RAMAIAH
College of Arts, Science
& Commerce - Autonomous

REF.: MSRCASC/MB/2024-2025/ Date: 15/10/2024

Circular

Department of Microbiology

Value Added Program in Analytical Techniques in Bacterial Proteomics

Department of Microbiology, is organizing value added program on **"Analytical Techniques in Bacterial Proteomics"** on October 2024.

The Add-on programme aims to train the students with hands on experience on the basic and advanced concepts of proteomics which involves- SDS PAGE analysis of proteins, Western Blotting, Protein sequence databases and analysis tools, Secondary structure prediction and analysis of tertiary structure of Proteins using bioinformatics tools.

The VAP will happen at Microbiology lab for 2 days' time.

The program is restricted for 30 students only

Date: October 2024
Venue: Microbiology (PG) Lab (Level -4)

HOD **Principal**

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Dr. Prasanna Srinivas R, Assistant Professor & Head (PG)
Dr. Snehalatha V, Associate Professor
Dr. Vemula Vani, Associate Professor
Mrs. Soumya S. Shanbhag, Assistant Professor & Head (UG)
Dr. Juliya Rani Francis, Assistant Professor
Dr. Nimitha Venugopal, Assistant Professor
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Dr. Vishal M, Assistant Professor
Dr. Prashanthi R, Assistant Professor
Dr. Vidya Jagadeeshan, Assistant Professor
Dr. Swathi D., Assistant Professor
Dr. Aravindganth, Assistant Professor
Dr. Swetha P, Assistant Professor
Dr. Tejaswini H K, Assistant Professor

Event Coordinators and Resource Persons

Dr. Vemula Vani,
Mrs. Soumya S. Shanbhag,
Dr. Nimita Venugopal C,
Dr. Swetha P,
Dr. Tejaswini HK
Department of Microbiology, MSRCASC.

REGISTRATION DETAILS

Total participants restricted to 30 (first-come-first-serve basis)
Registration: Registration is Free
Names should be given to the coordinator

RAMAIAH
College of Arts, Science & Commerce - Autonomous

सत्यमेव जयते
DEPARTMENT OF BIOTECHNOLOGY
Ministry of Science & Technology
Government of India

Value Added Programme

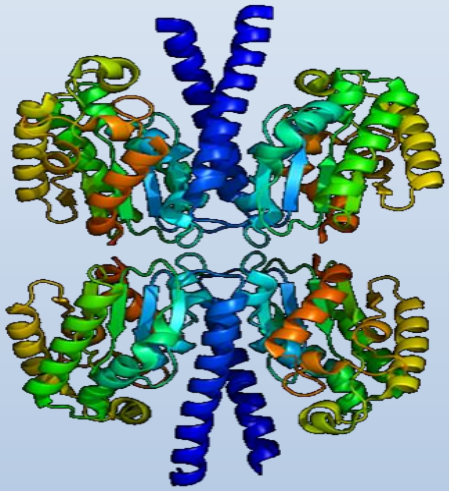
Under

DBT-Star College Scheme

on

ANALYTICAL TECHNIQUES IN PROTEOMICS

16th & 18th October, 2024



Organized by

Department of Microbiology

M. S. Ramaiah College of Arts, Science and Commerce-Autonomous

MSRIT Post, MSR Nagar, Bangalore - 560 054
www. msrcasc.edu.in
(Re-accredited "A" by NAAC,
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M. S. RAMAIAH COLLEGE OF ARTS, SCIENCE AND COMMERCE (MSRCASC)

Dr. M S Ramaiah, a visionary and philanthropist established "Gokula Education Foundation (GEF)", in the year 1962, to deliver education and healthcare for the betterment of mankind. Under the tutelage of GEF, Ramaiah College of Arts, Science and Commerce (RCASC) was established in 1994. MSRCASC is Re-accredited with "A" Grade by NAAC, Permanently affiliated to Bengaluru City University (BCU), and approved by AICTE. It is also recognized under section 2(f) & 12(B) of the UGC Act 1956. It has produced several rank holders and has alumni in distinguished institutions all over the world. The College has a legacy of organizing National Conferences and workshops in various disciplines of Science, Commerce and Management in addition to Quality Initiatives in Higher Education.

DEPARTMENT OF MICROBIOLOGY

The Department of Microbiology, established in the year 1999, offers both undergraduate and postgraduate courses. The faculty of the department are highly qualified with experience and expertise in various domains of Microbiology. The department has very good infrastructural facilities to carry out teaching and research activities. The theory and practical classes lay emphasis on 'problem based learning', knowledge content, utility value, application in real life, latest developments etc. The department is undertaking research projects in the major thrust areas of microbiology and attracted funds from various agencies. Also, the faculty of the department carry out multi-disciplinary research programs, encourage students to carryout in-house research projects, present papers, publish their research work and to participate in co-curricular and extra-curricular activities.

ABOUT THE ADD-ON PROGRAMME

The current 'omics' era, emphasizes the understanding and applications of molecular techniques, biological databases and computational tools for proteomics research.

Objective: The Value Added Programme aims to train the students with hands on experience on the basic and advanced concepts of proteomics which involves- SDS PAGE analysis of proteins, Western Blotting, Protein sequence databases and analysis tools, S and analysis of tertiary structure of Proteins using bioinformatics tools.

Outcome: This workshop envisions to make students well acquainted with emerging technologies in Proteomics which will help them to perform efficiently with better understanding and designing new approaches in the area of Proteomics research.

