BIOL-2125 Introduction to Cell & Molecular Biology Lab

Laboratory Notebook

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Section: 3

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Bright Field Microscopy and Prokaryotes

Date: 9/10/2025

Pre-Lab Questions:

1. Gram staining differentiates between two broad types of bacteria. What is the fundamental difference between these types of bacteria that make it possible for Gram staining to differentiate them?

Gram staining differentiates between Gram-positive and Gram-negative bacteria. The fundamental difference between these bacteria is within their cell walls. The peptidoglycan thickness is significantly greater in Gram-positive bacteria. In Gram-positive bacteria it is 50 to 90% of the cell envelope but it is only 10% of the cell envelope in Gram-negative bacteria. Since the Gram-positive bacteria have thicker peptidoglycan, the crystal violet stain is better seen in Gram-positive bacteria, making them look violet. Additionally, safranin makes all bacteria pink but the dark violet of the Gram-positive will still be seen even with the pink stain while the Gram-negative bacteria will only appear pink.

2. Lenses (the objective lenses, ocular lenses, and condenser lens), should ONLY be cleaned with what kind of paper? What will happen if the lenses are cleaned with something else, like a Kimwipe to paper towel?

The objective, ocular, and condenser lenses should only be cleaned with lens paper. If the lenses are cleaned with something else, the glass could become scratched since the lenses are very fragile.

3. What is a stage micrometer; what is its purpose? What is a scale bar; what is its purpose?

A stage micrometer is a small ruler put on a slide that one can see while using the microscope. Stage micrometers allow one to see the size and length of objects. Additionally, they aid in the creation of image scale bars. A scale bar is a part of a micrograph that is labeled with its length. Scale bars tell one the size of the object being examined under the microscope.

4. List out all bacterial cultures for Exercise 1 and indicate how you expect them to appear after Gram staining. Look for this information in your BIOL-2120 textbook or online. You can use this information to make hypotheses.

The bacterial cultures used in Exercise 1 are Escherichia coli, Bacillus megaterium, Bacillus cereus, Pseudomonas fluorescens, Bacillus subtilis, Serratia marcesens, Enterococcus faecalis, Staphylococcus saprophyticus, and Staphylococcus epidermidis. After Gram staining, I expect Escherichia coli, Pseudomonas fluorescens, and Serratia marcesens to appear pink. These are all Gram negative and should therefore appear pink. Additionally, I expect Bacillus megaterium, Bacillus cereus, Bacillus subtilis, Enterococcus faecalis, Staphylococcus saprophyticus, and Staphylococcus epidermidis to appear violet. These bacteria are all Gram positive and should therefore appear violet.

Objectives:

- To know the functions and names of each part of the microscope
- Use proper techniques to use, clean, and store the microscope
- Understand the terms resolving power, field of view, depth-of-field (focus), parfocal, and working distance
- Solve for the total magnification of a lens
- Perform and understand the science of Gram staining
- Create scale bars and different magnifications and label figures

Hypothesis:

After Gram staining, I expect Escherichia coli, Pseudomonas fluorescens, and Serratia marcesens to appear pink. These are all Gram negative and should therefore appear pink. Additionally, I expect Bacillus megaterium, Bacillus cereus, Bacillus subtilis, Enterococcus faecalis, Staphylococcus saprophyticus, and Staphylococcus epidermidis to appear violet. These bacteria are all Gram positive and should therefore appear violet.

Methods:

Pages 11-17 of Lab Manual

Exercise 1:

Materials:

- Glass slides
- Staining tray
- Wax pencil
- Transfer pipettes
- Disposable inoculating loops
- Biohazard bag
- Methanol
- Crystal Violet
- Gram's Iodine
- 95% ethanol
- Safranin
- dH2O squirt bottle

Control Cultures:

- *Escherichia coli (EC)*
- *Bacillus megaterium (BM)*
- Pre-mixed culture of Gram-positive and Gram-negative bacteria

Experimental Cultures:

- *Bacillus cereus (BC)*
- *Bacillus subtilis (BS)*
- *Enterococcus faecalis (EF)*
- *Staphylococcus epidermidis (SE)*
- *Pseudomonas fluorescens (PF)*
- *Serratia marcesens (SM)*
- *Staphylococcus saprophyticus (SS)*

Procedure:

1. Label two glass slides with your initials on the left side and slide #1 or #2, using a wax pencil. Put two dime-sized circles in the middle of the slides.
2. Put the slides in the staining tray.
3. Use an inoculation loop to put a full loop of *E.coli* into the left circle on slide #1. Spread the bacteria within the circle. Once you and your partner have completed this, put the loop into the biohazard bag.
4. Use a new loop, repeat step 3 with *B. megaterium* in the right circle.

5. Repeat steps 2 and 3 on slide #2. The left circle should have an experimental culture (the lab had run out of this culture and it was therefore not used) and the right circle should have the pre-mixed culture. Make sure your experimental culture is different from your lab partner's.
6. Let the slides air dry, fanning with a piece of paper (we also dried our slides in the lab dryer). This step is complete when the smears are hazy and not shiny.
7. Put one drop of methanol in each smear on both slides. Wait until the slides are dry again. Begin exercise #2 while waiting.
8. Put 2 to 3 drops of crystal violet on the smears and wait for 1 minute.
9. Decant the crystal violet into the staining tray. Put the slides at an angle and rinse the dH_2O until the water becomes colorless as it flows off. Carefully shake the slides so the water falls off but don't wait for them to dry.
10. Put 2 to 3 drops of Gram's iodine on the slides and wait 1 minute.
11. Repeat step 8.
12. Use 95% ethanol to rinse each slide for 5 minutes. Immediately rinse with water and do not dry the slides.
13. Put 2 to 3 drops of safranin on the slides and wait 1 minute.
14. Repeat step 8.
15. Wait for the slides to dry.

Exercise 2:

Materials:

- Bright-field microscope
- Lens paper
- Len cleaner (if needed)
- Fixed specimen slide (pig embryos)
- Immersion oil

Procedure:

1. Plug the microscope in, making sure the cord is not tangled.
2. Rotate the knob under the stage until the condenser lens is a few millimeters below the microscope stage.
3. Move the stage to the lowest position with the coarse adjustment knob.
4. Put the stage slide holder's lever arm back to put the pig embryo slide in the holder. The lever arm should snug the slide in the top right corner of the holder.
5. Use the nose piece to put the 4X objective lens over the stage micrometer.
6. Put the light source on at medium intensity.
7. Open the iris/diaphragm so that it allows the maximum amount of light to come through.
8. Using both eyes, look through the ocular lens and make the distance between the ocular lenses so that there is only one circle of light.
9. Use the X-Y movement control knobs on the mechanical stage to put the slide in the middle of the light, under the objective lens.
10. Use the coarse adjustment knob until the microscope is in focus. Adjust light intensity if needed.
11. Use the nosepiece to get to the 10X objective. Use coarse/fine adjustment knobs to come into focus.
12. Answer questions 1 and 2 in the lab questions section.
13. Use the nosepiece to put the 40X objective in place. Use only the fine adjustment knob to focus.

14. Put the nosepiece on the 4X objective and put one tiny drop of immersion oil onto the slide in the spot where the light is showing through.
15. Put the 100X objective in place. It should be touching the immersion oil.
16. Answer questions 3 and 4.
17. Use only the fine adjustment knob to focus. Turn light intensity to highest setting.
18. Answer question 5.
19. Use lens paper to wipe the oil off.
20. Put the pig embryo slide back in the drawer.

Exercise 3:

Materials:

- Lens paper
- Len cleaner (if needed)
- Immersion oil
- Stage micrometer slide
- Bacterial smears from Exercise 1
- AmScope ocular camera
- Laptop with AmScope software
- Kimwipes (not for cleaning lenses!)

Procedure:

1. Use the coarse adjustment knob to put the microscope stage at its lowest.
2. Put the stage slide holder's lever arm back in order to put the stage micrometer slide in the holder. The lever arm should snug the slide in the top right corner of the holder.
3. Use the nose piece to put the 4X objective lens on.
4. Set up the camera and calibrate the software for the 100X objective. Put a scale bar on the image of the micrometer slide. Add it to the lab notebook.
5. Use a kimiwipe to take the oil off of the stage micrometer slide. Put the slide back in the drawer.
6. Use the nose piece to put the 4X objective on and put slide #1 in. Put one wax circle directly under the objective with the X-Y knobs. Focus the microscope with the coarse adjustment knob.
7. Put immersion oil on one circle and put the 100X objective on. Use the fine adjustment knob to focus.
8. Use the camera to capture an image. Name the file with the bacterial culture and the objective power used.
9. Capture images for all other smears on the two slides.
10. Wipe the oil off the lens with lens paper.
11. Put the smears in the glass waste container.

Results/Assignments:

Assignment 1:

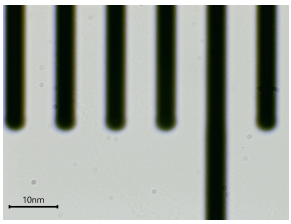


Figure 1: Stage Micrometer, 100X Objective

Assignment 2:

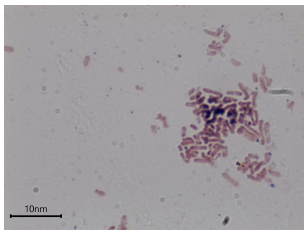


Figure 2: *Escherichia coli*, 100X Objective

Estimated Cell Size: 2 nm Notable Features: Pink, Bacilli, Orient in Clusters, Appears Gram-Negative

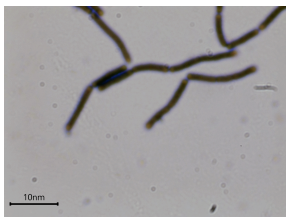


Figure 3: *Bacillus megaterium*, 100X Objective

Estimated Cell Size: 30 nm Notable Features: Purple, Bacilli, Orient in Chains, Appears Gram-Positive

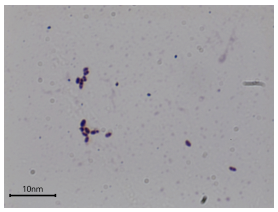


Figure 4: *Enterococcus faecalis*, 100X Objective

Estimated Cell Size: 1 nm Notable Features: Purple, Coccobacilli, Orient Solitarily, Appears Gram-Positive

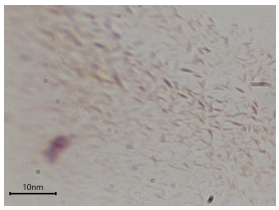


Figure 5: *Bacillus subtilis*, 100X Objective

Estimated Cell Size: 3 nm Notable Features: Pink, Bacilli, Orient in Clusters, Appears Gram-Negative

Lab Questions:

1. What is different about the field-of-view compared to the 4X objective?
   Field of view decreases as you go from the 4X objective to the 10X objective. This is because you are seeing a smaller area in more detail when using a higher objective.

2. How does this relate to the working distance of the objective lens?
   Working distance decreases as magnification increases and field of view decreases. The 10X objective is closer to the slide compared to the 4X objective.

