BITS Pilani
K K Birla Goa Campus

CERTIFICATE

OF COMPLETION

BITS Pilani
Pilani Campus
Department of Biological Sciences

THIS CERTIFICATE IS PROUDLY PRESENTED TO

Samuel Singh

For having actively completed a 1 month internship at BITS Pilani, KK Birla Goa Campus from 01-12-2022 to 31-12-2022

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25/04/2023

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REPORT OF INTERNSHIP

AT

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCES (BITS), GOA

1TH DECEMBER 2022 – 31TH DECEMBER 2022

SUBMITTED BY

SAMUEL SINGH

MSc MARINE BIOTECHNOLOGY

SCHOOL OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY

GOA UNIVERSITY

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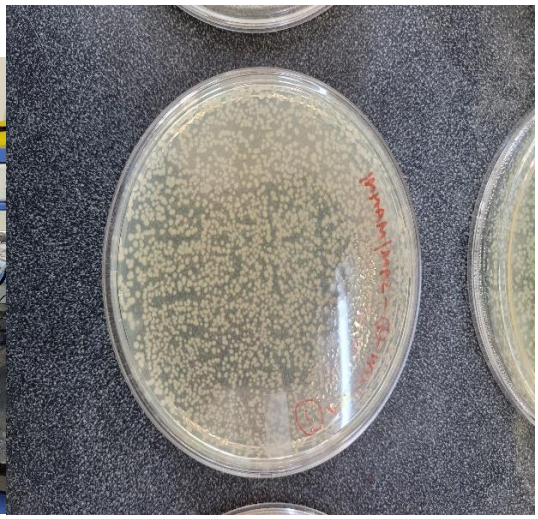
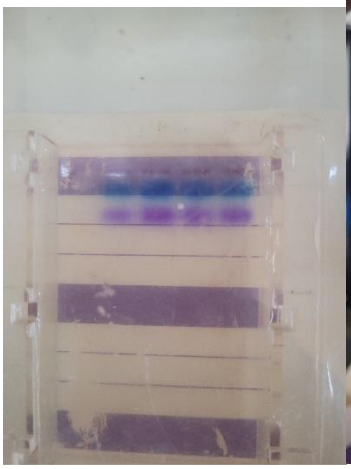


OVEREXPRESSION AND PURIFICATION OF A BACTERIAL PROTEIN IN A HETEROLOGOUS SYSTEM



- **CONTENTS**

Competency and Transformation

The bacterial cells that can take up the foreign DNA from the surroundings by a process called transformation are known as competent cells. Griffith first reported it in *Streptococcus pneumoniae. E.coli* cells are more likely to uptake the DNA if their cell walls are altered. The cells can be made competent by calcium chloride and heat shock treatment. The cells growing rapidly can be made competent more easily than those in other stages of growth.

The cells might express the acquired genetic information after transformation. The process is largely used to introduce recombinant plasmid DNA into competent bacterial cells. This process does not require a donor cell, but only a DNA in the surrounding environment. It is a common method in molecular biology with many applications, including cloning, DNA sequencing, and DNA library construction.

There are three basic steps in many protocols to transform bacterial cells

1. **The preparation step**: the bacterial cells are made competent to uptake foreign DNA by modifying the permeability of the cell membrane and the cell wall.
2. **The transformation step**: the transformation step is performed to allow DNA (usually plasmid DNA) to enter the cell. The most common transformation methods are electroporation or heat shock transformation.
3. **The recovery step**: the cells are incubated in a recovery medium to restore the cell membrane and the cell wall.

   The first protocol for artificial transformation of *E. coli* was published by Mandel and Higa in 1970 [3]. The procedure showed increased permeability of the bacterial cells to DNA after treatment with calcium (Ca^{2+}) and brief exposure to an elevated temperature, known as heat shock. This method became the basis for **chemical transformation.**

Day 1:

1. Preparation of Media/solutions

- Prepare LB-Agar medium for Petri plates (100 ml for 5 plates each group). Add 100 µg/ml kanamycin final concentration (100 µl from 100 mg/ml stock to 100 ml to cooled media to 40-45°C and pour 20 ml to each plate).
- Let the Petri plates cool down to room temperature and store them at 4-8°C in a refrigerator.
- Prepare LB-broth 20 ml.
- Prepare 100mM CaCl2 (100 ml) and autoclave.

2. Inoculum preparation

- Inoculate one colony from DH5-α petri plate using inoculating loop. Grow the cells overnight at 37°C at 120 rpm.

