Markelz Lab Manual

University at Buffalo

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Contents

1 Introduction

This lab manual is designed to provide an introduction to biochemistry for physics students. The manual is separated into 5 sections. After the introduction, you will find a list of lab equipment that you will be expected to know how to use. The third section is comprised of important laboratory practices and techniques. The fourth section is a list of useful lab procedures that you may be expected to replicate on your own. Finally, there is a collection of useful resources that you may refer to during your time in the lab.

2 Lab Equipment

When working in the lab, it is important to be familiar with the equipment that you will be using. Familiarity will help with understanding directions, asking questions, and being efficient.

- Scale-used to measure the mass of solid materials
- Micropipettes-used to sterilely transfer small volumes of liquid
- Serological Pipettes-used to sterilely transfer larger volumes of liquid
- Nanodrop-used to measure the absorbance or optical density of a liquid
- Centrifuge-used to separate components of liquids based on density
- Centrifuge Tube-capsule that is safe to use in a centrifuge
- Microcentrifuge Tube-capsule that is safe to use in a centrifuge
- Vortex-used to mix solutions in small tubes
- Incubator-used to maintain a constant temperature for cells to grow
- pH Meter-used to measure pH

3 Lab Practices

In addition to knowing and recognizing the equipment in the lab, it is also important to know how to keep the lab clean an organized.

3.1 Sterile Technique

Sterile technique is a collection of lab practices in order to ensure that an area is free of microorganisms. This is essential when working with bacteria as contamination may affect the results of your experiments.

- Wear gloves so that your cells do not contaminate the bacteria and so that the bacteria does not infect you. Wash your gloved hands with 70% ethanol. After working with bacteria, wash your hands.
- The lab bench must be washed down with 70% ethanol before and after use.
- Glassware is sterilized using the autoclave.
- A sterilized container should be kept sterile. In order to keep a container sterile when opening it, the lid and neck of the container must be repeatedly flamed in the following fashion:

1. Flame the entire circumference of the neck of the bottle.
2. Open the lid slightly. For a screw on cap, this could be about half a turn. For aluminum foil, unfold the edges slightly.
3. Repeat the first two steps until the cap is loose enough to easily lift off.
4. Flame the entire circumference of the lip of the container as well as the internal side of the cap.
5. Place the cap on the lab bench with the internal side facing up.

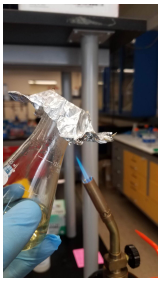


Figure 1: Flaming the neck of a sterile Erlenmeyer flask

- To close a cap, follow the previous procedure in reverse.

3.2 Clean Up After Yourself

In the lab, you generally want to return things to the way that you found them after use.

- After using the scale, brush down the scale with a paint brush, and wipe the surrounding lab bench with a wet paper towel.
- After using nanodrop or centrifuges, make sure that all cuvettes/tubes are removed and the devices are turned off.
- Wash lab equipment and wipe down lab bench after use.
- Return all lab equipment to where it was found.
- Place disposable items in the proper waste containers. i.e. disposable pipettes go in the sharps container.
- When washing lab equipment that has come into contact with bacteria, soak in a 5-10% bleach solution to ensure that all the bacteria is dead.

3.3 Communicate

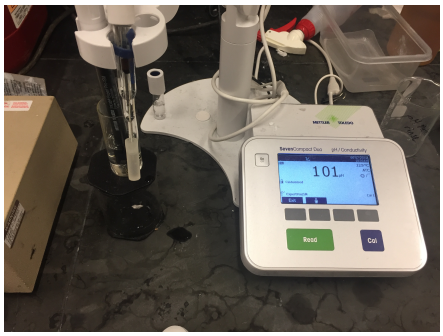
- Ask if you can use lab equipment before assuming that nobody else needs to use it.
- If the lab is running low on a certain item or material, notify somebody before it runs out.
- Notify someone if there is a spill.
- If you have questions, ask them.