3. What is the purpose of immersion oil?
   Immersion oil allows the slides to look clearer and have a greater resolution under the microscope. This happens because the oil causes more light to shine through since it limits refraction.

4. What is the meaning of the term parfocal and why is the parfocal arrangement of the objectives particularly helpful for the 100X objective?
   Parfocal means that magnification changes will not impact the focus of the microscope. This is helpful because it enables one to rotate objectives without needing to refocus every time. Additionally, this is

important for the 100X objective because it allows one to put the immersion oil on at the lower objective and not have to refocus when putting the microscope back on the 100X objective.

5. What is different about the depth-of-field compared to lower power objectives?
   Higher power objectives lead to a lower depth of field compared to the lower power objectives.

6. The *Escherichia coli* and *Bacillus megaterium* cultures are the controls for your technique; why are these good controls?
   Escherichia coli and *Bacillus megaterium* cultures are good controls because we know *Escherichia coli* is Gram-negative and *Bacillus megaterium* is Gram-positive. This makes it easier for us to compare the colors of the other samples to *Escherichia coli* and *Bacillus megaterium*, therefore enabling us to determine if the other samples are Gram-negative or Gram-positive.

7. Compare your Gram staining results to the expected results for each bacterium (this was the information you needed for Question 4 of the Pre-lab Questions). Did you get the results you expected?
   I received mostly the results I expected. The *Escherichia coli* was expected to be pink and the *Bacillus megaterium* and *Enterococcus faecalis* were expected to be purple. However, the *Bacillus subtilis* was expected to be purple but the image captured was mostly pink.

Conclusion/Discussion:

The objectives of the lab were achieved. Through use of the microscope, I have learned the terms and functions of the microscope. Additionally, I now know how to use and clean the microscope and how to put it away. Furthermore, I learned many terms associated with microscopes, which are described in the lab questions above. Knowledge about microscope magnification has also been obtained. I know the procedure of Gram staining as well as how to analyze the results of a Gram stain. Lastly, I have gained the ability to add scale bars to photos captured with the microscope.

As described in question 7 above, the majority of my predictions for the lab were correct. This means that the Gram staining was performed correctly to find the bacteria that are Gram-positive and Gram-negative. However, the color of *Bacillus subtilis* was inconsistent with my prediction. The image was mostly pink but *Bacillus subtilis* is Gram-positive and should appear purple. This could mean that the Gram staining was done incorrectly on this sample and in the future I would reattempt this lab with this sample, making sure that the step where the crystal violet is applied is done correctly.

Pipetting and Standard Curves

Date: 9/17/2025

Pre-Lab Questions:

1. What is the purpose of using a standard curve in determining protein concentration?

A standard curve is used to determine protein concentration from values that are arbitrary. One specific use is taking an absorbance value and finding that point on the curve, then looking at the other axis of the graph to find the protein concentration.

2. What is a serial dilution and how does it differ from other dilution schemes?

Serial dilution is when you use a stock solution of one concentration and dilute it, then you take the diluted solution and dilute it again, continuing the cycle. The same amount of water should be used in each dilution step. Serial dilution differs from other dilution schemes because you are continuously diluting the next sample with the same amount of water, typically when you dilute you use the original sample and do not participate in as many dilution steps compared to serial dilution.

3. Perform the serial dilution calculations required for Exercise 2 Part 1 using the table format shown in Table 2. Paste your serial dilution table below and in your notebook.

BSA Concentration	Volume BSA	dH_2O	Total Volume	Remaining Volume
1 mg/ml	10 µl	+10 µl	= 20 µl	10 µl
.5 mg/ml	10 µl	+10 µl	= 20 µl	10 µl
.25 mg/ml	10 µl	+10 µl	= 20 µl	10 µl
.125 mg/ml	10 µl	+10 µl	= 20 µl	20 µl

4. When using a micropipette, which “stop” do you go to when you want to uptake liquids?

When using a micropipette, you go to the first stop when you want to uptake liquids.

Objectives:

- Utilize a variety of micropipettes that are commonly used in cell and molecular biology
- Perform quantitative serial dilutions
- Create a standard curve and find values of unknown samples using the curve
- Use and understand the function of microplate readers as well as general spectrophotometry
- Know how protein samples are quantified and the limitations of the techniques used.

Hypothesis:

I believe the lower concentration proteins (.125 and .25 mg/ml) will appear brown and the higher concentration protein solutions will appear blue (.5 and 1 mg/ml). The standard curve line will appear roughly linear and R^2 will be around 1.

Methods:

Exercise 1:

Materials:

- P-10 micropipette
- P-20 micropipette
- P-100 or P-200 micropipette
- P-1000 micropipette
- Tips for each micropipette
- Weigh boat
- Balance (scale)
- dH2O (distilled water)
- Ethanol

Procedure:

1. Turn the balance on.
2. Place the weigh boat onto the balance and “zero”.
3. Choose the appropriate micropipette for the volume and set it to the designated volume. Never try to pipette a larger or smaller volume than printed on the micropipette!
4. Draw up water and transfer to the weigh boat, wait for the reading to stabilize.
5. Document the weight of the water in a table like the one shown.
6. Use the same weigh boat and re-zero (“tare”) the balance between readings. You do not have to dump the water in between measurements.
7. For the last sample, use 1 ml of ethanol.

Table 1: Pipetting Schematic

Pipet	Pipette Range	Volume	Sample	Mass(g)
P-10	.5 to 10 µl	5 µl	dH_2O	.003 g
	.5 to 10 µl	10 µl		.010 g
P-20	2 to 20 µl	10 µl		.010 g
	2 to 20 µl	20 µl		.019 g
P-100 or P-200	10 to 100 µl	20 µl		.020 g
	10 to 100 µl	100 µl		.089 g
P-1000	100 to 1000 µl	100 µl		.100 g
	100 to 1000 µl	500 µl		.498 g
	100 to 1000 µl	750 µl		.755 g
	100 to 1000 µl	1000 µl		.876 g

		1000 µl	Ethanol	.631 g

Exercise 2:

Part 1: Serial Dilution of BSA for a Standard Curve

Materials:

- Microfuge tubes
- Distilled water
- Microfuge tube with 2 mg/ml BSA
- Microfuge tube with BSA of unknown concentration

Procedure:

1. Write out a serial dilution scheme to dilute the 2 mg/ml stock BSA with water to 1, 0.5, 0.25, and 0.125 mg/ml. To run the Bradford assay, you will need at least 5 µl remaining after making the full serial dilution. To this end, calculate your dilution schedule so you have 10 µl remaining volume; this will give you a little extra to work with. The last dilution will have more remaining volume than this since you will not be taking anything from it to make subsequent dilutions – this is okay.
   a. Use the formula: C1V1 = C2V2
   b. Make sure to include units in this formula.
   c. First determine how much of the 2 mg/ml sample you need to make the 1 mg/ml standard. Then, determine how much of the 1 mg/ml sample you need to make the 0.5 mg/ml standard, and so on.

Table 2: Serial Dilution Schematic for BSA Standards (2 mg/ml in Stock)

BSA Concentration	Volume BSA	dH_2O	Total Volume	Remaining Volume
1 mg/ml	10 µl	+10 µl	= 20 µl	10 µl
.5 mg/ml	10 µl	+10 µl	= 20 µl	10 µl
.25 mg/ml	10 µl	+10 µl	= 20 µl	10 µl
.125 mg/ml	10 µl	+10 µl	= 20 µl	20 µl

2. Pipette the water first and then add the protein to the tube and mix by gently pipetting up and down. Do not introduce air bubbles. When mixing, stay within the mid-range when pressing the pipette plunger up and down. Also, do not press the plunger all the way to the second stop.
3. Briefly centrifuge the samples.
4. Set samples aside until you have finished Part 2 and are ready to start the Bradford Assay.

Part 2: Dilution of an Unknown

Procedure:

1. Write down which unknown you have.
   B
2. Write out a dilution scheme to directly dilute the unknown protein samples 1:2, 1:5, and 1:10 each in 10 µl final volume. Do not dilute these serially. Show your calculations to the instructor before proceeding.

Volume B	Volume dH_2O	Total Volume	Ratio

5 µl	+ 5 µl	= 10 µl	1:2
2 µl	+ 8 µl	= 10 µl	1:5
1 µl	+ 9 µl	= 10 µl	1:10

3. Perform the dilutions.
4. Mix and centrifuge briefly.

Part 3: Bradford Assay

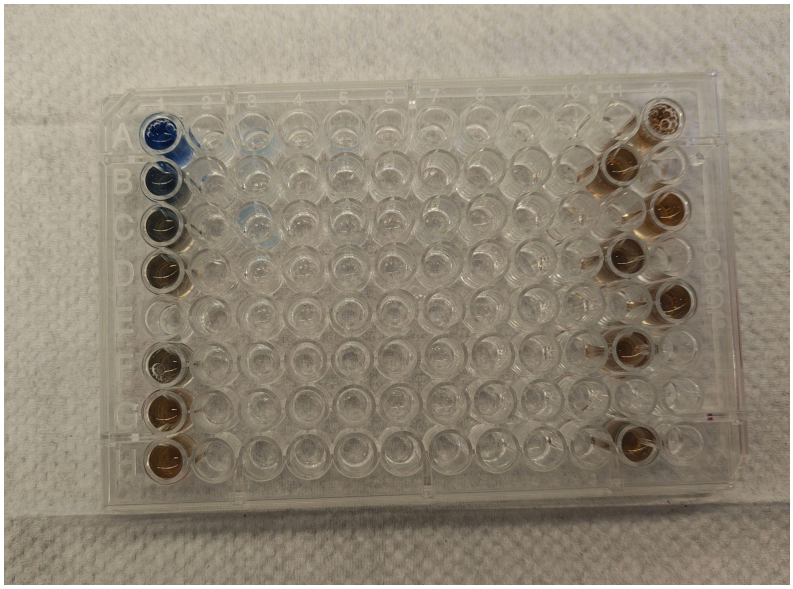
Materials:

- Diluted BSA samples (from Part 1)
- Diluted Unknown samples (from Part 2)
- 1X Bradford reagent
- 96-well microplate
- Microplate reader
- Computer with Excel or graphing program

Procedure:

1. Pipette 5 µl of each protein sample (the 4 standards and 3 unknown dilutions) into separate wells within the microplate. Make sure you record in your notebook where each sample is loaded.
2. Add 250 µl 1X Bradford dye into each of the wells of the microplate where you loaded protein samples. For each well, first pipette in the Bradford reagent, then mix carefully by pipetting up and down; change pipette tips before taking more Bradford reagent since the current tip is contaminated with protein from mixing. Be careful to not introduce bubbles.
3. Incubate at room temperature for 5 minutes. Take a picture for your notebook.
4. Read the absorbance at 595 nm for each sample. Record your results in your notebook.
5. Plot the concentration vs. absorbance in Sheets for your standard curve as shown below. Determine the R2 value. If R2 < 0.95, redo the dilutions and assay.
6. The values determined for the unknown sample will be used in the Assignment section below.