Modules

Module 1: Basic of Proteomics	Module 2: Advanced Concepts in Proteomics	Module 3: Experimental Techniques in Proteomics
•Introduction to proteomics •SDS PAGE analysis of proteins •Applications of SDS PAGE •Concepts of Blotting •Research approach for use of Western Blotting analysis •Bioinformatics in Proteomics	•Protein sequence Databases and Retrieval of sequences •Sequence alignment methods •Sequence similarity search •PDB and retrieval of protein structures •Rasmol analysis of protein 3D structures	•SDS PAGE analysis of proteins •Visualization of proteins on SDS PAGE gel •Western Blotting •Immuno-detection of proteins on membrane

Programme Schedule

Day 1	Introduction to the Workshop and Proteomics
	SDS PAGE analysis of proteins (Lecture)
	Applications of SDS PAGE
	Bioinformatics in Proteomics
	Protein sequence databases and Retrieval of Protein sequences from the protein databases
	Protein sequence alignment methods
	Protein sequence similarity search tools
	Protein Data Bank and retrieval of protein structures
	Visualization and analysis of 3D structure of proteins using Rasmol software
	SDS PAGE analysis of proteins (Experiment)
Day 2	Concepts of Blotting
	Research approach for use of western blotting analysis
	Western Blotting (Experiment)
	Visualization of proteins and Interpretation of Results
	Immuno-detection of proteins on membrane
	Discussion and Assessment
	Feedback and Valedictory

DEPARTMENT OF MICROBIOLOGY

REPORT ON

VALUE ADDED PROGRAM

ON

ANALYTICAL TECHNIQUES IN BACTERIAL PROTEOMICS

Title: Analytical Techniques in Bacterial Proteomics

Date: 16th, 18th, and 19th October 2024

Venue: 402 classroom & PG Microbiology Lab

Participants: III Year BSc students

Resource Persons: Dr. Vemula Vani, Mrs. Soumya S Shanbhag, Dr. Nimita Venugopal C, Dr. Swetha P and Dr. Tejaswini H K

No. of Participants: 29

Objectives:

The Value-Added Programme aims to train the students with hands on experience on the basic and advanced concepts of proteomics which involves- SDS PAGE analysis of proteins, Western Blotting, Protein sequence databases and analysis tools, analysis of tertiary structure of Proteins using bioinformatics tools.

Twenty-Nine students of III-year B.Sc. registered for the add on program. The sessions of the add on program were divided into 3 modules for 3 days.

On day one, the program was started by welcoming the students to the VAP by Mrs. Soumya S Shanbhag from department of Microbiology and giving the overview of the Value-Added Programs conducted by department of Microbiology for the academic year.

Day 1: Introduction to Bioinformatics in Proteomics

The workshop began with a comprehensive session on “Bioinformatics in Proteomics” delivered by Dr. Vemula Vani. The session included a presentation followed by hands-on training on various bioinformatics tools, which introduced the students to:

- Protein sequence databases
- Retrieval of protein sequences from these databases
- Protein sequence alignment methods
- Protein sequence similarity search tools
- Retrieval of protein structures from the Protein Data Bank
- Visualization and analysis of 3D protein structures using Rasmol software

Students gained valuable insights into the practical applications of bioinformatics tools for their research, enhancing their knowledge of protein sequence analysis.

Day 1: Afternoon Session - SDS PAGE Analysis of Proteins

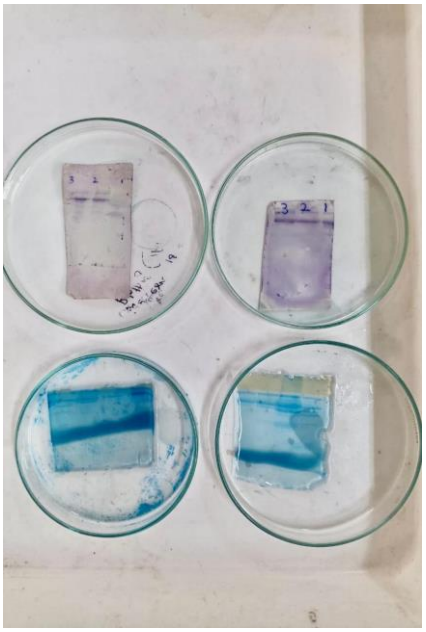
In the post-lunch session, Mrs. Soumya S. Shanbhag presented on “SDS PAGE Analysis of Proteins” and its applications. This was followed by hands-on training, where students performed SDS PAGE analysis under the guidance of Mrs. Soumya S. Shanbhag, Dr. Nimita Venugopal C, Dr. Swetha P., and Dr. Tejaswini H.K. The session provided students with practical experience in protein separation and analysis using SDS PAGE.

Day 2: Western Blotting Technique

The second day commenced with presentations on the “Concepts of Blotting” by Dr. Nimita Venugopal C. followed by the research approach for using Western Blotting analysis by Dr. Swetha P. This was followed by hands-on training in the Western Blotting technique, which continued throughout the day, giving students practical experience in the procedure. By the end of the day, students had acquired the skills necessary to perform Western Blotting for protein detection.

Day 3: Protein Visualization and Data Interpretation

The third day began with a continuation of the previous day's experiments, focusing on the visualization and interpretation of proteins. Students performed immuno-detection of proteins on a membrane and calculated Rf values for protein samples using a molecular marker. The sessions were interactive, allowing students to interpret their results and understand the relevance of their findings.



The day concluded with a discussion and assessment of the knowledge gained. Students were given a questionnaire related to the experiments they had performed, followed by a feedback session. The valedictory ceremony was presided over by Dr. Vemula Vani, Mrs. Soumya S. Shanbhag, Dr. Nimita Venugopal C, Dr. Swetha P., and Dr. Tejaswini H.K.

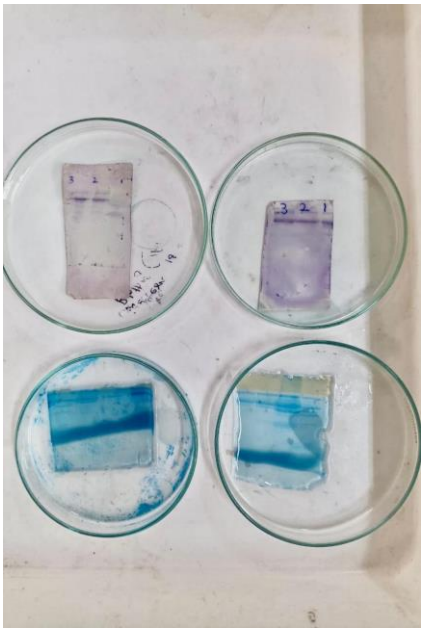
Outcomes of the Program

- This workshop envisions to make students well acquainted with emerging technologies in Proteomics which will help them to perform efficiently with better understanding and designing new approaches in Proteomics research.