4 Lab Protocols

4.1 Calibrating the pH Meter

1. Turn on pH meter.
2. Remove the electrode cap by gently unscrewing the white neck of the cap.
3. Place the cap on the table so that it is not in the way.
4. Place the pH rinse beaker under the pH probe.
5. Thoroughly rinse the entire black section of the probe with dH2O.
6. Dry the probe gently with a paper towel. Be sure to gently dab the tip of the probe.
7. Position the black stand beneath the probe of the pH meter.
8. Place the pH 1 standard on the stage.
9. Press the calibrate button.
10. Lower the arm of the pH meter so that the probe is completely submerged in the buffer solution. Your setup should look like Figure 2a at this point.
11. Wait until the pH meter is done calibrating. This is indicated by a radical symbol on the M on the top right of the pH meter screen. See Figure 2b.
12. Click the "read" button.
13. Raise the arm so that the probe is no longer in the buffer solution.
14. Repeat steps 4-12 with the other buffer solutions.

It may help to view the following video for additional instruction:
https://www.youtube.com/watch?v=7yuXjGdQRzM



(a) pH meter during calibration

(b) pH meter screen when it is ready to read (notice the M in the top right)

Figure 2

4.2 Preparing Buffer Solutions

A buffer is a solution which resists changes in pH. A buffer normally consists of a weak acid and its conjugate base or a weak base and its conjugate acid.

1. Calibrate pH meter.
2. Measure desired amount of weak acid/base needed for desired molarity at desired volume.
3. Measure 60% of the desired volume of distilled water into a beaker.
4. Mix weak acid/base in water with a stirbar.
5. Keeping the mixture stirring add the pH probe into the beaker.
6. Add HCl or NaOH until the desired pH is reached.
7. Once the desired pH is reached, remove and wash the pH probe.
8. Add distilled water to the solution until the desired volume is reached.

4.3 Filtering Solutions With a Syringe

1. Remove the syringe plunger from the syringe.
2. Attach the filter to the tip of the syringe.
3. Fill the syringe with distilled water.
4. Using the plunger, push all the distilled water through the filter.
5. Remove the filter from the syringe.
6. Remove the plunger from the syringe.
7. Attach the filter to the syringe.
8. Obtain a container (beaker, centrifuge tube, etc.) to store your filtrate.
9. Holding the filter syringe above the container, add the solution to the syringe.
10. Using the plunger, push the solution into through the filter.
11. Discard the syringe and filter in the proper waste container.

4.4 Preparing Growth

1. Measure 15 g of LB powder per liter of solution desired.
2. To a beaker or Erlenmeyer flask, add the LB powder, stir bar, and desired volume of dH2O.
3. Stir the mixture until all of the LB powder has dissolved.
4. Transfer the mixture to an autoclave-safe container or cover the lid with the container with aluminum foil.
5. Place a small piece of autoclave tape on the lid of the container.
6. Before placing the mixture in the autoclave, make sure that the lid is not airtight.

7. Autoclave the mixture on the liquid setting for 20 minutes.
8. Store the solution at room temperature.

LB agar is a solid growth medium for bacteria that can be melted and poured onto plates. The following is a procedure for how to prepare LB agar.

1. Measure 15 g of LB powder and 15 g of agar per liter.
2. Dissolve LB powder in dH2O.
3. Transfer LB solution to autoclave-safe container.
4. Add agar to solution.
5. Stir the mixture by swirling the autoclave bottles.
6. Autoclave the mixture on the liquid setting for 20 minutes.
7. Store the mixture in the refrigerator.