Results:



Column 1: Top to Bottom (My Solutions)

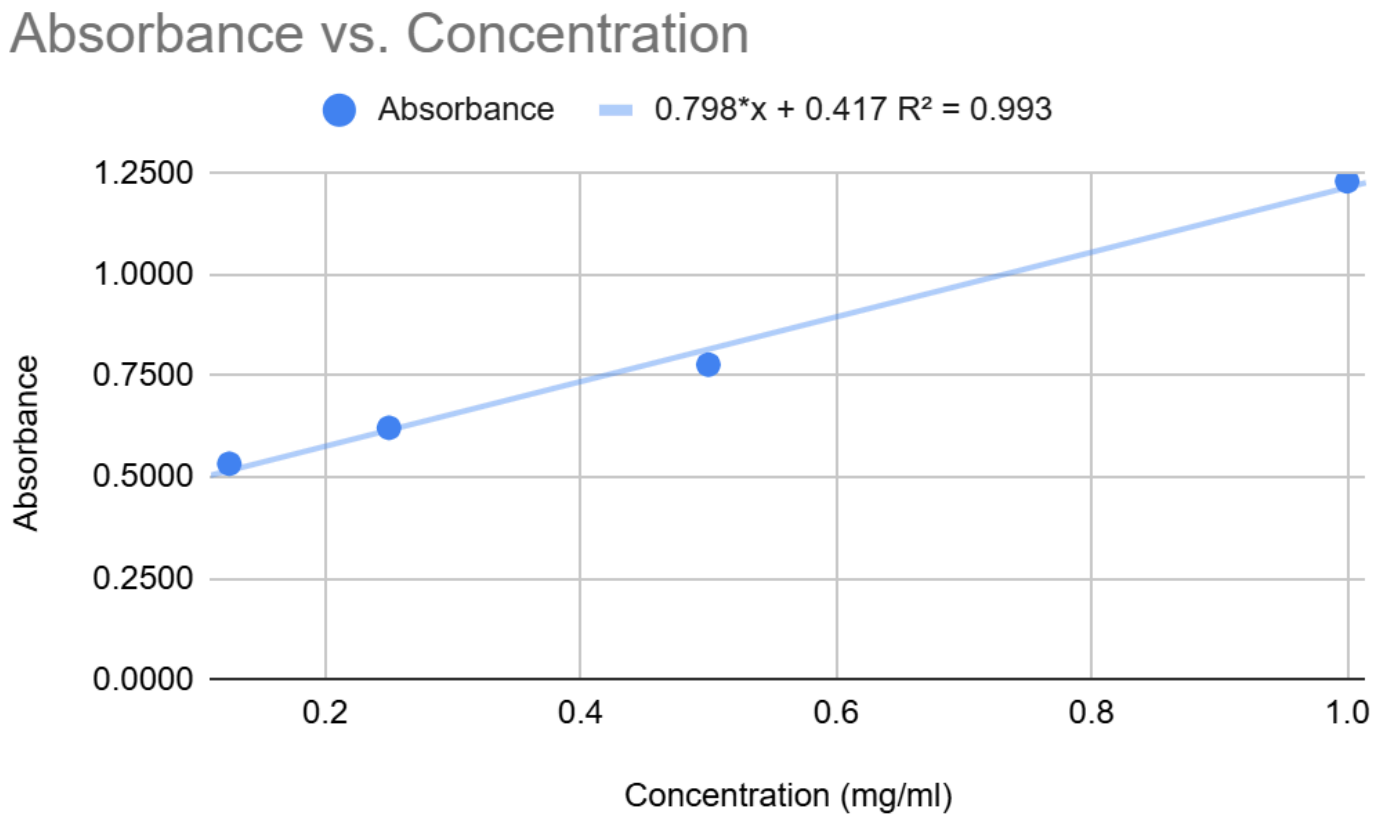
1 mg/ml Stock BSA, .5 mg/ml Stock BSA, .25 mg/ml Stock BSA, .125 mg/ml Stock BSA
.5 mg/ml B, .2 mg/ml B, .1 mg/ml B

Column 11: Top to Bottom (My Partner's Solutions)

1 mg/ml Stock BSA, .5 mg/ml Stock BSA, .25 mg/ml Stock BSA, .125 mg/ml Stock BSA

Column 12: Top to Bottom (My Partner's Solutions)

.5 mg/ml A, .2 mg/ml A, .1 mg/ml A



Concentration Stock BSA	Absorbance		Concentration B	Absorbance
1	1.2301		0.5	0.5000
0.5	0.7777		0.2	0.4695
0.25	0.6220		0.1	0.4637
0.125	0.5334			

Lab Questions:

Assignment 1:

- A. Which pipette should you use to pipette 100 µl?
  You should use the P-1000 pipette to pipette 100 µl.
- B. Which pipette should you use to pipette 195 µl?
  You should use the P-1000 pipette to pipette 195 µl (we didn't have a P-200 pipette)
- C. Do you trust your pipetting to be accurate?

I trust my pipetting to be accurate for the most part. Our P-100 pipette is inaccurate at 100 µl so I would use the P-1000 pipette for that instead.

- D. Why is the ethanol mass different from the mass of the same volume of water?
  Ethanol has a different mass from the mass of the same volume of water because ethanol is more dense than water. Therefore, it has more mass even though it is the same volume.

Assignment 2:

- A. How would you dilute a solution containing antibodies 1:1000 if the total final volume is 10 ml? Describe your pipetting scheme.
  I would use 10 µl of solution and 9990 µl of dH_2O. This would create 10000 µl, otherwise known as 10 ml, of solution.

Assignment 3:

- A. Include a labeled picture of the microplate with samples in it (do this on a white background).
  See Results Section Above
- B. Include labeled graph.
  See Results Section Above
- C. Include a table of absorbance values.
  See Results Section Above
- D. Get the R2 value for the standard curve.
  The R^2 value for the standard curve is .993.
- E. Get the equation for the trend line.
  x = Concentration
  .798x+.417 = Absorbance
- F. Determine the concentration of your unknown. Remember to take into account your dilutions. Use the equation from the trend line to calculate the concentration. See discussion below for further information.
  (.4695-.417)/.798 = .06578…
  .06578…*5 = **.3289 mg/ml**

Conclusion/Discussion:

The objectives of the lab were achieved. Throughout the lab, my partner and I gained knowledge in the utilization of micropipettes, performed serial dilutions, created a standard curve and determined a concentration from said curve, learned about microplate readers and spectrophotometry, and learned the methods of quantification of protein samples as well as the potential drawbacks of those methods.

My hypothesis was correct since the most concentrated solutions appeared blue in color while the less concentrated solutions appeared brownish. This means that my solution of B, which appeared brownish, was also less concentrated. This makes sense since the calculated concentration was only .3289 mg/ml. If I were to repeat this experiment, I would repeat the dilution of B to see if I would get a similar concentration value of B with a second trial. Additionally, the results of the standard curve were acceptable since the graph was roughly linear with an R^2 value of .993.

Enzyme Kinetics

Date: 9/24/2025

Pre-Lab Questions:

1. Perform the serial dilution calculations required for Part 1 and Part 2 using the table format shown in Tables 1 and 2. Paste your serial dilution tables below and in your notebook.

Part 1

Concentration	Substrate Volume	Volume 1X Reaction Buffer	Total Volume	Remaining Volume
4 mM	960 µl	+ 240 µl	= 1.2 ml	600 µl
2 mM	600 µl	+ 600 µl	= 1.2 ml	600 µl
1 mM	600 µl	+ 600 µl	= 1.2 ml	600 µl
.5 mM	600 µl	+ 600 µl	= 1.2 ml	600 µl
.25 mM	600 µl	+ 600 µl	= 1.2 ml	600 µl
.125 mM	600 µl	+ 600 µl	= 1.2 ml	1.2 ml

Part 2

	Product Volume	Volume 1X Reaction Buffer	Total Volume	Remaining Volume
.1000 mM	Stock			
.500 mM	125 µl	+ 125 µl	= 250 µl	125 µl
.250 mM	125 µl	+ 125 µl	= 250 µl	125 µl
.125 mM	125 µl	+ 125 µl	= 250 µl	125 µl
.0625 mM	125 µl	+ 125 µl	= 250 µl	250 µl
0 mM	Reaction Buffer Only			

2. Why can't we study cellobiase activity with cellobiose as a substrate?

We can't study cellobiase activity with cellobiose as a substrate because the product of the reaction with cellobiose as a substrate is glucose. Glucose and cellobiose both have no color and therefore cause difficulty observing the reaction.

3. What is the purpose of p-Nitrophenol glucopyranoside?

p-Nitrophenyl glucopyranoside is used in this lab because it is colorless and its products in the reaction with cellobiase as the enzyme are glucose and p-Nitrophenol. p-Nitrophenol is light yellow, which makes it easy to observe the reaction progress and the enzymatic activity of cellobiase.

4. How does temperature influence an enzymatic reaction?

Temperature influences an enzymatic reaction by increased heat initially causing the reaction to go faster because more reactants have the kinetic energy to go over the activation energy barrier. However, if the temperature gets too high, the enzymes will become denatured which will significantly reduce reaction speed since the enzymes are no longer able to lower the activation energy of the reaction.

Objectives:

- Perform Time Course Experiments
- Create a Standard Curve
- Evaluate Reaction Rates of Enzymes and Comprehend Michaelis-Menten Plots
- Find the Vmax and Km of an Enzyme

Hypothesis:

I predict that higher concentrations of substrate will turn more yellow. Additionally, the well plate should contain solutions that increase in yellow color as more time is given for the reaction.

Methods:

Part 1: Serially dilute the substrate

Materials:

- 5 mM substrate (p-Nitrophenyl glucopyranoside, PNG)
- 1X reaction buffer, pH 5
- Microfuge tubes

1. First, calculate the serial dilutions necessary for each concentration (Table 1).
2. Perform the necessary dilutions.

Table 1. Serial dilution schematic for substrate (5 mM stock)

Concentration	Substrate Volume	Volume 1X Reaction Buffer	Total Volume	Remaining Volume
4 mM	960 µl	+ 240 µl	= 1.2 ml	600 µl
2 mM	600 µl	+ 600 µl	= 1.2 ml	600 µl
1 mM	600 µl	+ 600 µl	= 1.2 ml	600 µl
.5 mM	600 µl	+ 600 µl	= 1.2 ml	600 µl
.25 mM	600 µl	+ 600 µl	= 1.2 ml	600 µl
.125 mM	600 µl	+ 600 µl	= 1.2 ml	1.2 ml

Part 2: Time courses for cellobiase reaction

Materials:

- Substrate dilutions from Part 1
- Cellobiase (diluted 1:30 by instructor, keep on ice)
- 1X stop solution
- Microfuge tubes
- Microplate and reader
- Timer

Table 2. 96-well plate diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	**4 mM 1 min**		**1 mM 1 min**		**.25 mM 1 min**							**1 mM SC**
B	**4 mM 2min**		**1 mM 2 min**		**.25 mM 2 min**							**.5 mM SC**
C	**4 mM 3 min**		**1 mM 3 min**		**.25 mM 3 min**							**.25 mM SC**
D	**4 mM 4 min**		**1 mM 4 min**		**.25 mM 4 min**							**.125 mM SC**
E		**2 mM 1 min**		**.5 mM 1 min**		**.125 mM 1 min**						**.0625 mM SC**
F		**2 mM 2 min**		**.5 mM 2 min**		**.125 mM 2 min**						**0 mM SC**
G		**2 mM 3 min**		**.5 mM 3 min**		**.125 mM 3 min**						
H		**2 mM 4 min**		**.5 mM 4 min**		**.125 mM 4 min**						

96-Well Plate Setup:

1. For each substrate concentration (6 total), add 50 µl of 1X stop solution to 4 wells of the microplate (1 well for each time point in the time course). The stop solution is a strong base, so avoid contact with it.
2. Setup 6 microfuge tubes and label them all “Rxn”. These are your reaction tubes; they are all the same for now, so you do not need to label them with anything other than “Rxn”.
3. Place 40 µl of enzyme in each Rxn tube and place the tubes on ice.
4. Record the temperature of the room.