Conclusion

The workshop successfully provided students with theoretical knowledge and practical experience in various proteomics techniques, including protein sequence analysis, SDS PAGE, and Western Blotting. The hands-on sessions and discussions helped reinforce the concepts learned, equipping participants with the tools to further their research in proteomics.

Glimpse of the Training Program




Silence
Please

RAMAIAH
College of Arts, Science
& Commerce - Autonomous

सत्यमेव जयते
DEPARTMENT OF BIOTECHNOLOGY
Ministry of Science & Technology
Government of India

VALUE ADDED PROGRAMME

On

Analytical Techniques

In

Bacterial Proteomics

16th & 18th October 2024

Organized by

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CONTENTS:

1. SDS PAGE analysis and visualization of proteins

Introduction

The separation of macromolecules in an electric field is called *electrophoresis*. A very common method for separating proteins by electrophoresis uses a discontinuous polyacrylamide gel as a support medium and sodium dodecyl sulfate (SDS) to denature the proteins. The method is called sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

SDS (also called lauryl sulfate) is an anionic detergent, meaning that when dissolved its molecules have a net negative charge within a wide pH range. A polypeptide chain binds to huge amounts of SDS in proportion to its relative molecular mass. SDS destroys most of the complex structure of proteins and imparts negative charge to the proteins which hence gets attracted towards anode (positively-charged electrode) in an electric field.

Polyacrylamide gels restrain larger molecules from migrating as fast as smaller molecules. Because the charge-to-mass ratio is nearly the same among SDS-denatured polypeptides, the final separation of proteins is dependent almost entirely on the differences in relative molecular mass of polypeptides. In a gel of uniform density the relative migration distance of a protein (Rf) is negatively proportional to the log of its mass. If proteins of known mass are run simultaneously with the unknowns, the relationship between Rf and mass can be plotted, and the masses of unknown proteins estimated.

Protein separation by SDS-PAGE can be used

1 To estimate relative molecular mass,

2 To determine the relative abundance of major proteins in a sample,

3 To determine the distribution of proteins among fractions.

4 To check the purity of protein samples and the progress of a fractionation or purification procedure can be followed.

5 For Western blotting applications

Chemical ingredients of the gel

- **Tris (tris (hydroxy methyl) aminomethane):** It has been used as a buffer because it is an innocuous substance to most proteins. Its pKa is 8.3 at 20°C and reasonably a very satisfactory buffer in the pH range 7.0 – 9.0.

- **Glycine (Aminoacetic Acid):** Glycine has been used as the source of trailing ion because the mobility of glycinate are such that the effective mobility can be set at a value below that of the slowest known proteins of net negative charge in the pH range.

- **Acrylamide:** It is a white crystalline powder. While dissolving in water, autopolymerisation of acrylamide takes place. It is a slow spontaneous process by which acrylamide molecules join together by head on tail fashion. But in presence of free radicals generating system, acrylamide monomers are activated into a free-radical state. These activated monomers polymerise quickly and form long chain polymers. A solution of these polymer chains becomes viscous but does not form a gel, because the chains simply slide over one another. Gel formation requires hooking various chains together.

- **Bisacrylamide (N,N'-Methylenebisacrylamide):** Bisacrylamide is the most frequently used cross linking agent for poly acrylamide gels. Chemically it is thought of having two-acrylamide molecules coupled head to head at their non-reactive ends.

- **Sodium Dodecyl Sulfate (SDS):** SDS is the most common dissociating agent used to denature native proteins to individual polypeptides. When a protein mixture is heated to 100 °C in presence of SDS, the detergent wraps around the polypeptide backbone. It binds to polypeptides in a constant weight ratio of 1.4 g/g of polypeptide. In this process, the intrinsic charges of polypeptides becomes negligible when compared to the negative charges contributed by SDS. Thus polypeptides after treatment becomes a rod like structure possessing a uniform charge density, that is same net negative charge per unit length. Mobilities of these proteins will be a linear function of the logarithms of their molecular weights.

- **Ammonium persulfate (APS)**: APS is an initiator for gel formation.

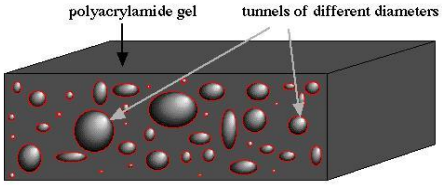
- **TEMED (N, N, N', N'-tetramethylethylenediamine**): Chemical polymerisation of acrylamide gel is used for SDS-PAGE. It can be initiated by ammonium persulfate and the quaternary amine, N,N,N',N'-tetramethylethylenediamine (TEMED). The rate of polymerisation and the properties of the resulting gel depends on the concentration of APS and TEMED.

- **Sample Buffer:** Most SDS PAGE sample buffers contain the following: SDS (sodium dodecyl sulphate, also called lauryl sulphate), β-mercaptoethanol (BME), bromophenol blue, glycerol, and Tris-glycine at pH 6.8. BME is added to prevent oxidation of cysteines and to break up disulfide bonds. Bromophenol blue is a dye that is useful for visualizing your sample in the well and tracking its progress through the gel. Glycerol is much denser than water and is added to make the sample fall to the bottom of the sample well rather than just flow out and mix with the entire buffer in the upper reservoir. The interesting components are the buffer and the SDS.