4.5 Pouring Agar Plates

1. Melt agar in a microwave.
2. Clean the lab bench with 70% ethanol.
3. Using sterile technique, open the lid of the agar container.
4. Transfer 30 ml of LB agar to a centrifuge tube.
5. Add 25 mg/ml chloramphenicol and 50 mg/ml kanamycin.
6. Cap the centrifuge tube and gently mix the solution by inverting the tube a couple times.
7. Label petri dish.
8. Pour the mixture into a petri dish.
9. Partially cover the petri dish with lid to allow some moisture to escape.
10. Repeat 5-9 until enough agar plates have been prepared.

4.6 Streaking Agar Plates With E. Coli

1. Select 5 isolated colonies from a plate. Circle the colonies and label them 1-5.
2. Sterilize an inoculating loop with a flame by holding the loop in the flame until red hot.
3. Allow the inoculating loop to cool. To make sure that loop is cool enough, gently touch the surface of the agar plate near the edge. If the agar sizzles, the loop is not cool enough.
4. Once the loop has cooled, gently touch the loop to the first colony being careful not to touch any other colonies with the loop.
5. Streak back and forth in the first quadrant of the agar plate. See arrow 1 in Figure 3b. Streak gently to not gauge the agar.
6. Flame the loop.

7. Streak the plate by dragging the loop from the first quadrant to the second quadrant of the plate four times. See arrow 2 in Figure 3b.

8. Flame the loop again.

9. Streak the plate by dragging the loop from the second quadrant to the third quadrant of the plate four times. See arrow 3 in Figure 3b.

10. Flame the loop again.

11. Streak the plate by dragging the loop from the third quadrant to the fourth quadrant of the plate four times. See arrow 4 in Figure 3b.

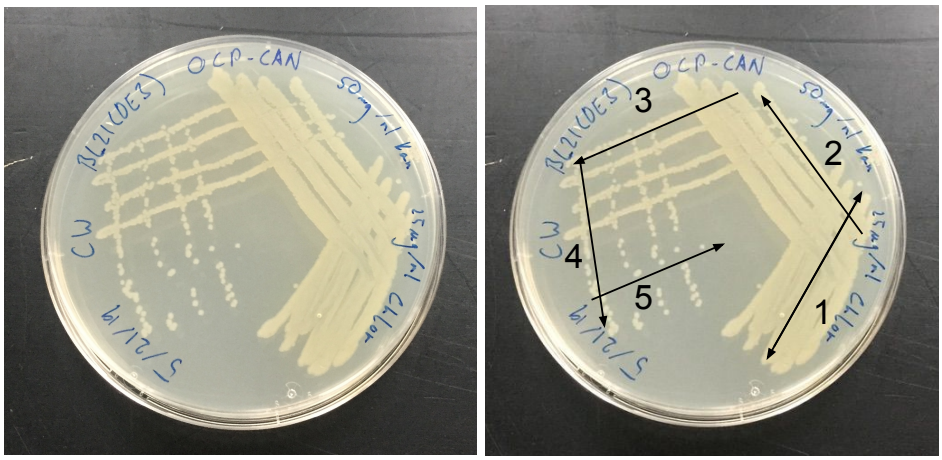
12. Flame the loop again.

13. Streak the place by dragging the loop from the fourth quadrant to the middle of the plate once. See arrow 5 in Figure 3b.

14. Cover the plate. Wrap parafilm around the circumference of the plate to hold the lid.

15. Store the plate with the lid side down in an incubator.

Refer to the following video for additional assistance:
https://www.youtube.com/watch?v=0heifCiMbfY



(a) (b)

Figure 3: A streaked plate after growing overnight

4.7 Preparing Glycerol Stocks

Glycerol stocks are used for long-term storage of plasmids. This allows you to easily obtain the desired bacterial strain with the desired plasmid without having to transform more cells.