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5. Read and understand the next steps fully before proceeding. These are time sensitive!

Enzyme Assay Protocol:

1. Remove one of the Rxn tubes from ice and place it in a microfuge tube rack – do NOT hold it in your hand for long.
2. Select a tube of substrate from Table 1 and transfer 550 µl of its contents to the Rxn tube. Mix by gently pipetting up and down once and START YOUR TIMER as soon as you Substrate dilutions from Part 1 Cellobiase (diluted 1:30 by instructor, keep on ice) 1X stop solution Microfuge tubes Microplate and reader Timer as soon as you begin mixing. Do NOT stop your timer until step 4.
3. At 1, 2, 3, and 4 minutes, transfer 100 µl of the reaction to the appropriate wells in the microplate and mix with the stop solution in the well by pipetting. Avoid making bubbles.
4. Stop and reset your timer.
5. Repeat steps 1 - 4 for each remaining substrate concentration.
6. Put the microplate aside until the end of Part 3. Time is not of concern after the enzyme reaction has been mixed with the stop solution in the microplate.

Part 3: p-Nitrophenol Standard Curve

Materials:

- 1 mM p-Nitrophenol stock solution
- 1X reaction buffer
- 1X stop solution
- Microplate and reader

1. In addition to the tube of 1 mM stock p-Nitrophenol, you will need 4 more microfuge tubes for p-Nitrophenol serial dilution and 1 more microfuge tube for "0 mM" (reaction buffer only). Label the tubes accordingly.

2. Serially dilute the product from the stock solution to the concentrations indicated on each tube in a total volume of 250 µl with 1X reaction buffer. Write out the dilutions before lab and show the TA/instructor (see Table 3).

Table 3. Dilutions for product standard curve (1 mM stock)

	Product Volume	**Volume 1X Reaction Buffer**	**Total Volume**	**Remaining Volume**
.1000 mM	Stock			
.500 mM	125 µl	+ 125 µl	= 250 µl	125 µl
.250 mM	125 µl	+ 125 µl	= 250 µl	125 µl
.125 mM	125 µl	+ 125 µl	= 250 µl	125 µl
.0625 mM	125 µl	+ 125 µl	= 250 µl	250 µl
0 mM	Reaction Buffer Only			

3. On the microplate, add 50 µl of 1X stop solution to the wells designated for the standard curve (6 total). Do NOT add more stop solution to the wells containing your time course samples.

4. For each tube from Table 3, transfer 100 µl from the tube to the designated well and gently mix with the stop solution in the well by pipetting. Avoid making bubbles. Do NOT add anything from Table 3 to the wells containing your time course samples

Microplate Reader Protocol:

1. Make sure you know the order and arrangement of your samples in the 96-well plate.

2. Pop any bubbles in the centers of wells with a fresh P-20 tip; bubbles around the edges of wells shouldn’t be a problem.

3. Read your plate at ABS 405 nm. Remember, the plate reader will give values for every well. You need to extract the useful ones for analysis.

Results:

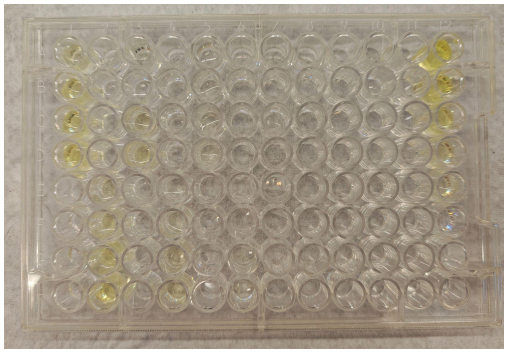


Figure 1: Solutions

Column 1: 4 mM p-Nitrophenol Top to Bottom
4 min, 3 min, 2 min, 1 min
Column 2: 2 mM p-Nitrophenol Top to Bottom (Starting in Well E)
4 min, 3 min, 2 min, 1 min
Column 3: 1 mM p-Nitrophenol Top to Bottom
4 min, 3 min, 2 min, 1 min
Column 4: .5 mM p-Nitrophenol Top to Bottom (Starting in Well E)
4 min, 3 min, 2 min, 1 min
Column 5: .25 mM p-Nitrophenol Top to Bottom
4 min, 3 min, 2 min, 1 min
Column 6: .125 mM p-Nitrophenol Top to Bottom (Starting in Well E)
4 min, 3 min, 2 min, 1 min
Column 12: Top to Bottom
1 mM SC, .5 mM SC, .25 mM SC, .125 mM SC, .0625 mM SC, 0 mM SC

Concentration p-Nitrophenol (nmol)	Absorbance at 405 nm
100	3.8534
50	2.7023
25	1.4135
12.5	0.6692
6.25	0.3427
0	0.0622

Figure 2: Concentration p-Nitrophenol Versus Absorbance

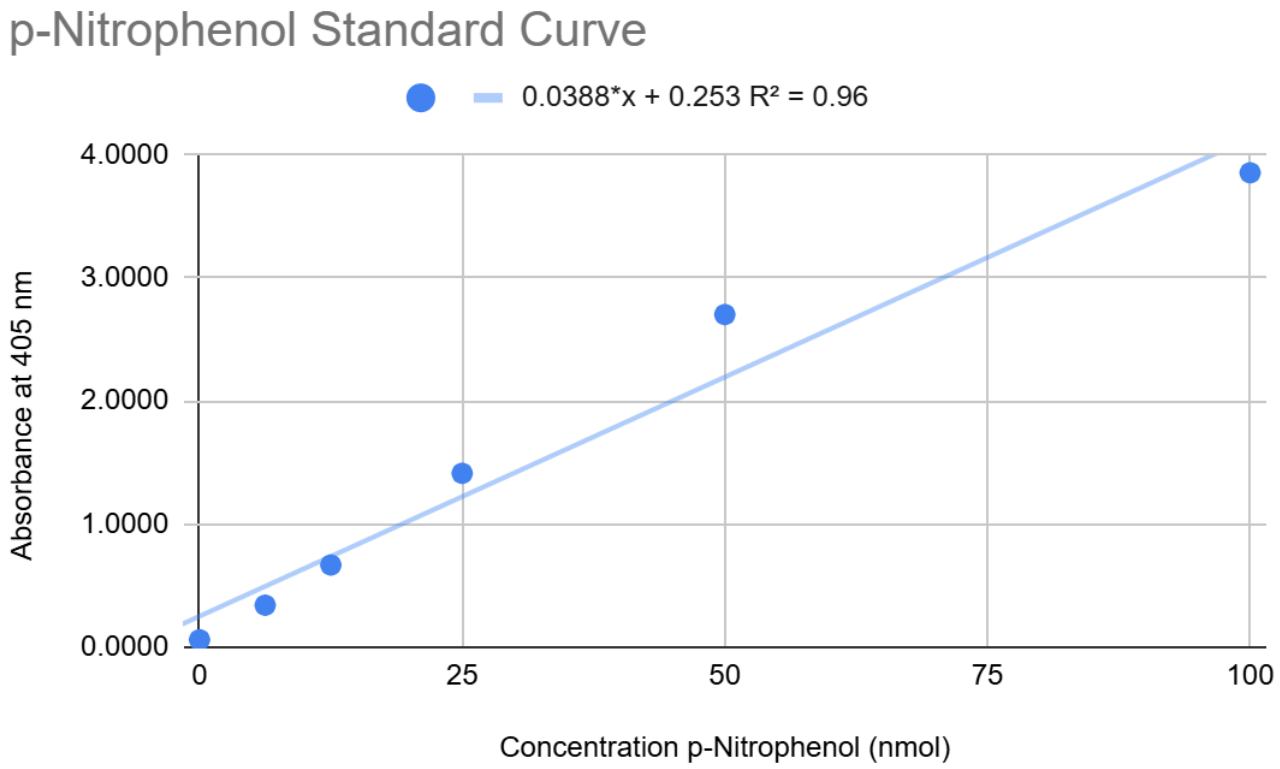


Figure 3: p-Nitrophenol Standard Curve

	p-Nitrophenol Concentration (nM)					
Time (mins)	**4 mM**	**2 mM**	**1 mM**	**0.5 mM**	**0.25 mM**	**0.125 mM**
1	6.193298969	3.059278351	-1.425257732	-2.603092784	-3.974226804	-4.146907216
2	15.66237113	9.489690722	-4.595360825	-0.2345360825	-3.105670103	-3.546391753
3	24.01804124	17.04381443	5.131443299	2.886597938	-2.043814433	-2.775773196
4	33.1314433	20.96134021	8.721649485	5.170103093	-1.237113402	-2.190721649

