University of Lethbridge

FIAT LUX

2023

# ULETH IGEM
# LAB PROTOCOLS

Protocols used by the University of Lethbridge iGEM team during the 2023 year for the Club$^2$ project

University of Lethbridge

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# Elution Buffer Preparation

## Materials

- 1M Tris (pH 8)
- 5M NaCl
- Imidazole
- Monosodium phosphate ($NaH_2PO_4$)
- pH Meter
- MilliQ Water
- 500mL glass bottle
- Balance and stir plate
- Graduated cylinder
- Vacuum filter
- Stir bar

## Protocol

1. In the 500mL bottle, combine the following components:

| **COAP-GFP** | **PbEL04** |
|---|---|
| 3.45g $NaH_2PO_4$ | 10mL of 1M Tris |
| 30mL of 5M NaCl | 25mL 5M of NaCl |
| 8.51g of imidazole | 10.21g of imidazole |

2. Add 250mL of MilliQ water and mix all components together
3. Test and adjust the pH of the solution to 8.0
4. Top up to 500mL with MilliQ water
5. Filter the solution using the bottle top vacuum filtration
6. Degas the solution using the vacuum filter with a stir bar for ~1hr (until there are no bubbles)
7. Label the bottle with your initials, date, and its components.

# Binding Buffer Preparation

## Materials

- 1M Tris (pH 8)
- 5M NaCl
- 100% Triton X-100
- Imidazole
- $NaH_2PO_4$
- Graduated cylinder
- Lysozyme
- pH Meter
- MilliQ Water
- 500mL glass bottle
- Vacuum filter
- Balance and stir plate

## Protocol

1. In the 500mL bottle with 400mL of MilliQ water, combine the following components:

| COAP-GFP | PbEL04 |
|---|---|
| 3.45g $NaH_2PO_4$ | 10mL of 1M Tris |
| 30mL of 5M NaCl | 25mL 5M of NaCl |
| 8.51g of imidazole | 340.4mg of imidazole |
| 2.5mL of 100% Triton X-100 | 0.5mL of 100% Triton X-100 |
| 1.5g lysozyme | |

2. Adjust volume to 500mL and mix all components together
3. Test and adjust the pH of the solution to 8.00
4. Filter the solution using the bottle top vacuum filtration
5. Degas the solution using the vacuum filter with a stir bar for ~1hr (until there are no bubbles)
6. Label the bottle with your initials, date, and its components.

# 10x SDS Running Buffer Preparation

## Materials

- Tris base
- Glycine
- SDS
- MilliQ Water
- 1L glass bottle
- Balance and stir plate
- Graduated Cylinder

## Protocol

1. In a 1L bottle with 800mL water, combine all components together:
    - 30.3g of Tris
    - 144.4g of Glycine
    - 10g of SDS
2. Adjust volume to 1L and mix all components together.

# Glycerol Stocks

## Materials

- Bacterial Cultures
- 50% Glycerol
- Sterile loop
- -80°C freezer
- 2mL screw top tubes
- LB Agar Plate
- Pipet and tips

## Protocol

1. Add 500µL of overnight bacterial culture to 500µL of 50% glycerol in a 2mL screw top tube or cryovial and gently mix.
2. Freeze the glycerol stock tube at -80°C.
3. To recover bacteria from your glycerol stock, open the tube and use a sterile loop, toothpick, or pipette tip to scrape some of the frozen bacteria off the top. Do not let the glycerol stock thaw! Streak the bacteria onto an LB agar plate.
4. Grow your bacteria overnight at the appropriate temperature. The next day you will be able to start an overnight culture for plasmid DNA prep the following day.

# Antibiotic Preparation

## Materials

- 100% Ethanol
- MilliQ water
- Chloramphenicol
- Kanamycin sulfate
- 1.5mL tubes
- Balance
- 0.22µM syringe filter
- 10mL falcon tubes
- -20°C freezer

## Protocol (Chloramphenicol Preparation)

1. In a 10mL falcon tube, dissolve 0.34g of chloramphenicol into 9mL of 100% ethanol
2. Adjust to final volume of 10mL using 100% Ethanol
3. Filter sterilize using a 0.22µM syringe filter
4. Aliquot 1mL into 1.5mL tubes, label each tube ‘CHL 34 mg/mL’ and the date it was made
5. Store in -20°C freezer and use within one month.