1. Prepare 5 1 ml cultures of desired bacteria.

2. Allow cultures to grow overnight.

3. Pipette 15% desired volume of glycerol into tubes.

4. Pipette 85% desired volume of bacterial culture into tube.

5. Tighten the caps of the tubes.
6. Shake/vortex tubes.
7. Pour liquid nitrogen into a container.
8. Use tweezers to submerge the first tube until frozen.
9. Store the tube in $-80°C$.
10. Repeat steps 7 and 8 with the remaining tubes.

https://www.addgene.org/recipient-instructions/myplasmid/

4.8 Inoculating Liquid Cultures With an Autoclaved Toothpick

1. Prepare a culture tube with 2 ml of LB with antibiotics.
2. Uncap the toothpick vial with your little finger.
3. Tilt the vial so that it is sideways.
4. Gently shake the toothpick vial so that some of the toothpicks are sticking out of the open end.
5. Carefully remove a single toothpick with your pointer and index finger.
6. Holding onto the toothpick, recap the vial.
7. Gently touch the tip of the toothpick to the first colony. Make sure that the tip does not touch any other colonies.
8. Uncap the first culture tube.
9. Drop the toothpick into the first culture tube.
10. Recap the tube.
11. Repeat steps 1-10 with the remaining colonies.

The following video provides a good explanation of this process:
https://www.youtube.com/watch?v=5XbLrXtWNOk

4.9 Inoculating Liquid With a Loop

1. Sterilize the loop with an open flame. Make sure to sterilize the neck of the loop with the flame as well.
2. Touch the loop to the first colony. Make sure that the loop does not touch any other colonies.
3. Uncap the first culture tube.
4. Touch the loop to the liquid in the culture tube. Try not to touch the sides of the tube with the loop. If possible, touch the loop to a drop on the side of the tube rather than the liquid at the bottom of the tube.
5. Remove the loop from the tube and recap the tube.
6. Holding the cap in place, shake the tube.
7. Repeat steps 1-6 with the remaining colonies.

Note: When inoculating cultures from glycerol stocks, the glycerol stock should be kept in a cold block when they are not in the freezer.

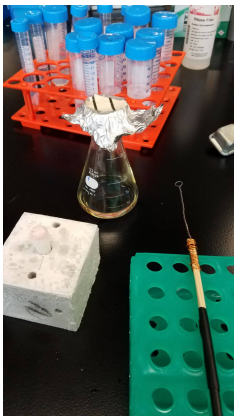


Figure 4: Glycerol stock in cold block about to be used to inoculate a sterile flask

4.10 Preparing 2 ml Liquid Cultures

1. Select 5 individual colonies from bacteria plate. Circle the colonies and label them 1-5.
2. Label 5 sterile culture tubes with the colony number, date, and initials.
3. Using a 10 ml serological pipette, transfer 12 ml of LB into a sterile centrifuge tube.
4. Using a p20 micropipette, transfer 12 μl of 25 mg/ml 25 mg/ml chloramphenicol and 12 μl of 50 mg/ml kanamycin.
5. Shake the test tube to mix the contents.
6. Using a serological pipette, transfer 2 ml of the mixture into each of the 5 culture tubes.
7. Inoculate each culture tube with their respective colonies using an autoclaved toothpick or a sterile loop.
8. Place the inoculated culture tubes in a shaking incubator.
9. Turn on the incubator. Set the temperature to 37°C and the RPM to 220. Make sure that the hr setting is off.

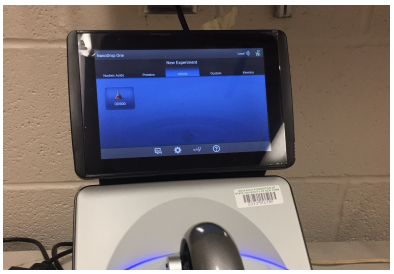
4.11 Inducing 1 ml Culture With IPTG

This process is used as a positive control to ensure that the desired protein is actually being produced by the bacteria. Therefore, an excess of IPTG is used to guarantee expression. The protein from this process is not to be analyzed by gel electrophoresis.