Figure 4: p-Nitrophenol Concentrations for Solutions over a Period of Time

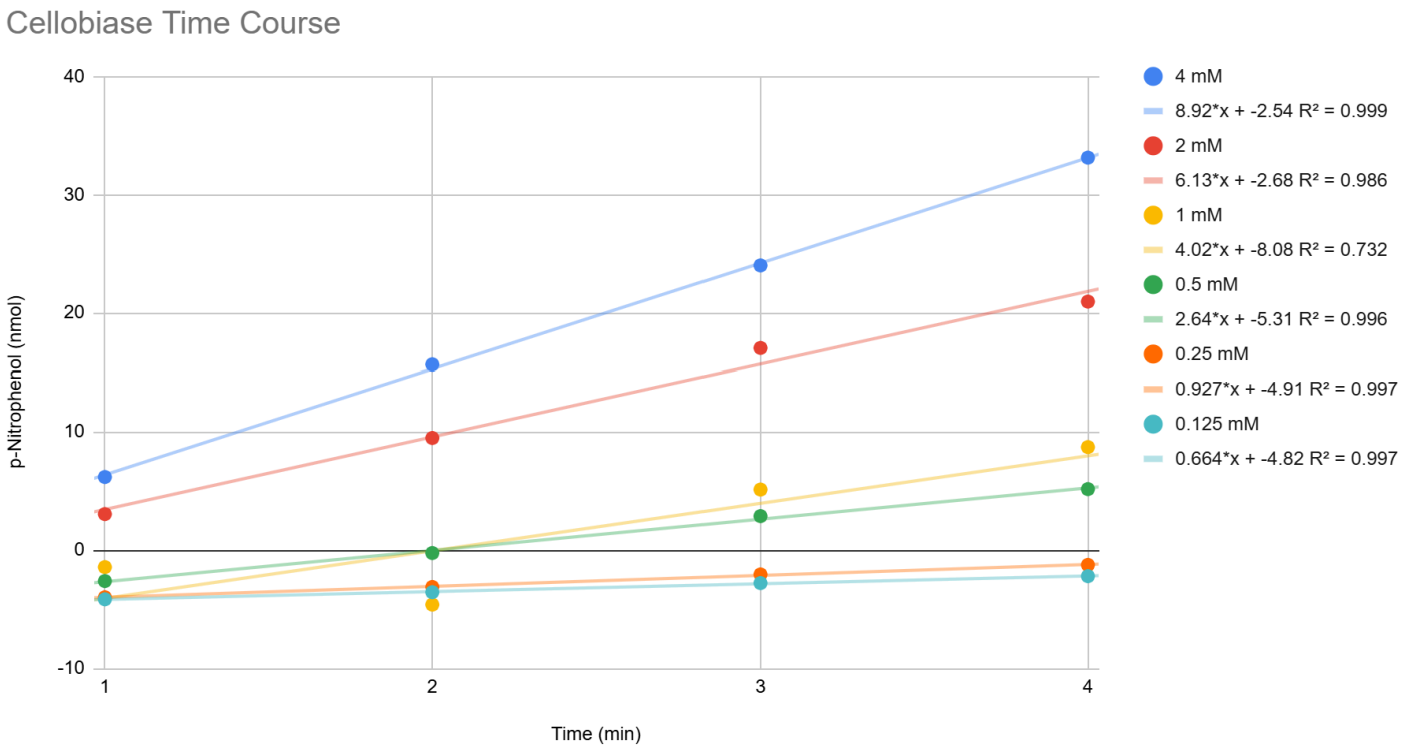


Figure 5: Cellobiase Time Course

p-Nitrophenol Concentration (mM)	Rxn Rate (nmol/min)
4	8.92
2	6.13
1	4.02
0.5	2.64
0.25	0.927
0.125	0.664

Figure 6: p-Nitrophenol Concentration Versus Reaction Rate

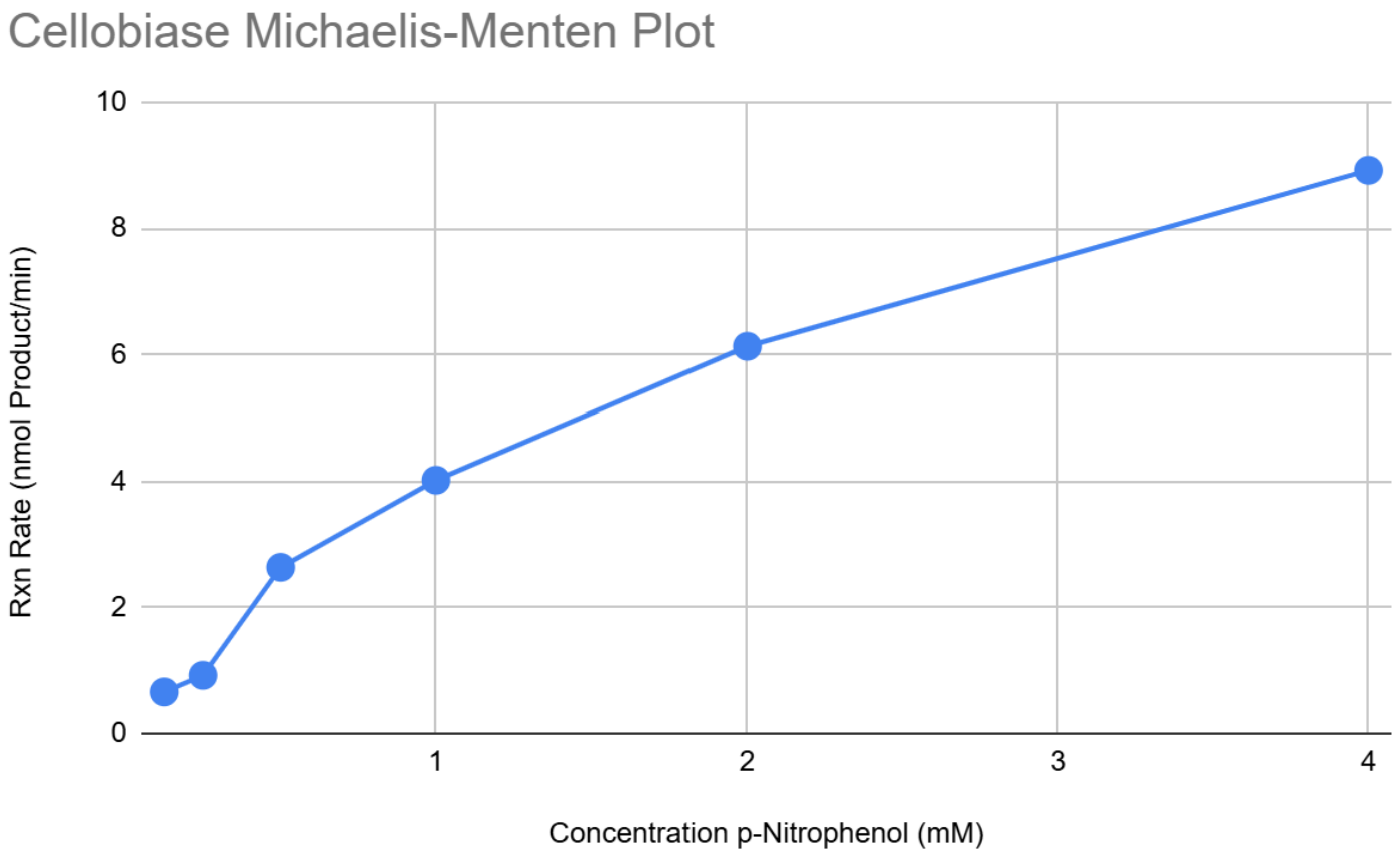


Figure 7: Cellobiase Michaelis-Menten Plot

1/[p-Nitrophenol]	1/Rxn Rate (min/nM)
0.25	0.1121076233
0.5	0.163132137
1	0.2487562189
2	0.3787878788
4	1.078748652
8	1.506024096

Figure 8: 1/[p-Nitrophenol] Versus 1/Reaction Rate

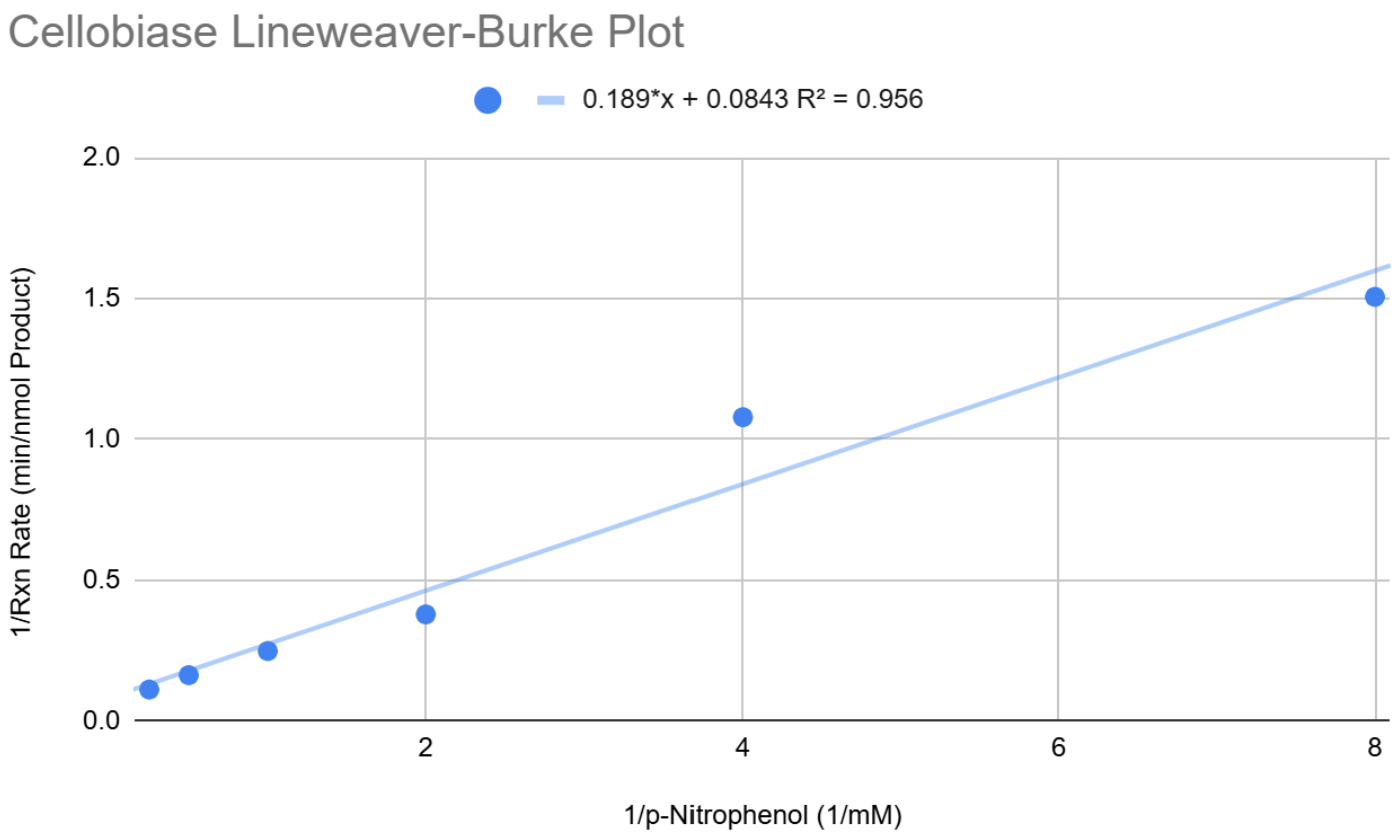


Figure 9: Cellobiase Lineweaver-Burke Plot

Lab Questions:

Part 1: Standard Curve

a)First, convert your standard curve concentrations (left column for Table 3) from miliMolar (mM) to nanomoles (nmol); this means you are converting from units of concentration (molarity) to units of quantity (moles).

See Graph Above

b). Next, make a scatter plot of nmol p-Nitrophenol versus the corresponding Abs values. This is a standard curve. The purpose of this is to correlate arbitrary absorbance values (Abs) with nmols of product. Add a linear trend line and display the equation and R2 .

See Graph Above

Part 2: Use Standard Curve to Convert Time Course Data to nmols Product

a. You need to convert each Abs value from the time course experiment to nmols of pNitrophenol. This is just like determining the concentration of BSA in the Bradford assay. Use the equation from the linear regression to solve for x by plugging in Abs values for y.