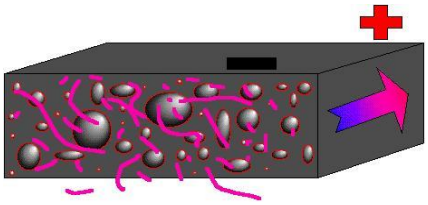
Discontinuous SDS-PAGE: SDS-PAGE gels are composed of two different gels (stacking gel and running gel), each cast at a different pH. In addition, the gel buffer is at a third, different pH. The running gel is buffered with Tris at a pH 8.8. The stacking gel is also buffered with Tris at a pH 6.8. The sample buffer is also buffered to pH 6.8 with Tris HCl. The electrode buffer is also Tris, but here the pH is adjusted to a few

tenths of a unit below the running gel (in this case 8.3) using only glycine – nothing else. Gels are run at a constant voltage.

Since a higher charge and the small size of a molecule increases the mobility of that molecule in the **stacking gel (pH=6.8)**, the resultant migration pattern of the molecules would be molecules with high charge and small size would be in the first row and in the last row would be the small charged large molecules. The rows in between would have the molecules organized by their mobility. The chloride ions in the stacking gel has the greatest mobility and highest charge. The proteins have a lower mobility than the chloride ions, but are faster than the glycine anions from the buffer. The resultant stack would be the chloride ions, proteins, and then the glycine anions. After the ions stack in the stacking gel, they enter the **resolving gel (pH=8.8)**, which has smaller pore sizes. The first event that occurs is that the higher pH places a greater negative charge on the small glycine anions. This results in the glycine anions migrating faster than the chloride ions. The second event is that the decrease in pore size creates a large frictional component on the mobility of each protein. **Since the SDS in the gel creates a charge to mass ratio that is equal, the proteins now migrate based on size.**



This cartoon shows a slab of polyacrylamide with tunnels exposed on the edge. Notice that there are many different sizes of tunnels scattered randomly throughout the gel.



Cartoon showing a mixture of denatured proteins beginning their journey through a polyacrylamide gel. An electric filed is established with the positive pole at the far end and the negative pole (black minus) at the closer end. Since all the proteins have strong negative charges, they will all move in the direction the arrow is pointing (run to red).

The bands created by the proteins of different size can be compared to a standard that is run along with the sample. A **standard curve** can be created from the standard's bands and the samples can then be compared to the standard.

2. WESTERN BLOTTING

Introduction:

Immunoblotting or western blotting procedures combine the resolution of gel electrophoresis with the specificity of antibody detection. Blotting can be used to ascertain a number of important characteristics of protein antigens, including the presence and quantity of an antigen, the molecular weight of the antigen, and the efficiency of antigen extraction. This method is especially helpful when dealing with antigens that are insoluble, difficult to label, or easily degraded, and thus not amenable to procedures such as immunoprecipitation.

Western blot analysis can detect **one** protein in a mixture of any number of proteins while giving you information about the size of the protein. It does not matter whether the protein has been synthesized in vivo or in vitro. This method is, however, dependent on the use of a high-quality antibody directed against a desired protein. Antibodies are widely used as detecting agents, and the procedure is called Western Blotting or Protein Blotting or Immunoblotting.

The Basic Blotting Procedure Can Be Divided into the Following Steps:

1. Sample Preparation
2. Gel Electrophoresis
3. Membrane Transfer
4. Antigen detection
    - Blocking Non-specific Binding
    - Addition of the Antibody
    - Detection

1. Sample Preparation:

A solution of proteins, frequently an extract of cells or tissues, is first prepared in a gel electrophoresis sample buffer. The buffer of the protein solution must be compatible with that of gel separation technique. For Tris/glycine SDS-Polyacrylamide Gels, the pH of the protein solution should be approximately neutral, salt concentration should be below 200 mM. But most other buffer components will not affect the running of the gel.

One of the most critical parameters that differentiate between a successful blot and a blotch is the quality of the sample whose constituent components are to be separated by electrophoresis prior to blotting. Partial protein degradation by proteolysis is normally reflected by the appearance of a large smear or a band of erroneous molecular weight. Total degradation will give the false impression that the protein is not present in the sample.

2. Separation of polypeptides by SDS-PAGE:

Prior to blotting the proteins are separated by SDS-PAGE. The samples are heated with sample buffer and loaded onto SDS-PAGE. Most SDS PAGE sample buffers contain the following: SDS (sodium dodecyl sulphate, also called lauryl sulphate), β-mercaptoethanol (BME), bromophenol blue, glycerol, and Tris-glycine at pH 6.8. BME is added to prevent oxidation of cysteines and to break up disulfide bonds. Bromophenol blue is a dye that is useful for visualizing the sample in the well and tracking its progress through the gel.

Glycerol is much denser than water and is added to make the sample fall to the bottom of the sample well rather than just flow out and mix with the entire buffer in the upper reservoir. The samples are then loaded into a vertical polyacrylamide gel electrophoresis system and proteins are separated according to their molecular weight.

3. Membrane transfer:

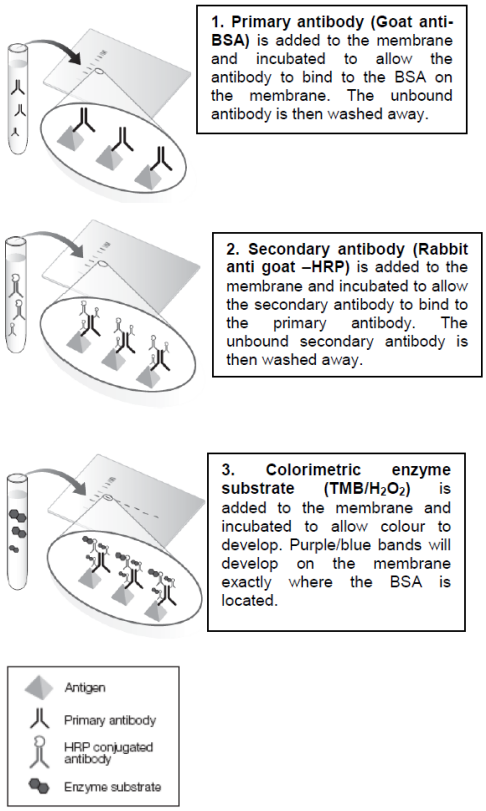
In order to make the proteins accessible to antibody detection, they are moved from within the gel onto a membrane made of nitrocellulose or PVDF. The membrane is placed on top of the gel, and a stack of tissue papers placed on top of that. The entire stack is placed in a buffer solution which moves up the paper by capillary action, bringing the proteins with it.