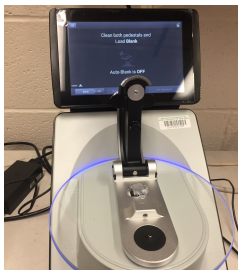
1. Transfer 1 ml of bacteria from the culture tube to a sterile culture tube.
2. Add IPTG to a concentration of 1 mM.
3. Repeat with remaining cultures.
4. Incubate cultures in a shaking incubator.

4.12 Measuring OD_{600} with Nanodrop

1. Turn on the nanodrop. The switch is in the back.
2. Set nanodrop to OD600
3. Lift the arm of the nanodrop. Figure 5a.
4. Insert a cuvette containing sterile LB media, and lower the arm.
5. Click the "blank" button.
6. Remove the blank cuvette and insert the cuvette to be measured.
7. Click the "measure" button.
8. Record the OD_{600}.



(a)



(b)

Figure 5

4.13 Growing Expression Cultures

1. Inoculate 25 ml of sterile culture medium containing both 25 μg/ml chloramphenicol and 50 μg/ml kanamycin in a 125 ml Erlenmeyer flask.
2. After growing the cultures overnight, inoculate a 2 L Erlenmeyer flask containing 400 ml of sterile media with antibiotics with 20 ml of the overnight culture. Inoculate a 250 ml Erlenmeyer flask containing 50 ml of sterile media and antibiotics with 2.5 ml of the overnight culture. This is your negative control.
3. Grow cultures at 37°C with vigorous shaking.
4. Measure and record the OD_{600} of the 2L cultures every 15-20 mins.
5. Once the OD_{600} is 0.6, induce the culture in the 2 L flask by adding IPTG to a final concentration of 0.05 mM. Do not induce the negative control!
6. Return the 2 L flasks to the shaking incubator.
7. Measure and record the OD_{600} of both the induced culture and the negative control every hour.
8. After 4-5 hours, transfer a 1 ml sample from each Erlenmeyer flask to a labeled 1.5 ml microcentrifuge tube.
9. Pellet the cells by centrifugation at 4000g for 20 mins.

10. Discard the supernatant into a waste container and store the cells overnight at $-20°C$.
11. Allow the rest of the flasks to grow overnight.

4.14 Harvesting Cells from Overnight Culture

1. Sterilize lab bench with 70% ethanol.
2. Remove overnight culture from the incubator.
3. Remove the lid of the culture using sterile technique.
4. Transfer all of the culture from 1 flask into 1 empty 500 ml centrifuge bottle. Repeat with the rest of the cultures.
5. Make sure that the bottles are balanced.
6. Place the A-4-81 roter in the centrifuge.
7. Centrifuge at 4000 g for 20 minutes.
8. Once the centrifuge is done, make sure that the cells are pelleted at the bottom of the bucket.
9. Carefully pour the supernatant into a waste beaker.
10. Add bleach to the waste beaker to a final concentration of 10%.
11. Label a 50 ml centrifuge tube with the name of the bacteria, the plasmid, the date, and your initials.
12. Add 5-10 ml of 1X lysis buffer to each bucket.
13. Using a spatula, scrape all of the cells off of the bottom of the buckets.
14. Pour the cells out of the buckets and into the 50 ml centrifuge tube.
15. Fill another 50 ml tube with water until it is the same weight as the first tube. This will act as a balance in the centrifuge.
16. Replace the A-4-81 rotor with the A-4-62 centrifuge rotor.
17. Centrifuge the 50 ml test tubes at 4000 g for 40 mins.
18. Discard the supernatant.
19. Store pelleted cells in freezer.
20. Sterilize culture flask with bleach.

Note: When pelleting multiple cultures, try to centrifuge as many cultures as possible per cycle to save time. Label each test tube with the culture number to keep track of which culture goes in which tube.