See Graph Above

b. You should now have values in nmol for each time point of each time course for a total of 24 data points (see examples in Table 4). You are done with the standard curve now; the only values that matter from here onwards are the 24 data points associated with the time course experiment.

c. Now, plot time versus product (in nmols) for each substrate concentration on the same graph, as shown below. See your lab instructor or a fellow classmate for help with this. Also, include the linear trend line and equation on the graph with R2 . You will need the equation and slope for each. The slope is the rate of the reaction in nmol/min.

See Graph Above

Part 3: Michaelis-Menten Plot

a. You should now have reaction rates for each substrate concentration (the slope of each line); the y-intercepts are not needed. Organize the substrate concentrations and reaction rates into a table as shown below (Table 5).

See Table Above

b. Plot each substrate concentration (mM) versus the corresponding reaction rate. This gives a Michaelis-Menten plot, which is typical for enzyme kinetics. This type of plot demonstrates that typical enzymes have a saturation limit, which makes their kinetics nonlinear. You can try to estimate the Km and Vmax by eye, but it won't be very accurate.

See Graph Above

Part 4: Lineweaver-Burke

a. Although it is possible to perform nonlinear regression to determine Km and Vmax from the Michaelis-Menten plot, for the reasons mentioned in the Background section we will instead determine Km and Vmax by the Lineweaver-Burke linear transformation. To do this, you need to calculate the reciprocal of the substrate concentration and the reciprocal of the reaction rates. Organize this data into a table as shown below (Table 6).

See Table Above

b. Plot these data and add a linear trend line; display the equation and R2 . This is called a Lineweaver-Burke plot.

See Graph Above

c. From the Lineweaver-Burke plot you can now calculate the Km and Vmax. First find Vmax by taking the reciprocal of the y-intercept. Then, multiply the slope by the Vmax to find the Km. Figure 7 illustrates the relationship between these values in the Lineweaver-Burke plot. Make sure you have the proper units for each value. Km should be in units of mM; Vmax should be in units of nmol/min.

Vmax=11.9 nmol/min

Km=2.24 mM

Conclusion/Discussion:

The purpose of the lab was achieved since my lab partner and I were able to perform time-course experiments, create a standard curve, find enzyme reaction rates, create and understand Michaelis-Menten plots, and find the Km and Vmax of an enzyme.

My hypothesis was correct since higher concentrations of p-Nitrophenol led to more of a yellow color in the substance. Additionally, longer reaction times led to an increase in yellow color as was expected. If I were to repeat this experiment, I would redo the 1 mM p-Nitrophenol time course since the R^2 value of .732 was much lower than it should be.

SDS-PAGE

Date: 10/1/2025

Pre-Lab Questions:

1. Explain what TEMED is. What is its purpose in SDS-PAGE?

TEMED is a catalyst that is used on polyacrylamide gels. In SDS-PAGE, TEMED is used to make the separation of larger and smaller molecules proceed faster during electrophoresis.

2. Explain why the protein samples travel to the positive pole when an electric current is applied.

Protein samples travel to the positive pole after an electric current is applied because the proteins are treated with SDS. The SDS denatures the protein and causes it to become negatively charged and therefore attracted to the positive pole.

3. Perform calculations for the gel solutions in Part 2 AND the BSA serial dilution in Part 3. Paste your calculations below and in your notebook.

Table 1: Resolving Gel (5 ml total volume)

Concentrated Stock	Final Concentration	Volume
1.5 M Tris pH 8.8	195 mM Tris pH 8.8	0.65 ml
40% Acrylamide	8% Acrylamide	1 ml
10% SDS	0.1% SDS	0.05 ml
10% APS	0.12% APS	0.06 ml
dH_2O (Q.S. to 5 ml)		3.24 ml

Table 2: Stacking Gel (2 ml total volume)

Concentrated Stock	Final Concentration	Volume
1 M Tris pH 8.8	125 mM Tris pH 8.8	0.25 ml
40% Acrylamide	5% Acrylamide	0.25 ml
10% SDS	0.1% SDS	0.02 ml
10% APS	0.12% APS	0.024 ml
dH_2O (Q.S. to 2 ml)		1.456 ml

Table 3: BSA dilution schematic

BSA Concentration	Volume BSA	dH_2O	Total Volume	Remaining Volume
400 µg/ml	8 µl	+32 µl	= 40 µl	20 µl
200 µg/ml	20 µl	+20 µl	= 40 µl	20 µl
100 µg/ml	20 µl	+20 µl	= 40 µl	20 µl
50 µg/ml	20 µl	+20 µl	= 40 µl	20 µl

25 µg/ml	20 µl	+20 µl	= 40 µl	40 µl

4. Explain what an IgG is and how it's structured. (Hint: think quaternary structure.) What do you predict the two different IgG samples will look on your gel?
IgG is immunoglobulin G, the antibody found most frequently in blood. It is a good model of most antibodies as well. Antibodies are y-shaped and contain two variable and two constant domains on each arm of the Y. The domains both contain antiparallel beta sheets with three and five strands. Additionally, a disulfide bond holds together the sheets. There is one heavy variable, heavy constant, light variable, and light constant per each arm of the Y. Antigens bind to the hypervariable loops in the variable domain. I predict that the IgG sample with DTT will have two lines, a lower line for the heavy domains and a higher line for the light domains. The IgG sample without DTT will have a singular line in between the two lines formed from the IgG sample with DTT.

Objectives:

- Create multi-component solutions
- Create and run SDS-PAGE gels
- Find detection limits of an SDS-PAGE
- Estimate molecular weight and amount of a protein sample

Hypothesis:
I predict that the IgG sample with DTT will have two lines, a lower line for the heavy domains and a higher line for the light domains. The IgG sample without DTT will have a singular line in between the two lines formed from the IgG sample with DTT.
Additionally, I predict that the higher concentrations of each protein will form lines lower than the lesser concentrations of each protein.

Methods:
Part 1: Setting up the gel casting assembly
Materials:

- Glass plates (1 tall, 1 short)
- Gel casting clamp
- Gel casting stand
- dH2O squirt bottle
- Absorbent pad

Procedure:
1. Clean both glass plates with dH2O and wipe dry with a Kimwipe. These should be spotless, especially where the spacers of the tall plate contact the short plate.
2. Identify the gel casting clamp – it's a green plastic apparatus (Figure 1). Set the casting clamp down directly on the lab bench – it must be on a solid surface; do NOT set it down on the blue absorbent pad or in the gel casting stand.
3. Make sure the casting clamp is resting on its feet and open the clamp by pulling the two levers open (towards you).
4. Insert the glass plates into the casting clamp; the short plate should be in front of the tall one; the tall plate should be oriented so the spacers leave a gap between the plates.

5. Ensure the glass plates are aligned; they should be resting on the surface of the lab bench. If the plates are slightly wet, they could stick together, so you might need to gently push the short plate down so it aligns with the tall plate.
6. Without lifting the assembly, close the clamp (Fig. 1 shows levers in the closed position).
7. Ensure that a gray foam pad is present on the gel casting stand (the large, transparent stand). Load the gel casting clamp assembly onto the casting stand – lift the clip at the top of the stand and place the assembly underneath it. The clip will push down on the tall plate to seal the bottoms of the plates against the gray foam pad.
8. Confirm the assembly is water-tight: use a squirt bottle to fill the space between the glass plates with dH2O and let it stand for several minutes. You can get other things prepared during this time.
a. If a leak appears, restart this procedure from step 2.
b. If there is no leak, remove the water from the assembly by tilting it against a Kimwipe so the water is pulled out from between the plates by capillary action (your instructor will demonstrate). DO NOT REMOVE THE GEL CASTING CLAMP ASSEMBLY FROM THE CASTING STAND – if you do this, you will need to test the water-tightness of the assembly again.
9. Place the entire casting stand assembly on the absorbent pad if you have not done so already.

Part 2: Prepare the SDS-PAGE gel solutions

Materials:

- Reagents listed in Tables 1 and 2
- Two transfer tubes with caps (white)
- Micropipettes and tips

Procedure:

1. Prepare the multi-component solution for the resolving gel; reagents and concentration are given in Table 1. Always add water to your tube FIRST, then you can add the other reagents. Q.S. (quantum satis, Latin for “the quantity that is enough”) means to add enough dH2O to make the final volume of the resolving gel 5 ml in our case.

Table 1: Resolving Gel (5 ml total volume)

Concentrated Stock	Final Concentration	Volume
1.5 M Tris pH 8.8	195 mM Tris pH 8.8	0.65 ml
40% Acrylamide	8% Acrylamide	1 ml
10% SDS	0.1% SDS	0.05 ml
10% APS	0.12% APS	0.06 ml
dH_2O (Q.S. to 5 ml)		3.24 ml

2. Add 12 µl TEMED to the resolving gel solution only when you are ready to cast the gel.
3. Close the cap and mix by gently inverting the tube. Do not introduce air into the solution since this will slow polymerization. Use a P1000 pipette to dispense the gel solution between the two plates from Part 1. Fill no higher than the bottom of the green bar of the gel casting clamp (see Figure 1). If any of it spills, clean it up immediately; remember that the gel solution contains acrylamide!
4. After pouring the solution, quickly but VERY GENTLY use a P1000 pipette to add a 1 ml layer of dH2O. This makes the resolving gel top level. Do NOT use a water squirt bottle.

5. After 5 minutes, check if the remaining gel solution in the tube has polymerized by capping and inverting the tube. Do not tilt or distribute the gel casting stand assembly to test for polymerization – this will disrupt the matrix that forms and will ruin your gel. If it has not polymerized, keep checking every 5 minutes for up to 20 minutes.
6. Once the gel solution in the tube has polymerized, confirm the gel in the casting stand has polymerized by tilting up one end of the stand; you should see only the water layer move. Remove the water layer with a Kimwipe; dispose of the Kimwipe in biohazardous waste (acrylamide hazard). Change your gloves.
7. Prepare the stacking gel; reagents and concentrations are given in Table 2. Note the different tris solution being used for the staking gel (this stock is 1 M Tris, pH 6.8).

Table 2: Stacking Gel (2 ml total volume)

Concentrated Stock	Final Concentration	Volume
1 M Tris pH 6.8	125 mM Tris pH 6.8	0.25 ml
40% Acrylamide	5% Acrylamide	0.25 ml
10% SDS	0.1% SDS	0.02 ml
10% APS	0.12% APS	0.024 ml
dH_2O (Q.S. to 2 ml)		1.456 ml

8. After the resolving gel has polymerized AND you are ready, add 6 µl TEMED to the stacking gel solution, mix, and transfer the solution onto the top of the resolving gel. Add enough stacking gel solution to it reaches the top of the short plate. DO NOT ADD TEMED TO THE RESOLVING AND STACKING GELS AT THE SAME TIME!!
9. Immediately after adding the stacking gel solution, insert the 10-well comb in between the plates. Avoid catching any bubbles when inserting the comb. Absorb any solution that flows over the plates with a Kimwipe; dispose of the Kimwipe in biohazardous waste (acrylamide hazard). Change your gloves.
10.Wait 10-20 minutes for polymerization and check by observing the remaining stacking gel solution in the tube. Do not remove the comb yet; that will be done later.