## Protocol (Kanamycin Preparation)

1. In a 10mL falcon tube, dissolve 0.50g of kanamycin sulfate into 9mL of MilliQ water
2. Adjust to final volume of 10mL using MilliQ water
3. Filter sterilize using a 0.22µM syringe filter
4. Aliquot 1mL into 1.5mL tubes, label each tube ‘KAN 50 mg/mL’ and the date it was made
5. Store in -20°C freezer and use within one month.

# 1M IPTG Preparation

## Materials

- Isopropyl ß-D-1-thiogalactopyranoside (IPTG)
- MilliQ water
- 10mL falcon tube
- 0.22µM syringe filter
- Balance
- 1.5mL tubes

## Protocol

1. In a 10mL falcon tube, dissolve 2.38g of IPTG in 8mL of MilliQ water by inverting the tube
2. Adjust to final volume of 10mL using MilliQ water
3. Filter sterilize using a 0.22µM syringe filter
4. Aliquot 1mL into 1.5ml tubes, label each tube ‘1M IPTG’ and the date it was made
5. Store in -20°C freezer and use within one month.

# LB Media Preparation

## Materials

- Tryptone
- Yeast extract
- NaCl
- MilliQ water
- 1L beaker
- 5M NaOH
- Graduated cylinder
- Balance and stir plate
- pH meter
- Scoopula and weigh boat
- Aluminium foil and autoclave tape

## Protocol

1. For a 500mL LB media, weigh each component using a scoopula and weigh boat:
    - 5g Tryptone
    - 2.5g Yeast extract
    - 5g NaCl
2. Combine all components in a 1L beaker
3. Measure 500mL of MilliQ water in a graduated cylinder and pour into 1L beaker
4. Mix all components together
5. Adjust pH to 7 with 5M NaOH using the pH meter
6. Cover media with aluminum foil and apply autoclave tape, and include the appropriate information (Initials, contents, date)
7. Autoclave* liquid media for 20 minutes on liquid cycle

    *If autoclave is not readily available, store media at 4°C
8. Store autoclaved media at room temperature, or at 4°C if not using within the next week

# 2xYT Media Preparation

## Materials

- Tryptone
- Yeast extract
- NaCl
- MilliQ water
- 2L beaker
- 5M NaOH
- Graduated cylinder
- Balance and stir plate
- pH meter
- Scoopula and weigh boat
- Aluminium foil and autoclave tape

## Protocol

1. For 1L 2x YT media, weigh each component using a scoopula and weigh boat:
    - 16g Tryptone
    - 10g Yeast extract
    - 5g NaCl
2. Combine all components in a 2L beaker
3. Measure 1000mL of MilliQ water in a graduated cylinder and pour into 2L beaker
4. Mix all components together
5. Adjust pH to 7 with 5M NaOH using the pH meter
6. Cover media with aluminum foil and apply autoclave tape, and include the appropriate information (Initials, contents, date)
7. Autoclave* liquid media for 20 minutes on liquid cycle

    *If autoclave is not readily available, store media at 4°C
8. Store autoclaved media at room temperature, or at 4°C if not using within the next week

# Restriction Digest

## Materials

- 10x CutSmart Buffer (New England Biolabs)
- Restriction enzymes (XhoI and NheI)
- Plasmid DNA
- Microcentrifuge tubes
- Pipette and tips
- Water bath
- Heat block

## Protocol

1. Take a 5µL sample of the plasmid DNA to be digested to run on an agarose gel later
2. Mix the following reaction in a microcentrifuge tube on ice:

| Component | Volume per reaction |
|---|---|
| 10x CutSmart Buffer | 5µL |
| Restriction enzymes | 1µL of each |
| Plasmid DNA | ~1µL |
| MilliQ water | Up to 50µL |
| **Total volume** | **50 µL** |

3. Incubate for 1.5 hours at 37°C
4. Heat inactivate the reaction at 65°C for 20 minutes
1. Run the pre-digest sample and post-digest sample on an agarose gel
2. Perform gel extraction on the successfully digested bands

# DNA Gel Extraction (New England Biolabs kit)

## Materials

- Monarch DNA Gel Extraction Kit (New England Biolabs)
- Razor blade or scalpel
- 1.5mL microcentrifuge tube
- Balance
- Monarch Gel Dissolving Buffer
- Water bath
- Monarch DNA Cleanup Columns
- Centrifuge
- DNA Wash Buffer
- DNA Elution Buffer
- Pipette and tips
- Biodrop