Percent	Stacking Gel 4%	Resolving Gel X%
30% Acrylamide	1.98 ml	$0.5 \times X$ ml
0.5M Tris-HCl, pH 6.8	3.78 ml	-
1.5M Tris-HCl, pH 8.8	-	3.75 ml
10% SDS	150 μl	150 μl
diH_2O	9 ml	$11.03 - (0.5 \times X)$ ml
TEMED	15 μl	7.5 μl
10% APS	75 μl	75 μl
Total Volume	15 ml	15 ml

Table 1: Polyacrylamide Gel Recipe

4.15 Handcasting Polyacrylamide Gels

1. Calculate the amount of 30% acrylamide and diH_2O needed for desired resolving gel percentage using table 1.
2. Prepare resolving and stacking gels without APS or TEMED.
3. Prepare 10% APS solution. Use 10 μl for every 1 mg of APS.
    - Note: APS slowly degrades in solution and therefore should be made fresh. Do not use old solutions of APS.
4. Assemble glass cassette sandwich, and place it in the clamp with a foam strip underneath to prevent the cassette from leaking.
5. Place the comb into the assembled sandwich. With a marker, draw a mark on the glass 1 cm below the teeth of the comb.
6. Measure 5 ml of resolving gel into a centrifuge tube.
7. Add 2.5 μl of TEMED and 25 μl of 10% APS to the tube.
8. Cap the tube and gently mix the solution by inverting the test tube.
9. Fill the glass cassette to the mark made in step 5 with resolving gel using a pippette.
10. Add about 100 μl of isobutanol to the glass cassette. Make sure that the top layer of the resolving gel is completely covered with the isobutanol.
11. Allow the gel to polymerize for 45-60 minutes.
12. Remove all of the isobutanol by inserting a piece of filter paper or a Kimwipe.
13. Place the comb into the glass cassette. Raise one end of the comb until there is enough room between the comb and the glass for a pipette tip to fit between the comb and the glass.
14. Measure 5 ml of stacking gel into a centrifuge tube.
15. Add 5 μl of TEMED and 25 μl of 10% APS to the tube.
16. Cap and gently mix the solution by inverting the test tube.
17. Slowly fill the remainder of the glass cassette with the stacking gel with a pipette.

18. Press the comb into the glass cassette. Make sure that there are no bubbles in the gel.
19. Allow the gel to polymerize for 30-45 minutes.
20. Remove the comb by pulling it up slowly and gently.

This protocol is revised from the following Bio-Rad guide:
http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6201.pdf

This video provides an example of how to cast a gel:
https://www.youtube.com/watch?v=EDi_n_0NiF4
Note that the video does not fill the stacking gel as described in the procedure.

4.16 Running Polyacrylamide Gels

1. Remove the gel from the clamp.
2. Rinse the wells with deionized water.
3. Shake the water out of the wells.
4. Place the glass cassette into the electrophoresis chamber with the short piece of glass facing inwards.
5. If only one gel is being ran, place a buffer dam on the other side of the electrophoresis chamber. If two gels are being ran, place the second gel on the other side of electrophoresis chamber with the short piece of glass facing inwards.
6. Using the green clamps, lock the glass cassttes/dam into the chamber.
7. Prepare 750 ml of 1X SDS-PAGE Buffer.
8. Place the inner chamber into the outer chamber making sure to align the red side with the red side and the black side with the black side.
9. Fill the inner chamber with 1X SDS-PAGE buffer, so that the buffer is above the top of the short plate, but below the top of the tall plate. Make sure that the inner chamber is not leaking. If it is, dump the buffer into the outer chamber then reassemble and refill the inner chamber.
10. Pour the remainder of the buffer into the outer chamber.
11. Load each well with 10 μl of sample.
12. Place the lid on the electrophoresis chamber making sure to align the red side with the red side and the black side with the black side.
13. Place the leads into the power supply matching red to red and black to black.
14. Set the power supply to a constant voltage of 200 V.
15. Turn on the power supply. You should see bubbles in the inner chamber at this point.
16. Let the gel run until the tracking dye reaches the bottom of the glass cassette. This should take about 45-60 minutes.