Part 3: Preparation of Protein Samples

Materials:

- Heat block set to 95°C
- dH2O
- 2 mg/ml Bovine Serum Albumin ("BSA")
- 100 µg/ml Immunoglobulin G ("IgG")
- 500 µg/ml E. coli lysate ("E")
- Unknown protein sample ("U")
- 2X SDS-PAGE Loading Buffer +DTT ("SLB+")
- 2X SDS-PAGE Loading Buffer -DTT ("SLB-")

Procedure:

1. Prepare the BSA dilutions shown in Table 3.

Table 3: BSA dilution schematic

BSA Concentration	Volume BSA	dH_2O	Total Volume	Remaining Volume
400 µg/ml	8 µl	+32 µl	= 40 µl	20 µl
200 µg/ml	20 µl	+20 µl	= 40 µl	20 µl
100 µg/ml	20 µl	+20 µl	= 40 µl	20 µl
50 µg/ml	20 µl	+20 µl	= 40 µl	20 µl

25 µg/ml	20 µl	+20 µl	= 40 µl	40 µl

2. Set up 9 tubes (8 if you aren't running an unknown), label them #1 through #9. Add 10 µl SBL+ to all tubes except tube #8. Add 10 µl SLB- to tube #8 (this is the tube for your IgG without DTT).
3. Transfer 10 µl of the following to each tube:

Tube #1: 400 µg/ml BSA
Tube #2: 200 µg/ml BSA
Tube #3: 100 µg/ml BSA
Tube #4: 50 µg/ml BSA
Tube #5: 25 µg/ml BSA
Tube #6: 500 µg/ml E. coli lysate
Tube #7: 100 µg/ml IgG
Tube #8: 100 µg/ml IgG
Tube #9: Unknown Protein

4. Once ALL PROTEIN SAMPLES are mixed with the appropriate SLB, heat them at 95°C for 5 minutes. Put one of the heating blocks on top of the tubes so they don't pop open while heating. Do NOT add SLB to the Kaleidoscope Molecular Weight Marker and do NOT heat it either.
5. Let samples cool and spin tubes down briefly.

Part 4: Assemble the SDS-PAGE apparatus

Materials:

- SDS-PAGE Running Buffer
- Electrophoresis apparatus
- Buffer dam
- Gel sandwich from Part 2

Procedure:

1. When you are ready to load the samples on your gel, remove the gel "sandwich" (glass plates with the gel inside) from the casting clamp.
2. Insert the gel sandwich into the gel electrophoresis apparatus. The short plate should be facing towards the inner chamber; make sure to gently push the sandwich up so the short plate touches the ridge of the gasket (Figure 2). If there's a gap, the inner chamber will not be properly sealed and the buffer will leak out.
3. Install the buffer dam as you installed the gel sandwich – do NOT leave a gap between the ridge of the dam and the gasket. Ensure the dam is flipped to the appropriate side.
4. Close the compression arms around the apparatus; this makes a tight seal. Ask your instructor to check this before proceeding to the next step.
5. Insert the apparatus into the electrophoresis tank in the correct slot (there are two slots) – the electrodes at the top of the apparatus should be able to fit through the lid of the tank. Do a test-fit to make sure the tank can be closed properly.
6. Pour running buffer into the inner chamber of the apparatus (the space between the gel sandwich and the buffer dam) until it overflows into the rest of the tank. Fill the rest of the tank only about halfway with a running buffer.
7. Slowly remove the comb from the gel. Rinse the wells with a buffer from the tank using a pipette set to 100 µl.

Part 5: Loading and running the gel

Materials:

- Power supply
- Samples from Part 3
- Kaleidoscope MW marker
- Electrophoresis apparatus from Part 4

Procedure:

1.Use the P20 pipette to slowly add each sample into a well. DON'T DEPRESS THE PIPETTE DOWN TO THE SECOND STOP – this will blow bubbles into your wells and likely blow out all of your carefully loaded sample! Use only 8 µl of MW marker in the first well.

Table 4: Gel loading scheme

Well #	Volume	Sample
1	8 µl	400 µg/ml BSA
2	20 µl	200 µg/ml BSA
3	20 µl	100 µg/ml BSA
4	20 µl	50 µg/ml BSA
5	20 µl	25 µg/ml BSA
6	20 µl	500 µg/ml E. coli lysate
7	20 µl	100 µg/ml IgG (+DTT)
8	20 µl	100 µg/ml IgG (-DTT)
9	20 µl	Unknown Protein
10	10 µl	Kaleidoscope MW marker

2. When all the samples are loaded, place the cover on the gel tank, connect the power supply, and set the voltage to 300 V. If you are sharing a power supply with another group, make sure to turn off the power supply before inserting or removing electrical leads. Turning the power supply off and on again will not hurt the other group's gel.
3. Press run on the power supply (the icon that looks like a person jogging). You should see bubbles being liberated from the electrode inside the inner chamber of the apparatus.
4. Begin cleaning up but keep an eye on the progress of your gel while it runs. Check the current (mA) occasionally. If the current goes to zero or the bubbles are no longer forming, this means the buffer has leaked out of the inner chamber, and you need to stop the gel and replace some buffer.
5. When the dye front has moved ~90% down the gel, turn off the power supply, and remove the electrical leads.
6. Take the lid off of the tank and pour the running buffer back into the bottle or large carboy that you got from (we can reuse it, let's not waste it). Remember that there is likely some non-polymerized acrylamide in the running buffer now, so be careful and do not spill it anywhere – work over the absorbent pad.
7. Disassemble the apparatus according to the instructor's directions and retrieve your gel sandwich.
8. Pour the running buffer from your tank back into the carboy (we can reuse the running buffer many times and don't want to waste it).
9. Wash all parts of the tank and inner gel apparatus in the sink with water and set it out to dry disassembled on the absorbent pad.

Part 6: Coomassie stain SDS-PAGE gel

Materials:

- Gel separator
- Gel Code Blue Coomassie stain
- Staining box
- dH2O
- Shaker (shaking platform)

Procedure:

1. To remove the gel from the glass plates, use the gel separator (green wedge) to GENTLY pry off one of the glass plates. The gel will remain stuck to the other glass plate. It's OK if some pieces of the stacking gel break off in this process – this is not where your proteins are located.
2. Fill a gel staining box about halfway with dH2O. Using tape, label the container with your names and section.
3. Turn the glass plate with the gel upside down so the gel peels off in one piece. If the gel does not move, use the end of a wet gel separator to pry a corner of the gel off of the plate, and let gravity assist the gel so it falls into the staining box.
4. Place the staining box on the shaker for 5 minutes. Then, pour off the water into the sink, being careful not to let the gel slip into the sink.
5. Add more dH2O and repeat Steps 2 and 3. These washes remove salts from the running buffer so the gel will stain better.
6. Once you have removed the water from the staining box, add at least 30 ml Gel Code Blue Coomassie stain and place the box back on the shaker.
7. Your instructor will do this part: after at least 1 hour, pour the stain into the designated bottle or conical tubes. Add dH2O to thoroughly cover the gel to de-stain overnight on the shaker.

Part 7: Image your gel (NEXT WEEK)

Materials:

- Your gel from last week (de-stained)
- GelDoc Imager with white transilluminator
- dH2O
- Fresh staining box (optional)

Procedure:

1. Retrieve your staining box, pour the water out, and replace with a small amount of fresh dH2O (just enough to cover the gel).
2. Place the entire staining box on the GelDoc imager. Make sure the lid is open. If stains adhered to the surface of your staining box, you might need to transfer your gel to a new staining box or you can simply lay your gel on the surface of the imager (no box).
3. Position the gel so no part is flopped over another – you want to see the entire gel! It's OK if the gel is crooked or not centered in the staining box; you can fix this by rotating and cropping the image later.
4. Take an image with the GelDoc and make sure it is saved to the proper location.
5. Dispose of the gel in biohazard waste and wash the staining box out with 70% ethanol to remove the remaining stain. Rinse the box out with water and set out to dry.

Results:

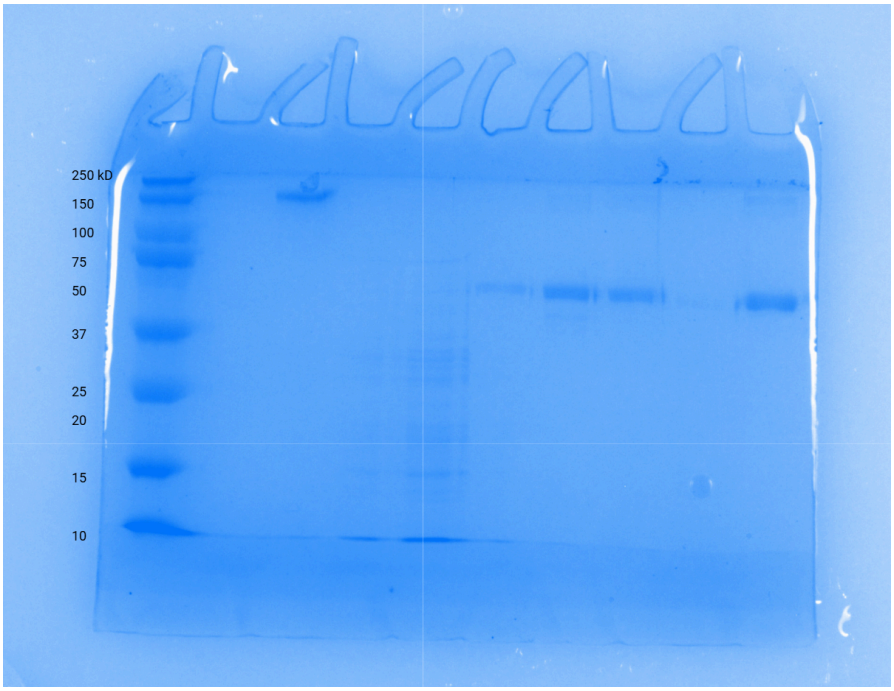


Figure 1 SDS-PAGE Gel

Lab Questions:

A. Make a labeled figure of your gel after destaining. Label each lane and every band in the MW marker lane. You will need to refer to Figure 3 to identify the molecular weight (in kDa) of each band in the MW marker lane. It is critical that these bands are labeled; otherwise, the figure will be considered incomplete for the next notebook check.

See Figure 1 Above

B. Using these molecular weight standards, estimate the size of each of the proteins that you ran on the gel, except for the E. coli lysate (too many proteins to assess).