Day 2:

1. Inoculation and Growth

- Inoculate 1 ml of inoculum (from overnight culture) to 20 ml LB in conical flask and allow the cells to grow at 37°C, 120 rpm.
- Allow the cells to grow till OD600 nm ~ 0.4-0.6 for about 2 hours.
- Transfer the flask to ice and cool the cells for 10 min.

2. Competent cells

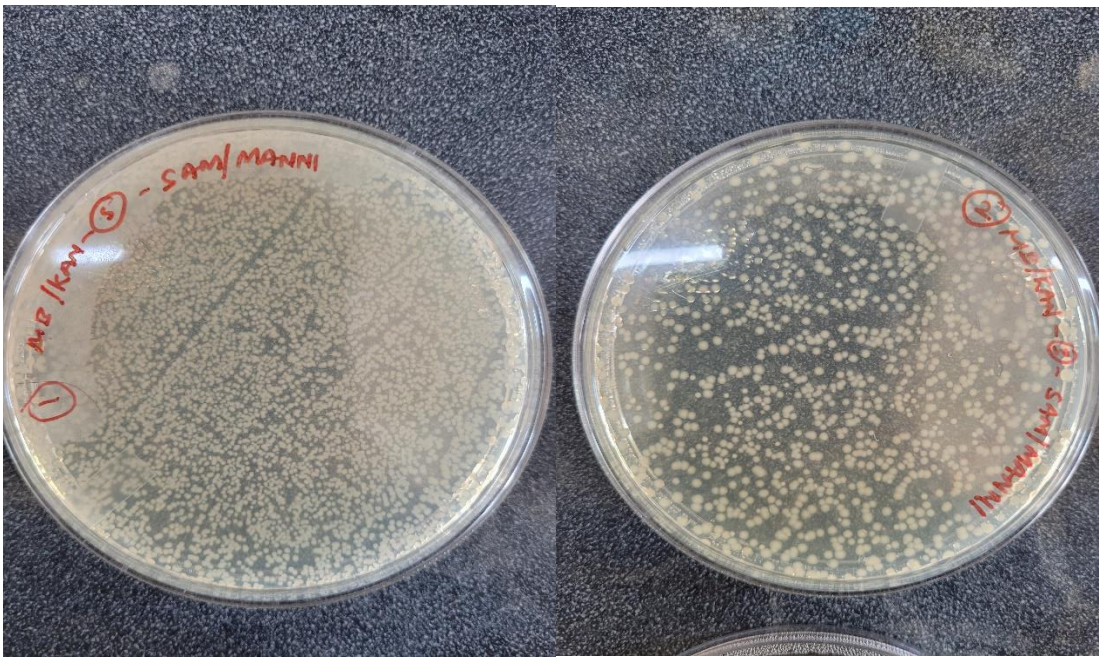
- Take 20 ml of culture, and put it in ice for 10 mins.
- Centrifuge 20 ml culture from the flask in a 50 ml centrifuge tubes at 7500 rpm, at 4°C for 10 min. Discard the supernatant.
- Resuspend the cell pellet gently in 10 ml of 100mM CaCl2 (Ice cold), and keep it in ice for 10 mins.
- Centrifuge at 7500 rpm, at 4°C for10 min. Discard the supernatant.
- Resuspend cells gently in 5 ml of 100mM of CaCl2 (Ice cold).
- Centrifuge at 7500 rpm, at 4°C for 10 min. Discard the supernatant.
- Resuspend cells gently in 100 µl of 100mM of CaCl2 (Ice cold).
- Leave the cells at 0°C (on ice) for 16h.
- The cells can be stored at 0- 4°C for a week or use directly for transformation.

Transformation:

- Add 2-3 µl of BL-21plasmid DNA to a 100 µl of competent cells.
- Place cells/DNA on ice for 1 hour.
- Heat shock cells at 42°C for 92 seconds.
- Add 500 µl LB to cells/DNA.
- Tape tube onto shaking incubator platform and incubate cells/DNA for 1 hour at 37°C while shaking at 120 RPM.
- Plate 100ul and 200ul of transformation onto 2 LB+kanamycin plate. Place plates inverted at 37°C overnight.

RESULTS –

The transformed colonies obtained for Lex-A are shown below. All the plates have shown almost similar transformation efficiency with respect to plasmid used.



Plasmid isolation

One of the most important techniques in molecular biology is the separation of plasmid DNA from bacteria, which is a necessary step in numerous processes like cloning, DNA sequencing, transfection, and gene therapy.
High purity plasmid DNA must be isolated in order to do these alterations.
All molecular biology techniques, including digestion with restriction enzymes, cloning, PCR, transfection, in vitro translation, blotting, and sequencing, can be done right away using the pure plasmid DNA.
Here, we have used Thermo Scientific GeneJET Plasmid Miniprep Kit for plasmid isolation.