## Protocol

1. Cut out the DNA fragment to be purified from the agarose gel with a clean razor blade or scalpel. Trim excess agarose away.
2. Transfer it to a new 1.5mL microcentrifuge tube.
3. Add 4 volumes of Monarch Gel Dissolving Buffer* to the tube with the excised gel (e.g., 400µL buffer per 100mg of agarose)
4. Incubate the sample between 37-55°C, inverting periodically until the gel slice is completely dissolved (generally 5-10 minutes)
   *If the gel slice is >150mg, considering reducing the amount of buffer to 3 or 3.5 volumes to minimize the guanidine salt present in the workflow
5. Insert the column into the collection tube and load sample onto the column
6. Spin for 1 minute at 16,000xg and room temperature (RT), and discard flow-through
3. Re-insert column into collection tube and add 200µL DNA Wash Buffer and spin again for 1 minute. Discard flow-through
4. Repeat wash (step 7)
5. Transfer column to a clean 1.5mL microcentrifuge tube. Use care to ensure that the tip of the column has not come into contract with the flow-through. If in doubt, re-spin for 1 minute before placing into clean tube.
6. Add ≥6µL* of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, and spin for 1 minute to elute DNA.
   *typical elution volumes are 6-20µL
7. Check DNA concentration on a Biodrop

# DNA Ligation

## Materials

- T4 DNA ligase (New England Biolabs)
- T4 DNA ligase buffer (10x)
- Vector DNA
- Insert DNA
- Incubator
- Microcentrifuge tubes
- Pipette and tips
- Ice
- MilliQ water

## Protocol

8. Mix the following reaction in a microcentrifuge tube on ice:

| Component | Volume per reaction |
|---|---|
| T4 DNA Ligase Buffer (10x) | 2µL |
| Vector DNA | 50ng |
| Insert DNA | 37.5ng |
| MilliQ water | Up to 20µL |
| T4 DNA ligase | 1µL |
| **Total volume** | **20 µL** |

9. Incubate overnight at 16°C
10. Heat inactivate the reaction at 65°C for 10 minutes
11. Chill on ice and transform 1-5µL of the reaction according to our transformation protocol

# Colony PCR

## Materials

- 0.5µM T7 forward primer
- 0.5µM T7 reverse primer
- Template DNA
- One*Taq* Quickload 2x Master Mix (New England Biolabs)
- 0.2mL PCR tubes
- Nuclease free water
- Pipet and tips
- Thermocycler

## Protocol

1. Prepare PCR mix on ice according to these volumes:

| **Reagent** | **Volume (per reaction)** |
|---|---|
| 10 µM T7 forward primer | 0.5 µM final concentration |
| 10 µM T7 reverse primer | 0.5 µM final concentration |
| Template DNA | 2µL |
| One*Taq* Quickload 2x Master Mix | 12.5µL |
| Nuclease free water | Up to 25µL |
| **Total volume** | **25 µL** |

2. Transfer a colony to a PCR tube using a sterile pipet tip and briefly stirring the pipet tip in the PCR tube to resuspend the colony
3. Amplify the DNA with the following thermocycling conditions:

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initial Denaturation | 95°C | 15 min | 1 |
| Denaturation | 95°C | 45 sec | 35 |
| Annealing | 48°C | 45 sec | |
| Extension | 72°C | 2 min | |
| **Final extension** | 72°C | 15 min | 1 |

4. After PCR, perform an agarose gel electrophoresis.

## Materials

- Agarose powder
- 1x TAE
- SYBR Safe DNA gel stain (Thermo Fisher Scientific)
- 250mL flasks
- Casting tray and well combs
- Pipette and tips
- Microwave
- Balance, scoopula, and weigh boat
- Gel box
- Voltage source
- UV light source
- 6x Gel Loading Dye, Purple (New England Biolabs)
- DNA sample to be visualized
- GeneRuler 1 kb DNA ladder (Thermo Fisher Scientific)

## Protocol

1. Measure 1.8g of agarose powder and mix with 100mL 1x TAE in a 250mL flask
2. Microwave flask in 30 second intervals until the agarose is completely dissolved
3. Let the solution cool down until you can comfortably hold the flask
4. Place the casting tray with the well comb in place into the gel box and pour the solution into the casting tray
5. Let the solution completely solidify
6. Once solidified, pour 1x TAE into the gel box until the gel is sufficiently covered
7. Add 2µL of 6x loading dye into 10µL of DNA samples
8. Load 3µL of 1 kb DNA ladder and 12µL of DNA samples into each well
9. Plug the gel box to the voltage source and run at 100V for 30 minutes
10. Once done, unplug from voltage source and remove the gel
11. Stain the gel with diluted SYBR safe in 1x TAE for ~10 minutes on a rocker
12. Visualize your gel under a UV light source

