expression of specific proteins in different tissues or under different conditions.
- Protein quantification: Western blotting can be used to quantify the amount of specific proteins in a sample.
- Protein-protein interaction analysis: Western blotting can be used to analyze protein-protein interactions, by detecting the presence of protein complexes.
- Disease diagnosis: Western blotting can be used to diagnose diseases by detecting specific disease-associated proteins.
- Western blotting is a widely used technique in molecular biology research, and it is a sensitive and specific method for protein detection. However, it requires a relatively large amount of starting material and can be time-consuming.

PCR - principle, types and application.

Polymerase chain reaction (PCR) is a widely used technique in molecular biology that allows the amplification of a specific DNA sequence in a sample. The technique was invented by Dr. Kary Mullis in 1983 and has revolutionized molecular biology research.

The principle of PCR involves the use of a heat-stable DNA polymerase enzyme, such as Taq polymerase, to amplify a specific DNA sequence through a series of temperature-controlled cycles. The basic steps of PCR include:

Denaturation: The double-stranded DNA template is denatured by heating to a high temperature (usually 95°C) to separate the two strands.

Annealing: The temperature is lowered to allow the primers, short single-stranded DNA sequences that are complementary to the flanking regions of the target DNA sequence, to anneal to the template DNA.

Extension: The temperature is raised to allow the DNA polymerase to extend the primers by adding nucleotides to the 3’ end of the annealed primer, creating a new strand of DNA.

The process is then repeated for multiple cycles, with each cycle doubling the amount of the target DNA sequence.

There are several types of PCR, including:

Standard PCR: The basic PCR technique described above.

- Reverse transcription PCR (RT-PCR): This technique is used to amplify RNA sequences by first converting them to complementary DNA (cDNA) using reverse transcriptase.
- Quantitative PCR (qPCR): This technique is used to quantify the amount of DNA or RNA in a sample, and is often used for gene expression analysis.
- Digital PCR: This technique allows the absolute quantification of DNA or RNA in a sample, and is useful for detecting low levels of target sequences.

The main applications of PCR include:

- Gene cloning: PCR is used to amplify specific DNA sequences that can be cloned into plasmids or other vectors for further analysis.
- Genetic diagnosis: PCR is used to diagnose genetic diseases by detecting specific mutations or variations in DNA sequences.
- Forensic analysis: PCR is used in forensic science to analyze DNA samples from crime scenes or other sources.
- Infectious disease diagnosis: PCR is used to diagnose infectious diseases by detecting the presence of specific pathogens in clinical samples.
- Overall, PCR is a powerful technique that has greatly advanced our understanding of genetics and molecular biology, and has numerous applications in research, medicine, and biotechnology.