Plasmid Isolation Protocol

Plasmid DNA purification using centrifuges

- Use 10 mL of culture of *E. coli* culture in LB media for purification of plasmids.
- Centrifuge at 8000 rpm, at 37°C for 10 min. Discard the supernatant.
- Resuspend the pelleted cells in **250 µl of the Resuspension Solution**. Transfer the cell suspension to a microcentrifuge tube.
- Add **250 µl of the Lysis Solution** and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear.
- Add **350 µl of the Neutralization Solution** and mix immediately and thoroughly by inverting the tube 4-6 times.
- Centrifuge for 5 min to pellet cell debris and chromosomal DNA.
- Transfer the supernatant to the supplied GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate.
- Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
- Add **500 µl of the Wash Solution** to the GeneJET spin column. Centrifuge for 30-60 seconds and discard the flow-through. Place the column back into the same collection tube.
- Repeat the wash procedure (step 8) using **500 µl of the Wash Solution**.
- Discard the flow-through and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.
- Transfer the GeneJET spin column into a fresh 1.5 mL microcentrifuge tube (not included).

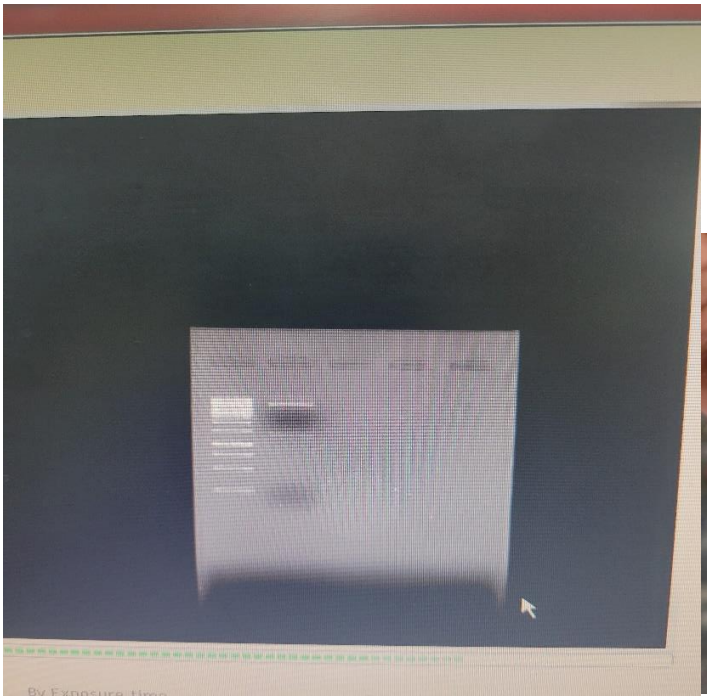
- Add **50 µl of the Elution Buffer** to the center of GeneJET spin column membrane to elute the plasmid DNA. Incubate for 2 min at room temperature and centrifuge for 2 min.

Note. An additional elution step (optional) with Elution Buffer or water will recover residual DNA from the membrane and increase the overall yield by 10-20%.

- Discard the column and store the purified plasmid DNA at -20°C.

RESULTS –

The Lex-A plasmids are isolated and shown below with agarose gel electrophoresis with bands of 27 kDa of molecular weight.