# Protocol Title New England Biolabs

## Materials

- Overnight culture
- Tabletop centrifuge
- 1.5mL and 2mL Eppendorf tubes
- Pipet and tips
- EZ-10 column
- Solution I, II, and III
- Wash solution
- Elution buffer
- Biodrop

## Protocol

1. Add 1.5-2 mL overnight culture to a centrifuge tube and centrifuge at 12 000 RPM for 2 minutes. Drain liquid completely.
2. Add 100 µL of Solution I to the pellet, mix well and keep for 1 minute.
3. Add 200 µL of Solution II to the mixture and mix gently inverting the tube 4-6 times. Keep at room temperature for 1 minute.
4. Add 350 µL of Solution III and mix gently. Incubate at room temperature for 1 minute. A fluffy white material should form and the lysate should become less viscous.
5. Centrifuge at 12,000 RPM for 5 minutes.
6. Transfer the supernatant to the EZ-10 column, then centrifuge at 10,000 RPM for 2 minutes.
7. Discard the flow through in the tube and add 750 µL of Wash Solution to the column, and centrifuge again at 10,000 RPM for 2 minutes. Repeat this wash procedure twice.
8. Discard the flow through in the collection tube and centrifuge at 10,000 RPM for an additional minute to remove an additional wash solution.
9. Transfer the column to a clean 1.5 mL centrifuge tube and add 50 mL of Elution Buffer into the center portion of the column and incubate at room temperature for 2 minutes, then centrifuge at 10,000 RPM for 2 minutes.
10. Check the DNA concentration on a Biodrop
11. Store purified DNA at -20° C.

# Sanger Sequencing

## Materials

- Purified plasmid DNA (50 ng/µL)
- Forward and reverse primers (0.25 µM)
- 1.5 mL microcentrifuge tubes
- Pipette and tips
- MilliQ water

## Protocol

1. Mix plasmid DNA and forward and reverse primers to a final volume of 15 µL into appropriately labeled 1.5 mL microcentrifuge tubes
2. Deliver samples to the Molecular Biology Facility (MBSU) at the University of Alberta

# Primary Cultures

## Materials

- Autoclaved LB (for PbEL04) or 2xYT media (for COAP-GFP)
- 50 mg/mL Chloramphenicol
- 50 mg/mL Kanamycin
- Erlenmeyer flask
- Transformed BL21 glycerol stock
- Pipet and tips
- Incubator

## Protocol

1. Prepare 50mL of autoclaved media.
2. Aseptically, add 50µL of 50mg/mL Chloramphenicol and 50µL of 50mg/mL Kanamycin.
3. Bring cryotube containing transformed BL21 glycerol stock, aseptically dip pipet tip into stock and eject tip into the flask.
4. Grow overnight at 37°C and 220rpm.

# Secondary Cultures

## Materials

- 2L flasks containing 500 mL LB or 2xYT media each
- 50mL falcon tubes
- 1M IPTG
- Incubator
- 50 mg/mL Chloramphenicol
- 50 mg/mL Kanamycin
- Cuvettes and Spectrophotometer
- Pipet and tips
- Centrifuge

## Protocol

1. Prepare two 2L flasks containing 500mL of media.
2. Into each flask add aseptically: 500µL of 50mg/mL Chloramphenicol, and 500µL of 50mg/mL Kanamycin.
3. Using a sterile 50mL falcon tube, measure and add 20-25mL of overnight grown primary culture to each secondary flask.
4. Grow flasks at 37°C and 180rpm.
5. Monitor the $OD_{600}$ reading every ~30min until the OD600nm nears 0.6. When the $OD_{600}$ reaches 0.6, aseptically add 250µL of 1M IPTG into each flask
6. Turn the temperature down to 18°C (Leave at 220rpm) and leave the culture for 4hrs.
7. Harvest cells in a centrifuge and store at -80°C.