Another method for transferring the proteins is called electroblotting and uses an electric current to pull proteins from the gel into the PVDF or nitrocellulose membrane. The proteins move from within the gel onto the membrane while maintaining the organization they had within the gel. As a result of this "blotting" process, the proteins are exposed on a thin surface layer for detection (see below). Both varieties of membrane are chosen for their non-specific protein binding properties (i.e. binds all proteins equally well). Protein binding is based upon hydrophobic interactions, as well as charged interactions between the membrane and protein. Nitrocellulose membranes are cheaper than PVDF, but are far more fragile and do not stand up well to repeated probings.

4. Antigen detection:

Once proteins are transferred to the nitrocellulose membrane (the 'blot'), the next step is to probe the blot with an antibody that is specifically engineered to detect a particular protein of interest. First the blot is incubated in a protein-rich solution like skim milk powder, gelatin etc. Blocking the membrane effectively coats the remaining surface area of the membrane (where no proteins have been blotted) and blocks nonspecific protein binding sites.
Next, the blot is incubated with a primary antibody. The primary antibody is designed to specifically recognize, or bind, the protein under study. In this kit, the primary antibody binds to the BSA on the membrane. Following a wash step, the membrane is incubated with an enzyme-linked secondary antibody that has been engineered to bind specifically to the primary antibody. A colourless colorimetric (colour-producing) enzyme substrate is added to the membrane in solution. The enzyme that is linked to the secondary antibody oxidizes the colorimetric substrate into an insoluble blue precipitate that leaves visible deposits on the membrane at the precise location of the proteins.

Antigen detection – Steps



Western Blotting Applications in the Real World

Western blotting provides information on the identity, size, and quantity of proteins. This information is useful for many applications including disease diagnosis, agriculture, and biomedical research.

Disease Diagnosis Western blotting is used to confirm positive ELISA results in case of HIV.

Agricultural Applications Western blotting is used extensively in agriculture to verify results obtained from ELISA tests. For example, western blotting may be used to confirm a positive ELISA test for bovine spongiform encephalopathy (BSE or mad cow disease). Western blotting is also an important tool in crop science used to detect and quantify protein associated with genetically modified crops. Protein markers in crop science are used to evaluate such things as crop yield, pest resistance, and nutritional value.

Biochemical and Biomedical Research Applications Because western blotting can determine the presence of a specific protein, its quantity and molecular mass, it is used extensively to understand protein structure and function in biochemical and medical research.

Objectives

1. To introduce the principles of SDS-PAGE for the separation of proteins.
2. To determine the molecular weight of proteins.
3. To learn the concepts and methodology involved in Western Blot Analysis.
4. In this test, proteins (Bovine serum and BSA) are separated on a denaturing SDS polyacrylamide gel and are blotted to a nitrocellulose membrane. Membrane is exposed to solutions containing primary antibody (goat anti BSA antibody), followed by a secondary antibody (rabbit anti goat IgG – HRP) coupled to an enzyme, membrane is then soaked in a substrate solution to develop the color reaction, which results in identifying the antigen as a band.

Materials Required

Micropipettes
Distilled water
Microtips
1.5 ml Vials
Techware for PAGE and electroblotting
Methanol
Agarose for sealing

Reconstitution

Buffer	Volume to be taken
1X Tris-Glycine-SDS buffer, 200 ml	20 ml of 10X Tris-Glycine-SDS buffer 180 ml of Distilled water
1X Blotting Buffer, 400 ml	20 ml of 20X blotting Buffer 20 ml of methanol 380 ml of Distilled water
1X Assay Buffer, 200 ml	10 ml of 20X assay Buffer 190 ml of Distilled water
5% Potassium per sulphate (KPS), 1ml Stable for a month if stored at 4°C	50 mg Potassium per sulphate 1 ml Distilled water

Precautions

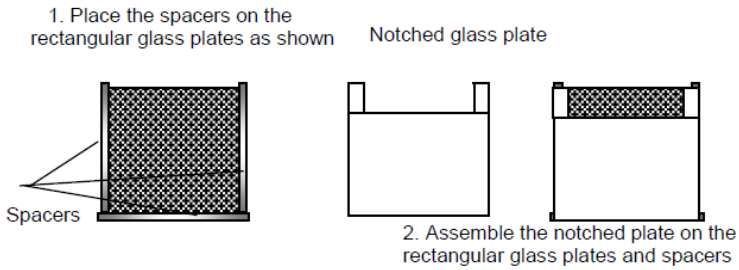
1. Handle the nitrocellulose membrane and set up the blot with gloves on.
2. Store the samples and primary antibody at -20°C.

Protocol

I. SDS-PAGE

Assembly of glass plates

1. Wipe the glass plates grease-free and assemble the glass plates and the spacers as shown below.



2. Assemble the gel casting apparatus, making sure that the sandwich of glass plates and spacers will make a good seal. Clamp the assembly of glass plates with the spacers and seal the three sides with molten agarose or tape. Ensure the assembly is leak proof by filling water between the plates. Follow the instructions of the manufacturer for assembly of the gel plates.
3. Leave the assembly on a leveled surface.

Preparation of resolving gel

1. Prepare 5 ml of 12% Separating Gel solution according to the following table. Mix well.

H2O	1.65 ml
30% acryl/bisacrylamide	2 ml
1.5M Tris-SDS pH 8.8	1.25 ml
5% Potassium per sulphate	100 µl
TEMED	5 µl
Total	5 ml

2. Pour the gel solution between the plates till the level is below 3- 4 cm from the top of notched plate. Immediately after pouring the gel mix, it must be overlaid with water to an additional height of 0.5 cm or so.
3. The gel takes around 30 min to polymerize. After polymerization, drain the water completely. Remove any water droplets between the plates using paper towel.