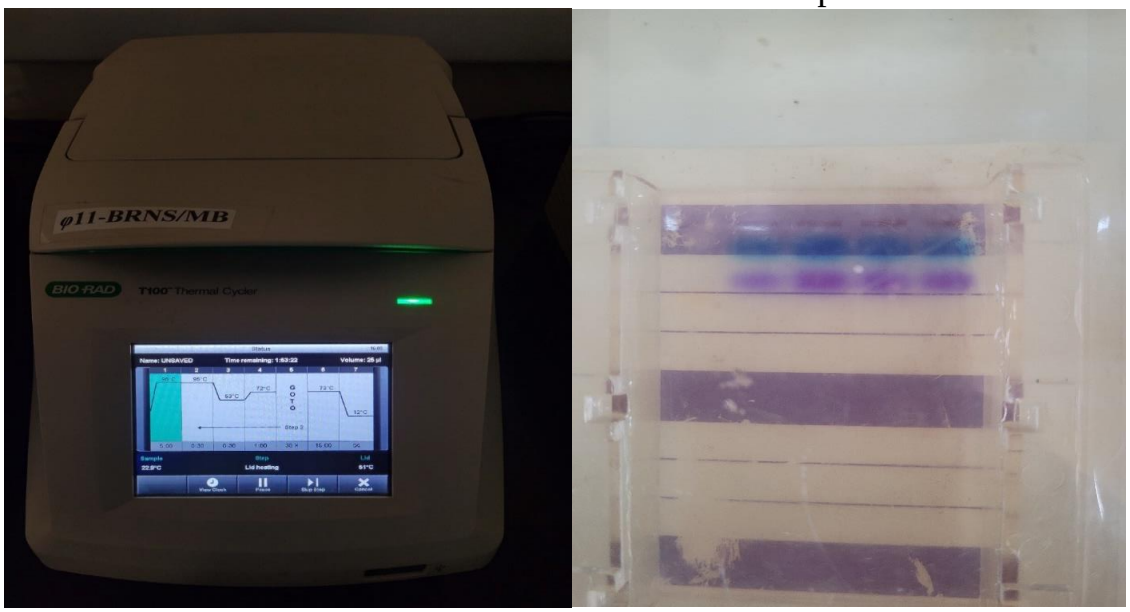
Colony PCR

Colony PCR is a simple and quick method for verifying correct assembly of a cloned DNA construct. Traditionally, verification of DNA insertion during cloning was time-consuming, following bacterial transformation with laborious DNA purification and subsequent restriction enzyme digest. Colony PCR is a **Direct PCR** method that bypasses both DNA isolation and restriction digest, providing a fast, easy, and inexpensive solution for screening cloned constructs. Meaning, it works only when the transformation is successful. The main advantage is using bacterial colonies directly as template DNA. We can use bacterial colonies directly as template DNA. Cyclic-enzymatic-temperature-dependent reaction only amplifies successfully transformed plasmids.

- Nuclease free water – 87 µl
- Buffer - 12.5 µl
- dNTPs - 2.5 µl
- Forward primer - 3.75 µl
- Reverse Primer - 3.75 µl
- Template - 25 µl
- Taq polymerase - 2.5 µl

❖ RESULTS –

Running agarose gel electrophoresis shows the bands of 27kDa for all wells which shows successful transformation in the isolated plasmids.



Overexpression of proteins

You need a sizable amount of a particular protein available for your intended experiments in order to research its structure or function.
Some proteins are abundantly present in nature and are simple to separate from their host organism. However, the majority of proteins are either found in extremely minute amountsor in organisms that are difficult to effectively purify proteins from. Protein overexpression techniques enable researchers to examine lowquantity, rare, dangerous and even altered proteins by producing huge quanti ties of the required proteins for further study.

Over expression protocol

- Inoculate one colony from transformed petri plate using sterile inoculum loop to 20 ml of LB broth. Grow the cells overnight at 37°C at 120 rpm.
- Inoculate 1% (2.5ml) of inoculum (from overnight culture) to 250 ml LB in conical flask and allow the cells to grow at 37°C, 120 rpm.
- Allow the cells to grow till OD 600 nm ~ 0.4-0.6 for about 2 hours.
- Add 0.5 Mm IPTG to 250 ml LB in conical flask and allow the cells to grow at 32°C, 140 rpm.
- Centrifuge 250 ml culture from the flask in a centrifuge tubes (250ml, 125*2ml) at 7500 rpm, at 4°C for 10 min. Discard the supernatant.
- Add 10 ml 0.9% NaCl wash to both the bottle, and resuspend cells gently
- Centrifuge at 7500 rpm, at 4°C for 10 min. Discard the supernatant.
- Keep the pellet in -20°C.

Purification of Proteins

The Ni-NTA Purification System is designed for purification of 6xHis-tagged recombinant proteins expressed in bacteria, insect, and mammalian cells. The system is designed around the high affinity and selectivity of Ni-NTA Agarose for recombinant fusion proteins that are tagged with six tandem histidine residues.

The Ni-NTA Purification System is a complete system that includes purification buffers and resin for purifying proteins under native, denaturing, or hybrid conditions. The resulting proteins are ready for use in many target applications. Ni-NTA Agarose uses nitrilotriacetic acid (NTA), a tetradentate chelating ligand, in a highly cross-linked 6% agarose matrix. NTA binds Ni2+ ions by four coordination sites.

Preparing cell lysate

- Take the pellet from -20°C and keep it in ice for thawing.
- Add 10 ml lysis buffer and incubate on ice for 30 minutes.
- Using a sonicator, sonicate the solution on ice using 5-6 cycles of one minute bursts at high intensity with a 2 minute cooling period between each burst.
- Centrifuge the lysate at 10,000 rpm at 4°C for 30 minutes to pellet the cellular debris. Transfer the supernatant to a fresh tube.