# Transformation

## Materials

- Competent *Escherichia coli* cells
- Plasmid DNA
- LB media
- Water bath
- Centrifuge
- Ice
- LB media agar plate w/ appropriate antibiotics
- Glass Beads
- Pipette and Tips
- Incubator
- 1.5mL microfuge tubes

## Protocol

1. Set the water bath to 42°C.
2. Thaw competent cells and DNA to be transformed on ice
3. Add 10-100 ng of DNA into the competent cells and gently mix
4. Incubate the mixture on ice for 5-15 minutes
5. Heat shock the samples in the 42°C water bath for 30 seconds
6. Place the samples back directly on ice for 5 minutes
7. Add 1mL of LB media, then incubate for 1 hour at 37°C and at 250rpm
8. Centrifuge the samples for 10 minutes at 5000rpm.
9. Discard 900µL of the supernatant
10. Resuspend the pellet in the remaining 100µL of media in the tube.
11. Spread the 100 µL onto an agar plate with the appropriate antibiotics
12. Add 5-10 sterile glass beads to aid in spreading the culture
13. Incubate overnight (for approximately 16 hours) at 37°C

# Expression Screening

## Materials

- Bacterial culture
- Appropriate antibiotics
- Lysis buffer
- 1M IPTG
- 20mg/mL lysozyme
- 8M Urea
- 25mg/mL sodium deoxycholate
- Cuvettes and spectrophotometer
- Incubator, Sonicator, and Centrifuge
- Pipet and tips
- 2mL microcentrifuge tubes
- 15mL falcon tubes

## Protocol

1. Grow roughly 50mL of overnight culture with the correct antibiotics. Inoculate using BL21 cells transformed with the correct vector. Do not allow the cultures to grow for over 24hrs.
2. Inoculate 500mL flasks with 25mL of overnight culture (Also 500µL of the appropriate antibiotics)
3. Grow cultures at 37°C, periodically monitoring the $O.D._{600}$ reading until it reaches 0.6. At this point add 250µL of IPTG to the culture and adjust the incubator to the desired expression temperature.
4. After 2, 4, 8 and 16 hours remove a 2mL of culture aseptically in a 2mL microcentrifuge tube. Spin at 10,000 x g for 2 min, then discard the supernatant. Repeat for all cultures and timepoints.
5. Using GFP-1 lysis buffer (or any other lysis buffer) resuspend the cell pellet from each time point in 1mL by pipetting. Then transfer the resuspended pellet from the 2mL microcentrifuge tube into a 15mL falcon tube. **Label the 15mL falcon tubes ahead of time.**
6. **Put the 15mL falcon tubes on ice.** Prepare a solution of 20mg/mL of lysozyme (weigh out 20mg of lysozyme and dissolve in 1mL MilliQ) and 25mg/mL Sodium deoxycholate. Then add 50µL of each solution to each tube. Invert several times, leave on ice for 10min, inverting periodically.

7. Sonicate each tube for 20 cycles (on/off is one cycle), then wait 5s and sonicate again for 15 cycles.
8. Transfer 750µL from each falcon tube into a NEW 2mL microcentrifuge tube. **Label the 2mL microcentrifuge tubes ahead of time.**
9. Spin the 2mL microcentrifuge tubes in the microcentrifuge at 16,000 x g for 20 min.
10. Pipette off the supernatant from each tube into a second, labelled tube. Make sure you differentiate between the supernatant and the pellet somehow. Then resuspend the pellet from step 9 with 750µL of 8M Urea
11. Run the samples on a SDS-PAGE and then perform a western blot

# COAP-GFP Protein Purification

## Materials

- Binding Buffer (50 mM $NaH_2PO_4$ pH 8.0, 300 mM NaCl, 0.5% Triton X-100, 20 mM imidazole, 3 mg/mL lysozyme)
- Equilibrium Buffer (50 mM $NaH_2PO_4$ pH 8.0, 300 mM NaCl, 20 mM imidazole)
- Elution Buffer (50 mM $NaH_2PO_4$ pH 8.0, 300 mM NaCl, 250 mM imidazole)
- Wash Buffer (50 mM $NaH_2PO_4$ pH 8.0, 300 mM NaCl, 30 mM imidazole)
- Lysozyme
- 50 mL Falcon tubes
- *Escherichia coli* pellet with overexpressed COAP-GFP
- Sonicator
- Benzonase (250 U/µL)
- 0.22 µM syringe filter
- Gravity flow column
- Ni-NTA resin
- MilliQ water
- 15 mL Falcon tubes
- Erlenmeyer flasks

## Protocol

**Cell Lysis:**

1. Resuspend cell pellet (from secondary culture) in Binding Buffer (3 mL per gram of cells)
2. Sonicate the cell lysate on ice at 60% output and 60% duty cycle for 30 seconds on and 30 seconds off, for a maximum of 5 minutes
3. Add 5 µL benzonase and leave to digest at room temperature while shaking for 30 minutes
4. Clarify the cell lysate by centrifugation at 30,000xg for 45 minutes at 4°C
5. Pour the supernatant into a new tube and properly dispose of the cell pellet
6. Filter the supernatant with a 0.22 µM syringe filter into a fresh tube