Preparation of Stacking gel

1. Prepare the 2 ml Stacking Gel as follows solution. Mix well

H2O	1.39 ml
30% acryl/bisacrylamide	0.33 ml
1.0M Tris-SDS pH 6.8	0.25 ml
5% Potassium per sulphate	50µl
TEMED	5µl
Total	2 ml

2. Pour the mix carefully onto the polymerized resolving gel. Clean and wipe the comb and carefully insert the comb without trapping any air bubbles into the stacking gel till 1 cm above the surface of the resolving gel. (The protein has to travel at least 1 cm before it enters the resolving gel for it to stack). Allow it to polymerize for 20 mins.

Sample preparation and electrophoresis

1. Label three vials from 1-3 and aliquot as follows:

vial 1: 40 µl of protein sample 1+ 20 µl of sample loading buffer

vial 2: 40µl of protein sample 2+ 20 µl of sample loading buffer
vial 3: 10µl of protein standard marker+ 10 µl of sample loading buffer

2. Heat the samples in a water bath at 95°C for 5 min.

3. Remove the gel sandwich from the casting apparatus.

4. Wash the wells immediately with distilled water to remove non-polymerized acrylamide. Straighten the teeth of the wells using a needle, if necessary. Remove the bottom spacer.

5. Clip the sandwich to the electrophoresis apparatus filled with 1X Tris-glycine-SDS Buffer in the lower chamber. Take care not to introduce any air bubbles between the bottom of the gel and the buffer. Any bubbles caught between the plates at the bottom of the gel can be removed by squirting running buffer through a syringe fitted with a bent needle. Carefully remove the comb from the gel and fill the top of the apparatus with 1XTris-glycine-SDS Buffer.

6. Leave the first and last lane empty. Load the samples prepared in step 9 into the bottom of the wells using microlitre syringe or micropipette fitted with long tip as follows:

Lane 2: 20 µl processed Protein standard marker from vial 3
Lane 3: 30 µl of processed protein sample 1 from vial 1
Lane 4: 30 µl of processed protein sample 2 from vial 2
Lane 5: 30 µl of processed protein sample 1 from vial 1
Lane 6: 30 µl of processed protein sample 2 from vial 2.

7. Connect the apparatus to the power supply start electrophoresis at 50 V for the first 30 min and then increase the voltage to 100V.

8. When the dye front comes to 0.5 cm above the bottom of the gel, turn off the power pack. Remove the gel plates and gently pry the plates apart. Use a spatula or similar tool to separate the plates. Cut a corner from the bottom of the gel that is closest to the well #1 for identification. Cut the gel along lane 4 and keep the lane 5 and 6 (protein sample 1 & 2) in blotting buffer and proceed for electroblotting immediately.

9. Transfer the gel containing lanes 2, 3 & 4 to a glass tray and wash the gel with deionized water 3 times 3 minutes each. This can be preferably done on a rocker. Presence of SDS in the gel will give background staining.

10. After the third wash add 15 ml of gel stainer and stain it overnight.

11. Destaining with water will enhance the sensitivity and makes the background clear.

Results & Interpretation (SDS-PAGE)

- The purity of the protein can be determined by the number of bands obtained in lane.
- Protein sample 1(lane 2) is serum and contains several bands as serum contains several proteins.
- Protein sample 2(lane 3) is Bovine serum albumin and shows a single band.
- Protein sample 3(lane 4) is ovalbumin and shows a single band.
- Protein standard marker (lane1) has four bands and the molecular weights are given in the table **below:**

Calculation of Molecular weight

Construct a standard curve to calculate protein molecular weights

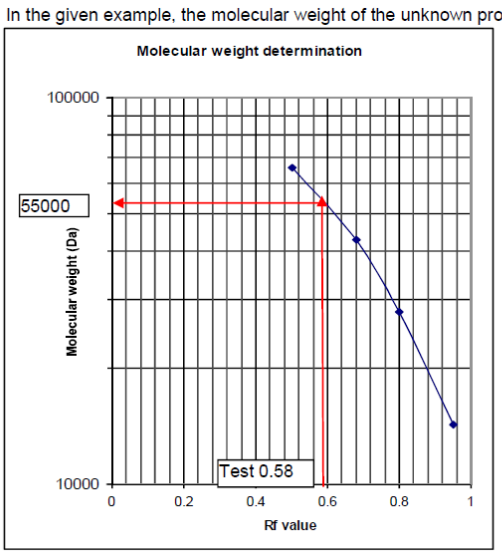
1. First measure the distance each of the 4 protein bands contained in the protein standard migrated from the well from the largest protein band down to the smallest protein band. Accuracy to 0.5 mm is sufficient. Also measure the distance between the well and the dye front. Record the distance of each protein in the protein standard migrated and plot that distance against the molecular weights of each.
2. In the same manner measure the distances protein 2 and 3 has travelled.

Protein	Distance travelled (mm)	Rf	Molecular wt in Da
Dye front			
Bovine Serum Albumin			66000
Ovalbumin			45000
Carbonic Anhydrase			29000
Lysozyme			14,300
Protein Sample 2			
Protein Sample 3			

Formula for relative mobility

Rf= Distance travelled by protein (A)/ Distance travelled by dye front(B)

3. Plot these Rf values of the protein standard on the x axis against the molecular weight of the bands in daltons on the y axis as a scatter plot on the logarithmic graph paper provided. Then connect the dots to produce a linear curve, as shown in the illustration. Students can then determine the molecular weights of unknown proteins in their samples by measuring the distance of each band migrated from its well of origin. By drawing a line up from the x-axis to the curve, they can estimate the molecular masses from the y-axis by extrapolation as shown in the sample curve.