Preparing Ni-NTA Column

When preparing a column as described below, make sure that the snap-off cap at the bottom of the column remains **intact**. To prepare a column:

1. Resuspend the Ni-NTA Agarose in its bottle by inverting and gently tapping the bottle repeatedly.
2. Pipet or pour 1.5 mL of the resin into a 10-mL Purification Column. Allow the resin to settle completely by gravity (5–10 minutes) or gently pellet it by low-speed centrifugation (1 minute at 800 × *g*). Gently aspirate the supernatant.
3. Add 6 mL sterile, distilled water and resuspend the resin by alternately inverting and gently tapping the column.
4. Allow the resin to settle using gravity or centrifugation as described in Step 2, and gently aspirate the supernatant.
5. For purification under **Native Conditions**, add 6 mL Native Binding Buffer (recipe on previous page).
6. Resuspend the resin by alternately inverting and gently tapping the

column.
7. Allow the resin to settle using gravity or centrifugation as described in Step 2, and gently aspirate the supernatant.
8. Repeat Steps 5 through 7.

Using the native buffers, columns and cell lysate, follow the procedure below to purify proteins under native conditions:

1. Add the supernatant to Ni-NTA column and keep it for 1 hour incubation.
2. Remove supernatant in a beaker and wash column by adding 5 ml of wash buffer.
3. Repeat the above step five times.
4. Add 250-500 µl elution buffer
5. Remove 5 µl of the lysate for SDS-PAGE analysis.
6. Store the remaining lysate on ice or freeze at -20°C.

SDS PAGE

SDS PAGE or Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis is a technique used for the separation of proteins based on their molecular weight. It is a technique widely used in forensics, genetics, biotechnology and molecular biology to separate the protein molecules based on their electrophoretic mobility.

The principle

When proteins are separated by electrophoresis through a gel matrix, smaller proteins migrate faster due to less resistance from the gel matrix. Other influences on the rate of migration through the gel matrix include the structure and charge of the proteins. The system actually consists of two gels - a resolving (aka running) gel in which proteins are resolved on the basis of their molecular weights (MWs) and a stacking gel in which proteins are concentrated prior to entering the resolving gel.

Compared to DNA molecules, proteins are structurally very diverse. Proteins show tremendous variation in their amino acid compositions and in the distribution of amino acids in their folded structures, features with important implications for electrophoresis. Because of the hydrophobic effect, the surfaces of proteins proteins have a higher frequency of polar and charged amino acids than the interiors, where hydrophobic residues predominate. Folded proteins assume many different geometries and their surfaces are mosaics with respect to the distribution of R groups with different chemistries. Because proteins are so diverse with respect to their surface charges and geometries, the molecular weights of folded proteins cannot be simply determined by their migration rate in an electric field. Postively and negatively charged proteins would migrate in different directions.

To resolve the proteins in a sample according to their size, we must convert the proteins to a uniform geometry and impart a uniform charge/mass ratio to the proteins. In SDS PAGE, the solution is to denature the proteins by boiling them with the anionic detergent, sodium dodecyl sulfate (SDS) and 2-mercaptoethanol. The combination of heat and detergent is sufficient to break the many noncovalent bonds that stabilize protein folds, and 2-mercaptoethanol breaks any covalent bonds between cysteine residues. Like other detergents, SDS is an amphipathic molecule, consisting of a hydrophobic 12-carbon chain and a hydrophilic sulfate group. The SDS Acrylamide gel polymerization. Ammonium persulfate and TEMED catalyze the polymerization of acrylamide and bis-acrylamide monomers into a crosslinked network.

SDS-PAGE system can be considered a 3-component system. The stacking and running (resolving) gels have different pore sizes, ionic strengths and pHs. The third component is the electrophoresis buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, pH ~8.3), which contains large amounts of glycine. The ionization state of the glycine is critical to the separation. At neutral pH, glycine is a zwitterion, with a negatively charged carboxyl group and a positively charged amino group. The pKa of the amino group is 9.6, considerably higher than the pH of the chamber buffer. Consequently, very little glycine has a negative charge in the chamber buffer or stacking gel, and significant ionization does not occur until the glycine enters the more alkaline pH 8.8 environment of the running gel.