**Chromatography:**

1. Load 4 mL of Ni-NTA resin (50% slurry) onto a column
2. Let the resin settle and rinse with 1 column volume (CV) of MilliQ water and 1 CV of Equilibrium Buffer
3. Fill column with cell lysate and gently resuspend the beads
4. Leave on tube rocker at room temperature for 1 hour

5. Drain lysate into new falcon tube and save the unbound fraction
6. Fill column with 5 CVs of Equilibrium Buffer and drain into clean Erlenmeyer flask
7. Fill column with 5 CVs of Wash Buffer and drain into another clean Erlenmeyer flask
8. Fill column with 5 mL of Elution Buffer at a time, collecting 5 mL fractions in 15 mL falcon tubes
9. Run samples on an SDS-PAGE to determine where COAP-GFP has eluted
10. Properly rinse and store the $Ni^{2+}$ column in 70% ethanol

# PbEL04 Protein Purification

## Materials

- Binding Buffer (20 mM Tris pH 8.0, 250 mM NaCl, 0.1% Triton X-100, 10 mM imidazole)
- Elution Buffer (20 mM Tris pH 8.0, 250 mM NaCl, 0.1% Triton X-100, 300 mM imidazole)
- *Escherichia coli* pellet with overexpressed PbEL04
- 50 mL Falcon tubes
- Deoxycholic acid
- 1 mg/mL lysozyme
- DNase I
- Sonicator
- High speed centrifuge
- 0.22 µM Syringe filters
- HisTrap HP 5 mL Column (Cytiva)
- ÄKTA pure™ chromatography system (Cytiva)
- Fraction collector

## Protocol

**Cell Lysis:**

1. Resuspend cell pellet (from secondary culture) in Binding Buffer (10 mL per gram of cells) on ice
2. Add 1 mg/mL of lysozyme crystals and incubate the cell suspension on ice for 30 minutes
3. Add 1.25 mg/mL deoxycholic acid and 2 µL DNase I
4. Sonicate the cell lysate at 60% output and 60% duty cycle for 30 seconds on and 30 seconds off, for a maximum of 5 minutes
5. Clarify the cell lysate by centrifugation at 30,000xg for 45 minutes at 4°C
6. Pour the supernatant into a new tube and properly dispose of the cell pellet
7. Filter the supernatant with a 0.22 µM syringe filter into a fresh tube

**Chromatography (at 4°C):**

1. Equilibrate a HisTrap HP 5 mL column in 5 column volumes (CVs) MilliQ water and 5 CVs Binding Buffer

2. Equilibrate the sample loop in Binding Buffer
3. Incubate the cell lysate on the column for 1 hour
4. Wash the column with 10 CVs Binding Buffer
5. Apply a linear gradient to the column of 10 CVs of gradually increased Elution Buffer
6. Based on the chromatogram, run fractions on an SDS-PAGE gel
7. Pool fractions containing clean PbEL04
8. Ensure that the column is cleaned and stored properly in 20% ethanol

# SDS-PAGE gel premixes

## Materials

- 40% Acrylamide (29:1)Materials
- 
- 1.5M Tris (pH 6.8 and 8.8)
- 10% SDS
- 250mL and 500mL screw-cap bottle
- Graduated cylinder
- Stir plate

## Protocol

**15% SDS-PAGE resolving premix (400mL)**

1. To a 500mL screw-cap bottle, combine the following components:
    - 150mL of 40% Acrylamide (29:1)
    - 100mL of 1.5M Tris* (pH 8.8)
    - 4mL of 10% SDS
    - 146mL MilliQ Water
2. Mix all components together

**6% SDS-PAGE stacking premix (200mL)**

1. To a 250mL screw-cap bottle, combine the following components:
    - 30mL of 40% Acrylamide (29:1)
    - 25mL of 1.5M Tris* (pH 6.8)
    - 2mL of 10% SDS
    - 143mL MilliQ water
2. Mix all components together

*Ensure that the pH of Tris solution used is correct for the premix desired.