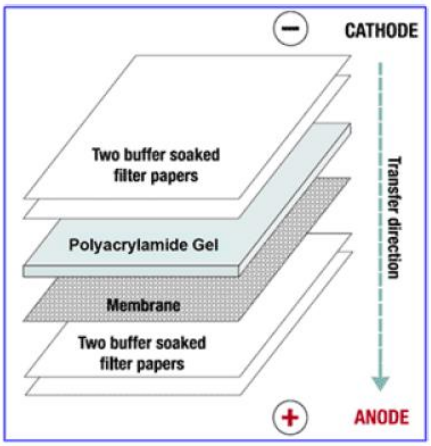


Sample standard curve for determination of molecular weight of protein

II. Western Blotting or Electroblotting

1. Cut the filter paper and the nitrocellulose membrane exactly to the size of gel. Prewet the sponges and filter papers in 1X blotting buffer.

2. Assemble the blotting sandwich within the blotting cassette as shown in the figure. Arrange the blotting sandwich from cathode to anode in the following order. Cathode-Sponge – 3 layers of filter paper - gel – nitro cellulose membrane – 3 layers of filter paper – sponge- Anode. Take care to avoid air bubbles between the gel and Nitrocellulose membrane. Tighten the screws of the cassette well.



3. Insert the cassette into the apparatus filled with blotting buffer such that the gel faces the cathode.

4. Connect the power supply. Set voltage to 50 V and electrophorese for 5 hours or electrophoresis can be continued overnight for blotting.

NOTE: The gel used for transfer can be put in the same stainer used for staining and can be checked for the completion of transfer. Any development of the band indicates incomplete transfer.

III. Immunodetection

1. After the transfer open the blotting cassette and remove the nitrocellulose membrane with the help of forceps and transfer to a petriplate.

2. Wash the membrane once with distilled water to remove any gel pieces.

3. Soak the membrane in 10 ml of blocking solution for 1-2 hours at room temperature or overnight in the cold.

4. Wash the blot by immersing in 1X assay buffer for 2 minutes X 3 times with gentle agitation.

5. Prepare1:1000 dilution of primary antibody in 1X assay buffer. (10µl of antibody in 10 ml of assay buffer). Immerse blot in the primary antibody solution and agitate gently for 45 min.

6. Wash the blot by immersing in 1X assay buffer for 2 minutes X 5 times with gentle agitation.

7. Prepare1:1000 dilution of Rabbit anti goat-HRP conjugate in 1X assay buffer (10µl in 10 ml of assay buffer). Immerse the blot and agitate gently for 30 min.

8. Wash the blot in 1X assay buffer for 2 minutes x 5 times with gentle agitation.

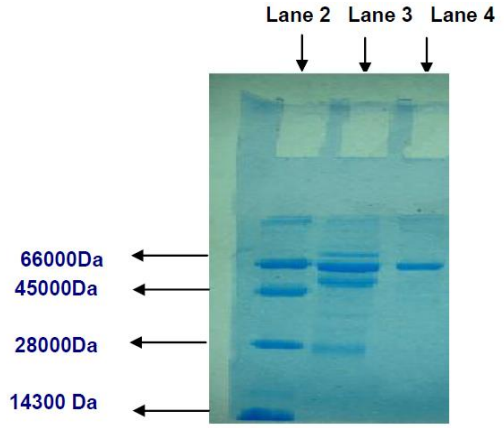
9. Dilute the substrate 10 times just before use. (0.5 ml of 10X substrate in 4.5 ml of distilled water).

10. Immerse the washed blot in 5 ml of substrate solution with gentle shaking. Bands should develop sufficient color within 5-10 minutes.

11. Remove the blot, wash with distilled water and dry.

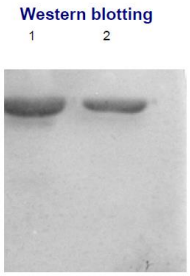
Results and Interpretation (Western Blotting)

Appearance of protein bands after Staining:



Lane 2: Protein standard marker
Lane 3: Bovine serum showing several bands indicating the presence of many proteins
Lane 4: Bovine serum albumin

Western blotting



Lane 1: Bovine serum after blot development shows a single band identified by the anti-BSA antibody
Lane 2: BSA

3. PROTEIN SEQUENCE ANALYSIS TOOLS

Protein Sequence Retrieval from Protein sequence Databases

Principle:

UniProtKB/Swiss-Prot is the manually annotated and reviewed section of the UniProt Knowledgebase(UniProtKB). It is a high quality annotated and non-redundant protein sequence database, which brings together experimental results, computed features and scientific conclusions. Since 2002, it is maintained by the UniProt consortium and is accessible via the UniProt website.

Protocol:

- Go to the Uniprot website, www.uniprot.org .
- Type query in search column and click on the search button.
- Select the required entry from the hit list of the Uniprot files.
- Click on FASTA option to view and retrieve the sequence in FASTA format.
- Use the sequence for further analysis.

Pair wise Sequence Alignment

Principle

Pair wise alignment of two protein sequences can be carried out using EMBOSS pair wise alignment tool.

EMBOSS Needle reads two input sequences and writes their optimal global sequence alignment to a file. It uses the Needleman-Wunsch alignment algorithm to find the optimum alignment (including gaps) of two sequences along their entire length.

This tool can be used for protein as well as nucleotide sequence alignment.

Protocol

- Type www.ebi.ac.uk/emboss.align and enter the website.
- Select the type of sequence (protein/ nucleotide) alignment.
- Retrieve the two protein sequences that are to be aligned from the protein sequence database in FASTA format.
- Paste the above two sequences in the space provided.
- Click the run button of the program to align these two sequences.
- Analyze and interpret the results.

Sequence Similarity Search Using FASTA

Principle

FASTA takes a given nucleotide or amino acid sequence and searches a corresponding sequence database by using local sequence alignment to find matches of similar database sequences.

The FASTA program follows a largely heuristic method which contributes to the high speed of its execution. It initially observes the pattern of word hits, word-to-word matches of a given length, and marks potential matches before performing a more time-consuming optimized search using a Smith-Waterman type of algorithm.