Let's follow the progress of protein samples during SDS-PAGE to see how differences in the composition of these three components generate the high resolving power of SDS-PAGE gels. The sample buffer used for SDS-PAGE contains a tracking dye, bromophenol blue (BPB), which will migrate with the leading edge of the proteins being separated on the gel. The sample buffer also contains glycerol, which allows the protein samples to settle into the bottom of the gel wells. The gel is vertically positioned in the electrophoresis apparatus and covered with chamber buffer containing glycine (right, shaded). Once a voltage is applied, the chloride ions in the sample buffer and stacking gel move rapidly toward the positive pole, forming the leading edge of a moving ion front. Glycine molecules have very little charge in the stacking gel, so they migrate at the rear of the moving ion front. This difference in chloride and glycine mobility sets up a steep voltage gradient in the stacking gel that sweeps along the negatively charged protein-SDS complexes. The large pores of the stacking gel present very little resistance to the movement of protein-SDS complexes, which then "stack up" into a very concentrated region at the interface between the running and stacking gels (right). Protein-SDS complexes remain concentrated at the interface until the slowly migrating glycine molecules reach the boundary between the two gels. not occur until the glycine enters the more alkaline pH 8.8 environment of the running gel. Let's follow the progress of protein samples during SDS-PAGE to see how differences in the composition of these three components generate the high resolving power of SDS-PAGE gels.

To visualize the positions of proteins after electrophoresis is complete, we stain the gels with various dyes that bind noncovalently and with very little specificity to proteins. During the staining process, proteins are also "fixed" in the gel, meaning that proteins become insoluble and unable to diffuse out of the gel. In our experiments, we will use Simply Blue, a colloidal suspension of Coomassie Brilliant Blue G-250. Brilliant Blue G-250 binds proteins non-specifically through a large number of ionic and Van der Waals interactions.

In this procedure, gels are rinsed with water to remove the buffer salts used for electrophoresis and then treated with the colloidal G-250 suspension. Protein bands appear rapidly, and when necessary, the gels can be destained with deionized water to lower the gel background. Brilliant Blue staining intensity is considered to be a quantitative procedure, because with some exceptions, the intensity of a stained band is directly proportional to the amount of protein in a band.

The sizes of proteins in an extract can be calculated by comparing their migration to a set of standard proteins run on the same gel. Researchers select standard proteins that will be well resolved on the particular gel that they are running.

These instructions are designed for constructing two 12% SDS-PAGE gel.

Assemble the gel casting apparatus

1. Assemble the components that you will need for casting the gel: a tall glass plate with attached 1 mm spacers, a small glass plate, a green casting frame and a casting stand.

2. Place the green casting frame on the bench with the green “feet” resting firmly against the bench and the clamps open (perpendicular to the frame) and facing you.

3. Place the two gel plates in the frame. Insert the taller spacer plate with the “UP” arrows up and the spacers facing toward you into the casting frame Insert the short glass plate in the front of the casting frame. There should be a space between the plates.

4. Secure the plates in the casting frame by pushing the two gates of the frame out to the sides.

5. Clamp the casting frame with glass plates into the casting stand, with the gates of the casting frame facing you. Repeat steps 1-5 to prepare a second gel in the casting frame.

6. Check to see if the assembled plates in the casting stand are sealed properly by pipetting a small amount of deionized water into the gap between the plates. If the glass plates hold water and don’t leak, you are ready to make the gels. Pour the water out by holding the entire casting platform over a liquid waste container or sink. Use paper towels or tissues to absorb any residual water. If the gel leaks, disassemble the frame, dry the plates and go back to step 3.

Assemble the chemicals that you will need to pour the gels.

Reagent	Resolving gel	Stacking gel
Deionized water	3.5 mL	2.1 mL
30% acrylamide:bis-acrylamide (29:1)	4.0 mL	0.63 mL
1.5 M Tris-HCl, 0.4% SDS, pH 8.8	2.5 mL	------
0.5 M Tris-HCl, 0.4% SDS, pH 6.8	------	1.0 mL
10% ammonium persulfate (catalyst)	100 µL	30 µL
TEMED (catalyst)	10 µL	7.5 µL