# Run an SDS-PAGE

## Materials

- Clamp, gel holder, and well combMaterials
- 
- 0.75mm or 1.0mm glass plate
- Kimwipe and MilliQ water
- 15% resolving gel premix
- 6% stacking gel premix
- 10% APS
- TEMED
- Isopropyl alcohol
- 15mL falcon tubes

## Protocol

1. Take the stand for the clamps, the clamp, a comb, a piece of rubber for the bottom of the stand and clean all pieces with water.
2. Depending on your experiment choose either a 0.75mm or a 1.0mm glass plate, along with a short glass plate, wash both with water then ethanol, drying with a kimwipe.
3. Place the rubber piece at the base of the stand
4. Take the two pieces of glass and place them together so that there is a gap between them, place the pieces of glass in the clamp and ensure that the glass is even at the bottom, clamp the clamp.
5. Wrap a piece of parafilm around the bottom of the glass pieces, and attach the glass and clamp to the stand
6. Pour 6mL of 15% SDS-PAGE resolving gel premix into a falcon tube, to a separate falcon tube pour 3.5mL of 6% stacking gel
7. When ready, transfer 60µL of 10% APS and 6µL of TEMED to the falcon tube with the resolving gel (once the TEMED is added the solution will begin to polymerize so only add the TEMED when ready to use) invert the falcon tube a couple times to ensure adequate mixing
8. Pour the resolving gel in the gap between the two glass pieces until it reaches about 1cm below the bottom of the comb, fill to the top of the gap with isopropyl alcohol ensuring that the liquid is even (ensure that the faint line from the resolving gel is straight).

# Run an SDS-PAGE (continued)

## Materials

- Materials
- Comb
- Electrophoresis chamber
- 1x SDS Running buffer
- 6x SDS loading dye
- 0.5mL microcentrifuge tube
- Pipet and tips
- Voltage source
- Heat block

## Protocol

9. Use the remaining resolving gel solution in the falcon tube as an indicator of polymerization, once polymerization has finished and the resolving gel mixture has solidified (around 15-20 minutes), pour the isopropyl out of the gap.
10. Transfer 35µL of 10% APS and 3.5µL of TEMED to the test tube containing the 6% stacking gel and invert a few times to mix the contents.
11. Pour the stacking gel mix into the gap between the two pieces of glass until it reaches the top or it begins to spill slightly over the top of the gap
12. Quickly insert the comb from left to right
13. Wait for the gel to fully polymerize
14. Place gels in the gel holder then into the electrophoresis chamber
15. Add 1x running buffer inside the gel holder then into the electrophoresis chamber
16. Prepare samples to be loaded by adding 2µL SDS loading dye to a microcentrifuge tube, then add 10 µL sample and mix by pipetting up and down.
17. Heat samples at 95℃ for 5 minutes
18. Add the sample/dye mix to the wells (place elbow on the table and steady wrist with hand not holding the pipette)
19. Cover the chamber and connect the anode and cathode, set the voltage to 200V, and allow to electrophoresis for ~45-90 minutes.
20. Disconnect the anode and cathode once the dye front has migrated out of the gel.

# Staining and Visualization of an SDS gel

## Materials

- Dehydration bufferMaterials
- Tupperware container
- 
- Coomassie stain
- Microwave
- Imager/Visualizer

## Protocol

1. Remove SDS-PAGE gel from glass and rinse once in dehydration buffer in a suitable container with a lid. Try not to use a container much larger or much smaller than the gel.
2. Add enough Coomassie Stain to cover the gel by 1/2 inch (~1.5cm).
3. Microwave on high power for 40 seconds to 1 minute (until the Coomassie Stain boils).
4. Incubate the gel in the Coomassie stain for 5 to 10 minutes on a rocking table. If you did not microwave the Coomassie/gel, incubate for at least 1 hour.
5. Pour off the Coomassie Stain. The Coomassie Stain can be recycled a couple of times by filtering it.
6. Use imager to take a picture of the gel

# Western Blot

## Materials

- Protein samples
- SDS-PAGE gel loading buffer
- SDS-PAGE gel
- Prestained molecular weight marker
- Transfer buffer and blocking buffer
- Appropriate antibodies
- PBST
- Nitrocellulose membrane, filter paper, and foam pad
- Heat block
- Voltage source
- Imager/Visualizer
- SuperSignal West Pico PLUS Substrate (Thermo Fisher Scientific)

## Protocol

1. Prepare your protein samples, ensuring they are denatured by heating at 95°C for 5 minutes in SDS-PAGE gel loading buffer.
2. Prepare a 15% SDS-PAGE gel and load the protein samples (usually 20µL) along with a pre-stained molecular weight marker. Run the gel at a constant voltage for 80V for 15 minutes and then 110V for 75 minutes.
3. Prepare the transfer apparatus and pre-equilibrate the membrane in transfer buffer. Assemble the gel/membrane sandwich and transfer the proteins onto a nitro-cellulose membrane by wet or semi-dry transfer methods. For a wet transfer, run the transfer for 110V for 70 minutes.
4. Block the membranes in the chosen blocking buffer (5% milk powder) for 1-2 hours at 4°C on a shaker.
5. Incubate the membrane with the primary antibody (diluted in blocking buffer and 0.1% PBST) specific to your target protein. Incubation time and temperature may vary but typically overnight at 4°C or 1-2 hours at room temperature.
6. Wash the membrane multiple times (3 times for 5-10 minutes each with PBST).