https://www.youtube.com/watch?v=SlbMhI2Fw_s

4.17 Staining/Destaining Gels

1. Remove the inner chamber and pour the buffer solution into the outer chamber.
2. Remove the glass cassette from the inner chamber.
3. Using a plastic scraper, carefully separate the planes of glass and remove the gel from the glass.
4. Use the scraper to chop off the stacking gel.
5. Use the scraper to chop off the top left corner of the resolving gel.
6. Place the gel in a container that is large enough to allow the gel to lie flat.
7. Pour enough stain solution to cover the gel.
8. Allow the gel to stain overnight on a rocker.
9. Save the stain solution.
10. Rinse the gel and container with deionized water.
11. Place the gel and a rolled up piece of paper towel in the container.
12. Pour enough destain solution into the container to cover the gel.
13. Wait until the lines on the gel are visible, then take a picture of the gel.

https://www.sciencedirect.com/science/article/pii/B9780124201194000136#f0005

4.18 Testing a Glycerol Stock for Protein Over-Expression

1. Have at least 10 ml of sterilized LB media available.
2. Transfer 10 ml of LB media to a 15 ml centrifuge tube.
3. Add 10 μl of antibiotics to the centrifuge tube.
4. Mix the contents of the tube by inverting the tube.
5. To each of 5 culture tubes, add 2 ml of media.
6. Inoculate the 2 ml cultures at the end of the day (4:00 p.m. - 5:00 p.m.) with a glycerol stock.
7. Allow the cultures to grow overnight in a shaking incubator at 200-250 rpm and 37°C.
8. The next morning (10:00 a.m.) transfer 1 ml from each culture tube into 5 new culture tubes.
9. Induce the new culture tubes with 1 mM IPTG.
10. Allow the old and new cultures to grow at 200-250 rpm and 37°C for 4-5 hours.
11. Transfer the 1 ml cultures into 10 different 1 ml centrifuge tubes.
12. Centrifuge the cells at 10,000 g for 10 mins.
13. Pour the supernatant into a waste container (a beaker or 50 ml centrifuge tube) to be sterilized later.
14. Freeze the cells at -20°.

	Final Concentration	Component	Amount per 10 ml
Part 1	0.225M Tris-Cl (pH 6.8)	1 M Tris-Cl, pH 6.8	2.25 ml
	50% glycerol	99% Glycerol	5ml
	5% SDS	SDS	0.5 g
	0.05% bromophenol blue	Bromophenol blue	5mg
		diH_2O	2.25 ml (or to 9.5 ml)
Part 2	0.25 M dithiothreitol (DTT)store at -20°C	5M DTT	0.5 ml

Table 2: 5X Sample Buffer

Handcasting Polyacrylamide Gels

1. See 4.15 for instructions.

Preparing Cells for Running

1. Prepare part 1 of 5X sample buffer without DTT.
2. Transfer 1 ml of the 5X sample buffer without DTT to a 1 ml centrifuge tube.
3. Prepare part 2 of 5X sample buffer.
4. Combine 450 μl of part 1 with 50 μl of 5M DTT.
5. Add 100 μl of 1X sample buffer to pelleted cells.
6. Resuspend cells by vortexing.
7. Heat the cells for 5 mins at 90°C.
8. Freeze the cells at -80°C.
9. Repeat the previous 2 steps 3 times.
10. Centrifuge the cells at 10,000 g for 10 mins.

Running Polyacrylamide Gels

1. See 4.16 for instructions.

Staining/Destaining Gels

1. See 4.17 for instructions.

5 Resources

- Addgene provides additional useful techniques and protocols. https://www.addgene.org/
- The QIAExpressionist provides information about expression and purification of proteins. http://kirschner.med.harvard.edu/files/protocols/QIAGEN_QIAexpressionist_EN.pdf
- This Bio-Rad pdf is a guide on electrophoresis. http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6040.pdf