1. Label two 15 mL conical tubes "Resolving gel" and "Stacking gel".
2. 2. Prepare ONLY the resolving gels at this time. Mix the acrylamide solution, pH 8.8 Tris buffer and water, as shown in the chart above. Mix the ingredients gently, trying not to introduce air. Oxygen inhibits polymerization of acrylamide gels.
3. To the resolving gel mixture, add 100 µL of a 10% ammonium persulfate (APS) solution. Gently mix the solution, trying not to introduce air. Oxygen inhibits acrylamide polymerization.
4. Add 10 µL of TEMED catalyst. Once again, gently mix in the catalyst trying not to introduce air bubbles.
5. Working quickly, use a plastic transfer pipette to fill the space between the two plates until the resolving gel solution reaches a height just above the green clamps on the gel casting frame. Draw up any remaining acrylamide into the transfer pipette.
6. Using a transfer pipette, add deionized water so that it gently flows across the surface of the polyacrylamide mixture. The water layer ensures that the polyacrylamide gel will have a level surface once it polymerizes.
7. Allow the gel to polymerize, which takes ~15-20 minutes. You will note that the interface between the polyacrylamide and water overlay disappears temporarily while the gel polymerizes. A sharp new interface then forms between the two layers, indicating that polymerization is complete.
8. When polymerization is complete, remove the water from the top of the resolving gel by tilting the gel to the side and using a paper towel or Kimwipe to wick out the water.

Pour the stacking gels

1. Prepare the stacking gels. Mix the acrylamide solution, pH 6.8 Tris buffer and water, as shown in the chart above.

2. Add 30 µL 10% APS and 7.5 µL TEMED to the stacking gel acrylamide mixture. Mix the contents by gently inverting the tube twice.

3. Use a transfer pipette to pipette the stacking gel on top of the resolving gel between the two glass plates. Add enough stacking solution until it just reaches the top of the small plate.

4. Carefully, but quickly, lower the comb into position, being careful not to introduce air bubbles. Adding the comb will force some solution out of the gel, but this is fine. If air bubbles become trapped below the comb, remove the comb and reposition it.

Save the SDS-PAGE gels

1. Carefully remove the gels from the casting stand and then from their green frames.

2. Keeping the combs in the gel, wrap the gels in a wet paper towel. Then wrap the gels in plastic wrap to be used in later labs. The gels will be ruined if they are not kept wet and properly wrapped!

Running SDS-PAGE gels Set up the electrophoresis apparatus

1. Retrieve one of the SDS-PAGE gels from the refrigerator.

2. Carefully remove the comb from the spacer gel.

3. Remove the casting frame from the gel cassette sandwich and place the sandwich against the gasket on one side of the electrode assembly, with the short plate facing inward. Place a second gel cassette or a buffer dam against the gasket in the other side of the electrode assembly.

4. Clamp the green clamps on the sides of the electrode assembly (below).

5. Lower the chamber into the electrophoresis tank.

6. Fill the space between the two gels with Tris-glycine running buffer. This forms the upper chamber for electrophoresis.

7. Add Tris-glycine running buffer to the outer (lower) chamber until the level is high enough to cover the platinum wire in the electrode assembly.

Load and run samples on the SDS-PAGE gel

1. Retrieve your cell extracts from the freezer. Recall that the samples have already been mixed with a tracking dye and glycerol. Allow the extracts to thaw and vortex vigorously for ~ 10 seconds to thoroughly mix the contents.

2. Using gel loading micropipette load up to 15 µL of sample into each well. Load 5 µL of a molecular weight standard into one lane of the gel. Load samples slowly and allow the samples to settle evenly on the bottom of the well.

3. Connect the tank to the power supply. Fit the tank cover onto the electrodes protruding up from the electrode assembly. Insert the electrical leads into the power supply outlets (connect black to black and red to red).

4. Turn on the power supply. Run the gel at a constant voltage of 120-150 V. Run the gel until the blue dye front nearly reaches the bottom of the gel. This may take between 45-60 min.

Staining SDS-PAGE gels

1. Turn off the power supply.

2. Remove the gel apparatus from the tank. Open the clamping frame and remove the gel cassette sandwich. Carefully, pry the two plates apart with a spatula. With the spatula, remove the lower right or left corner of the gel to serve as an orientation marker. Be sure to indicate in your lab notebook whether the notched corner corresponds to lane 1 or lane 10 of the gel. You may also remove the stacking gel with the spatula, if you desire.

3. Place the gel in a small plastic tray and label the tray with your initials on a piece of tape. To do this, fill the tray about halfway with deionized water. Gently free the gel from the glass plate, allowing it to slide into the water. The gel should move freely in the water. Place the gel and tray on a rocking platform. Rock the gel for ~2 minutes.

4. Drain the water from the gel and add enough Simply Blue to cover the gel, while allowing the gel to move freely when the tray is rocked. Cover the gel container with saran-wrap and rock overnight. Make sure that the gel does not stick to the bottom of the tray.

5. In the morning, drain the Simply Blue stain into an appropriately labeled waste container in the hood of the lab room.