7. Incubate the membrane with the secondary antibody conjugated to an enzyme (e.g., HRP) or fluorescent marker for 1 hour at room temperature. Wash the membrane again with PBST.
8. Combine equal parts of the substrate and stable peroxide components.
9. Incubate the membrane with the substrate working solution for 5 minutes.
10. Use chemiluminescent or fluorescent detection reagents according to the manufacturers' instructions. Use a chemiluminescence imaging system to capture the signal.
11. Analyze the Western blot data using image analysis software to quantify protein band intensities. Compare and interpret your results and consider using a housekeeping protein as a loading control.

# pH Meter Calibration

## Materials

- pH meter
- Waste Beaker
- Kimwipes
- Calibrating Buffers
- MilliQ water

## Protocol

1. Turn on the pH meter and allow it to warm up for a few minutes before calibrating. While this is happening, unscrew the bottle containing the storage solution and rinse the probe with MilliQ water into an empty waste beaker. Once rinsed, dab dry with a Kimwipe.
2. Choose the required calibrating buffers. You will generally need more than one buffer for calibrating a pH meter. However, the first will always be a “neutral” buffer with a pH of 7.
3. Choose the second calibrating buffer. This choice should be based on the expected pH of the sample. The second should be near the expected pH of the sample. Once the buffers are selected ensure there is an adequate amount of the buffer in their respective sampling containers.
4. The probe is then going to gently be placed in the buffer with a pH of 7 and the “calibrate” button will be pressed to begin reading the sample. Allow the pH reading to stabilize and then allow it to sit for approximately 1-2 minutes to ensure the value isn’t fluctuating anymore.
5. Once a stable reading is achieved, set the pH meter to the value of the calibrating buffer pH by pressing the “enter” button. Then remove the probe from the pH 7 buffer and rinse ethe probe with MilliQ into a waste beaker.
6. Place the probe in the appropriate second selected buffer for your sample and repeat steps 3-4.
7. Calibration is now complete, and your sample may be measured. Push “measure” to take pH meter out of calibration and into measuring mode. Repeat steps 3-4 with your sample.

# Using a Centrifuge

## Materials

- Sample Tubes
- Centrifuge
- Appropriate PPE

## Protocol

1. Determine the correct type of centrifuge needed (i.e., microcentrifuge, tabletop centrifuge, ultracentrifuge, etc.). This decision should be based on the size of tubes being used and the speed at which the samples need to be spun at.
2. Wear appropriate PPE for the lab space, even if the samples may not require specific PPE.
3. Before using the centrifuge, ensure that the centrifuge is clean, and everything is in working order.
4. Place your sample tubes into the rotor so that they are distributed evenly around the center. Even distribution ensures that the centrifuge is balanced. Using the centrifuge in an unbalanced state can damage the centrifuge and be dangerous for the user. If you do not have a balanced number of samples, you may need to add a blank. Blanks are often an extra tube filled with water to the same volume as the sample. If your samples are significantly higher density than water, then you may need to fill the blank based on weight instead of volume.
5. If the centrifuge has an internal lid on the rotor, secure it and close the external lid.
6. Adjust the centrifuge settings accordingly and push start.
7. The centrifuge will come up to speed. If there re noises or the centrifuge begins to wobble, immediately stop as something is unbalanced or improperly loaded.
8. Once completed, carefully remove the samples to not dislodge any pellets or remix any liquids.

# Direct Fluorescence ELISA

## Materials

- ELISA microplate
- Purified COAP-GFP
- Bovine serum albumin (BSA)
- Blocking buffer (0.3% BSA in PBS)
- PBS
- Purified PbEL04
- ELISA plate reader
- Pipette and tips

## Protocol

1. Add 15-20µg COAP-GFP to microplate well in triplicate and incubate overnight at room temperature
2. Aspirate wells to remove liquid and wash the plates 4 times with PBS
3. Add 300µL blocking buffer to each well and incubate for 2 hours at room temperature
4. Aspirate and wash plate 4 times with PBS
5. Add varying concentrations of PbEL04 (3-10µg) to each well and incubate 3 hours at room temperature
6. Monitor fluorescence with an ELISA plate reader