Protocol

- Go to EMBL website www.ebi.ac.uk and select FASTA tool.
- Retrieve the query sequence from the corresponding database.
- Input the query sequence in the space provided by the tool.
- Run the program to get the output
- Analyze the similar sequences with respect to the query sequence.

Sequence Similarity Search Using BLAST

Principle

Basic Local Alignment Search Tool, or BLAST, is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold. Different types of BLASTs are available according to the query sequences. For example, following the discovery of a previously unknown gene in the mouse, a scientist will typically perform a BLAST search of the human genome to see if humans carry a similar gene; BLAST will identify sequences in the human genome that resemble the mouse gene based on similarity of sequence.

Protocol

- Go to NCBI website www.ncbi.nlm.nih.gov and select BLAST tool.
- Retrieve the query sequence from the corresponding database.
- Input the query sequence in the space provided by the tool.
- Run the program to get the output
- Analyze the similar sequences from the database with respect to the query sequence.

Multiple Sequence Alignment using CLUSTAL W

Principle

Multiple Sequence Alignment (MSA) is generally the alignment of three or more biological sequences (protein or nucleic acid) of similar length. From the output, homology can be inferred and the evolutionary relationships between the sequences can be studied.

Multiple Sequence Alignment tools are used to identify regions of similarity that may indicate functional, structural and/or evolutionary relationships of biological sequences.

ClustalW is a general purpose multiple sequence alignment program for DNA or proteins.

Protocol

- Type www.ebi.ac.uk/clustalw/index.html and open the program.
- Retrieve four different nucleotide sequences from the protein sequence database.
- Paste the sequences one by one in the spaces provided in FASTA format.
- Click the run button to execute the program and get the output
- Analyze and interpret the result.

4. Protein Structure Analysis Tools

Secondary structure prediction of proteins

Principle

Secondary structure prediction predicts the conformational state of each residue in three categories, helical, extended or strand, and coil. Many methods are based on ideas related to secondary structure propensity, which is a number reflecting the preference of a residue for a particular secondary structure. Examples of secondary structure prediction tools are PHD, DSC, PREDATOR, NNSSP etc. The accuracy of all these methods is above 70%.

Protocol

- Go to the web page of www.npsa-pbil.fr
- Select the secondary structure prediction tool, secondary structure consensus prediction.
- Input the aminoacid sequence to be analysed in FASTA format
- Click on submit button to execute the program
- Analyse and interpret the result.

Analysis of tertiary structure of proteins by Rasmol

Principle

Rasmol is the molecular visualization software, which is used to view and analyse the three dimensional (3D) structure of proteins. The pdb file of the protein needs to be given as input for this software. It is used for the structural analysis of proteins.

Protocol

- Go to PDB web site www.rcsb.org.
- Type the query in search column to get the required protein
- Select the protein required from the hit list of protein structures
- Download the required protein 3D structure in pdb format
- Input the protein structure in Rasmol software and analyse the structural properties like hydrogen bonds, disulfide bonds, secondary structure, no. of chains present etc.

References:

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M S RAMAIAH COLLEGE OF ARTS, SCIENCE AND COMMERCE-AUTONOMOUS
DEPARTMENT OF MICROBIOLOGY
VALUE ADDED PROGRAMME ON ANALYTICAL TECHNIQUES IN BACTERIAL PROTEOMICS
16TH AND 19TH OCTOBER 2024
ATTENDANCE

Sl. No.	Name of the Students	Reg. No.	Mobile Number	Class	Section	16th Oct 2024	18th Oct 2024	19th Oct 2024
1	Sandeep Kumar Behera	U18EV22S0116	8900051927	V BSc	C			
2	Nihaarika SN	U18EV22S0167	9945865657	V BSc	A			
3	Laharika V	U18EV22S0314	9182711791	V BSc	A			
4	Pralayakaveri Devipriya	U18EV22S0386	9972715152	V BSc	B			
5	Sakshi Sunil Advitote	U18EV22S0123	8380909798	V BSc	B			
6	Monisha M	U18EV22S0350	8971085446	V BSc	B			
7	Sri Lalitha Hiranmai mula	U18EV22S0103	7093549383	V BSc	C			
8	Chandni Gupta	U18EV22S0389	7006675752	V BSc	A			
9	Tanya Chiripal S	U18EV22S0264	9487905424	V BSc	C			
10	Daisy Dominic	U18EV22S0153	8073885253	V BSc	A			
11	Snigdha Amarnath	U18EV22S0097	9538451922	V BSc	C			
12	Hafsa HS	U18EV22S0261	8431252327	V BSc	A			
13	R vaishnavi	U18EV22S0391	7009143126	V BSc	A			
14	G Baby Tejaswini	U18EV22S0114	7382522522	V BSc	C			
15	Yuktha L	U18EV22S0369	938030092	V BSc	C			
16	Hastha.D	U18EV22S0168	9108880189	V BSc	A			
17	Mandara K	U18EV22S0099	9611671662	V BSc	C			
18	Panuganti Harshitha	U18EV22S0296	8897253707	V BSc	B			
19	B. Swetha Reddy	U18EV22S0366	9440288849	V BSc	B			
20	M N Bhavana	U18EV22S0101	6360603902	V BSc	C			
21	V. Vibha Sai	U18EV22S0102	8790133991	V BSc	C			
22	Beesam Navadeep	U18EV22S0361	8790529639	V BSc	B			
23	Saman Tahniet	U18EV22S0351	6299007254	V BSc	A			
24	Ananya Bharadwaj	U18EV22S0273	7022165014	V BSc	C			
25	N Mohan	U18EV22S0118	6301739626	V BSc	B			
26	Arya Arun Kumar	U18EV22S0378	9605011215	V BSc	B			
27	Disha HM	U18EV22S0380	8618461600	V BSc	B			
28	Akshitha Prakash	U18EV22S0262	9538211164	V BSc	C			
29	Radhakrishnan Sreedhanya	U18EV22S0345	9490578922	V BSc	B			
~~30~~	~~Kashish Salian~~		7045646788	V BSc	C			