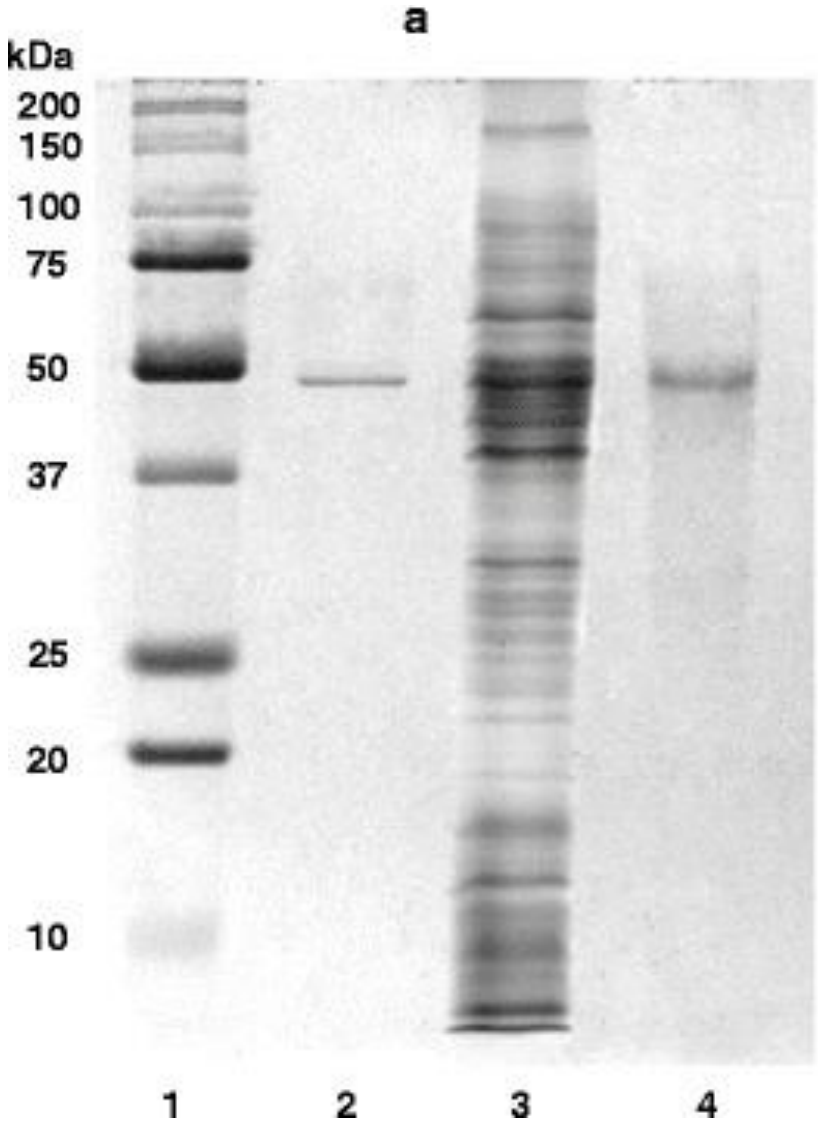
6. Destain the gel by filling the container about half full with deionized water. Shake the gel in the water for ~2 minutes. Pour off the water and add new deionized water. Repeat, if necessary, until protein bands become visible.

7. When individual bands are detectable, record your data. You may photograph the gel with your cell phone camera against a white background. Alternatively, place the gel in a clear plastic page protector and scan the gel.

8. After recording the data, dispose of the gel in the Biohazard waste container.

RESULTS

The purification method of surface enolase-like protein involved the use of sonication, extraction, and preparative electrophoresis. The first step of isolating the surface proteins was sonication of bacteria suspended in a buffer containing a protease inhibitor. Next, the undisrupted cells were removed by centrifugation, and the resulting supernatant was ultracentrifuged with the aim to separate the cellular membrane fraction from the cytosolic one. The resulting sediment contained protein fractions bound to external and cytoplasmic membranes. After undergoing extraction cycles in detergent buffers containing a complexing compound a mixture of proteins was received Preparative electrophoresis for separation of membrane proteins for detection allowed obtaining a purified membrane protein of electrophoretic mobility of 47 kDa.



CONCLUSION

From my internship at BITS PILANI, Goa, I gained hands on experience in Molecular biology techniques.

I gained practical exposure to various instruments used in research laboratory.

I got a clear view of what it meant to be in the research world.

I got a better understanding of how the scientific research work goes on and how effective it is.

I gained a lot more interest in research after completing the internship.

Overall, I found the winter internship experience to be positive, and I'm sure I will be able to use the skills I learned in my career later.