Protein (In Opposite Order of Wells in Picture)	Estimated Size
400 µg/ml BSA	75 kD
200 µg/ml BSA	75 kD
100 µg/ml BSA	75 kD
50 µg/ml BSA	75 kD
25 µg/ml BSA	75 kD
500 µg/ml E. coli lysate	SKIP
100 µg/ml IgG (+DTT)	37 kD, 10 kD
100 µg/ml IgG (-DTT)	200 kD
Unknown Protein	SKIP
Kaleidoscope MW marker	Labeled in Photo

C. Using the BSA protein titrations, estimate the amount of the unknown sample. To do this, qualitatively compare the thickness of the titration bands to that of the unknown.
We did not have an unknown protein.
D. Write in your notebook the purpose for each component and for each buffer and gel solution used today.
Gel Code Blue Coomassie Stain is used to make the lines formed in the gel via gel electrophoresis more discernable for viewing. SDS-PAGE Running Buffer is used to allow electricity to travel through the solution and allow gel electrophoresis to occur. Stacking gel is used to allow the proteins to correctly align. Resolving gel helps the proteins of different sizes to be separated. Kaleidoscope MW marker is used to show the differing molecular weights of proteins so that the proteins used in the lab can be compared to the known weights of the Kaleidoscope MW marker. The different concentration BSA samples were used in order to compare the different concentrations via gel electrophoresis. Lastly, the IgG + and - DTT was used to show how DTT breaks the disulfide bonds and causes two separate bands to occur on the gel.
E. What is the final amount (µg) of BSA samples used to load lanes 2-5? Remember to take into account that a loading buffer was added to the samples.
4 µg of BSA was used to load lane 2, 2 µg for lane 3, 1 µg for lane 4, and .5 µg was used for lane 5.
This is found by concentration in µg/ml times volume in ml.
F Did your two IgG lanes produce the same pattern of bands or were they different? Are the results what you expected? Explain.
The two IgG lanes produced different patterns of bands that mostly aligned with what I expected. Since the DTT separates the heavy and light domains of the protein, the IgG +DTT will have two lines and the IgG -DTT will only have one line. However, this one line should be located in the middle of the two lines but it was not in my gel.
G. Does the gel look as you would expect? Discuss any problems you had with running the gel.
The gel does not look as I would expect. Many of the proteins seemed to have similar molecular weights even though they technically do not. Additionally, as stated in part F, the lines for my IgG samples were not as expected. This most likely stems from issues loading the gel into the solution. Although we attempted to load all of the sample into the solution, sometimes the sample fell into the buffer or we were not sure if it went into the correct well. This could help to account for the inaccuracies found within our gel.

Conclusion/Discussion:
The objectives of the lab were achieved since a multi-compenent solution was created, an SDS-PAGE gel was created and run, detection limits were seen in an SDS-PAGE gel, and the molecular weights of protein samples were estimated.

Due to issues with our SDS-PAGE gel, my hypothesis was not found to be correct. The two IgG lanes produced different patterns of bands that mostly aligned with what I expected. Since the DTT separates the heavy and light domains of the protein, the IgG +DTT will have two lines and the IgG -DTT will only have one line. However, this one line should be located in the middle of the two lines but it was not in my gel. Additionally, many of the proteins seemed to have similar molecular weights even though they technically do not. These issues most likely stem from issues loading the gel into the solution. Although we attempted to load all of the sample into the solution, sometimes the sample fell into the buffer or we were not sure if it went into the correct well. This could help to account for the inaccuracies found within our gel.

If I were to repeat this lab, I would make sure to properly load the solutions into the gel so that they can be more easily seen in the final gel. Additionally, I would make extra of each solution so that if an issue arises where the solution falls into the buffer, I would still be able to attempt putting that solution in the gel again.

Bacterial Transformation

Date: 10/8/25

Pre-Lab Questions:

1. Explain why you will be heat shocking cells this week.

Heat shocking the cells is necessary because the DNA/plasmid enters the cell easier when heat shocking occurs. This is because heat shocking makes the membrane of the cells more permeable.

2. What are the purposes of araC and PBAD specifically in the pGLO plasmid?

The pGLO plasmid utilizes AraC to interact with the GFP gene promoter to cause the GFP gene to be expressed depending on how high or low the arabinose levels of the surroundings are. If there is arabinose, an allosteric change occurs in Arac causing the GFP gene to be transcribed and expressed, UV light will then make the bacteria glow green. PBAD is the promoter that the AraC gene binds to. The PBAD promoter helps with GFP gene expression.

3. What are the purposes of the antibiotic ampicillin and bla gene in the pGLO plasmid?

The bla gene in the pGLO plasmid is resistant to antibiotics, including ampicillin. This means that ampicillin will not kill it. During the lab we are going to attempt to incorporate the bla gene, which allows the formation of beta lactamase enzymes, into some plasmids. If the bla (which is always expressed) is incorporated into the plasmid, it will not die in the ampicillin and we will be able to tell which plasmids contain the gene and have been transformed with the pGLO plasmids. You can grow the cells via plasmid amplification in ampicillin only if they have the bla gene.

4. On which agar plate(s) do you expect to see both cell growth and green fluorescence? Explain why.

I expect to see both cell growth and green fluorescence only on agar plate 2. This is because it has pGLO and arabinose in it. The pGLO will make the cells glow in the dark. The arabinose enables the transcription of the GFP gene and will therefore allow the cells to grow in ampicillin and glow at the same time. The other plates do not contain both and will therefore not see both cell growth and green fluorescence.

Objectives:

- Perform Sterile/Aseptic Technique
- Understand a Bacterial Plasmid's Functional Components
- Perform a Bacterial Transformation
- Know the Difference Between Genetic Selections and Screens

Hypothesis:

I expect to see both cell growth and green fluorescence only on agar plate 2. This is because it has pGLO and arabinose in it. The pGLO will make the cells glow in the dark. The arabinose enables the transcription of the GFP gene and will therefore allow the cells to grow in ampicillin and glow at the same time. The other plates do not contain both and will therefore not see both cell growth and green fluorescence.

Methods:

Exercise 1 - Transformation

Materials:

- Two 12 µl aliquots E. coli DH5α (CaCl2 competent)
- pGLO plasmid (50 ng/µl)
- Ice and ice bucket
- 42°C heat block
- P10 pipette tips (sterile)
- P200 or P1000 pipette tips (sterile)
- 1 ml aliquot of LB liquid broth (sterile)
- 1 ml aliquot of 1% Arabinose (sterile)
- 3 LB/ampicillin 60-mm plates (sterile)
- 1 LB only 60-mm plate (sterile)
- Inoculation Beads (sterile)

Part 1: Transformation of E. coli with pGLO

Procedure:

1. The instructor or TA should have gently thawed the E. coli DH5α competent cells on ice at the start of the lab. Retrieve two of these tubes (each contains 12 µl cells).
2. Gently re-suspend the cells by "flicking" the tubes with your finger.
3. Label both tubes with your group #; label one tube "+" (this receives pGLO plasmid) and the other tube "-" (this tube is a negative control and will not receive plasmid).
4. Add at least 1 µl pGLO plasmid (max 5 µl) to the tube marked "+" and gently mix.
5. Incubate the tubes on ice for 20 minutes. You should do Part 2 (preparing agar plates) during this incubation.
6. To heat shock the cells, place the tubes in the 42°C heating block for exactly 90 seconds.
7. Remove the tubes from the heat block; place the tubes on ice for 2 minutes.
8. Aseptically add 200 µl LB broth to each tube.
9. Place the tubes in the microfuge tube rack that your instructor has set out. Once all students have loaded their tubes, the instructor will move the rack to the 37°C incubator in 3W12 and incubate for 1 hour to allow the cells to recover.

Part 2: Preparing Agar Plates

1. While the tubes are incubating on ice, prepare the agar plates. Retrieve 3 LB/ampicillin agar plates and 1 LB only agar plate. Do not get these plates confused; they will look similar to each other.
2. Label the bottoms of the plates (not the lids!) by writing along the rim of the plate bottom. Label with your initials, group number, section number, and as follows:

Plate #1: + pGLO, – Ara (LB/ampicillin)
Plate #2: + pGLO, + Ara (LB/ampicillin)
Plate #3: – pGLO, + Ara (LB/ampicillin)
Plate #4: – pGLO, + Ara (LB only)

3. Aseptically add 300 µl of the 1% arabinose to plates 2, 3, and 4.
4. Spread the arabinose evenly around each plate using a sterile inoculation loop.
5. Let the plates sit and absorb the arabinose until you are ready for Part 3.

Proceed to Exercise 2 and return to finish Part 3 of this exercise when your transformed cells have finished their 1 hour recovery.

Part 3: Inoculate Agar Plates with Transformed Bacteria

1. Prepare 4 new inoculation beads, one for each plate. While still in their sterile packaging, bend the last ~1 cm of each loop so they resemble tiny hockey sticks (Figure 4). Do not remove them from their packaging until you are ready to inoculate the plates.
2. Any excess arabinose solution still on the agar plates must be removed. Tip the plates at ~30° and remove the excess solution with a pipette (use sterile tips). Lay each plate flat.
3. Lift the cover of Plate 1, add 100 µl of the "+" transformation onto the agar, spread the bacteria evenly over the entire surface of the agar using a inoculation beads, close the lid of the plate immediately after spreading the bacteria, and dispose of the loop in biohazard waste. You might be provided with inoculation beads instead

of inoculation loops; follow your instructor's demonstration on how to use plating beads to inoculate your plates.
4. Repeat the previous step for Plate 2, which also gets 100 µl of the “+” transformation.
5. Repeat the previous steps for plates 3 and 4, each getting 100 µl of the bacterial labeled "-" transformation this time.
6. Do not disturb the plates. Let them sit for 1-5 minutes, then invert them (with the agarside up); you should be able to see the labels on the bottom of each plate facing up towards you.
7. Dispose of the tubes containing the remaining bacteria in biohazardous waste.
8. Stack the four plates, secure the stack using tape, and place them in the designated location for incubation at 37°C overnight. Double check that the plates are inverted (agar side up). Do not tape the lids onto individual plates; use the tape just to keep the 4 plates together so they will be easier to find next time.
9. After incubation, your instructor or TA will move your plates to 4°C

Exercise 2 - Protein Extraction

Part 1: Preparing Cell Lysates

Materials:

- Prepare culture: pGLO/DH5α + Arabinose
- Prepare culture: pGLO/DH5α – Arabinose
- B-PER cell lysis reagent (Thermofisher)
- Two extra-large (2 ml) microfuge tubes
- Scale (balance)
- Microfuge tube rotator

Procedure:
1. Label 2 extra-large (2 ml) microfuge tubes with your group number and the following:
a. pGLO + Ara
b. pGLO – Ara
2. Weigh each empty tube. Write the mass on the side of the tube.
3. Add 2 ml of the appropriate cell culture to each tube.
4. To pellet the cells, centrifuge for 3 minutes at max speed in the large centrifuge (the centrifuge where you can set the speed and time). Do not use the small, single-speed centrifuges at your bench to pellet cells; they are not powerful enough.
5. Using a pipette, remove as much of the supernatant (liquid) as possible, but leave the cell pellet. Pipette off the supernatant into a waste container.
6. Weigh each tube again and determine the weight of the cell pellet (current weight – previous weight = pellet weight in grams).
Pellet Weights:
+ .034 g
- .042 g
7. Determine the amount of B-PER needed (3 µl B-PER per mg of cell pellet) and add this to each tube. If your pellet weighs less than 10 mg, just add 30 µl B-PER; 30 µl is the minimum volume of B-PER that should be added.
8. Set your pipette to a volume smaller than the amount of B-PER you just added. Resuspend each pellet by gently pipetting between the top and first stop. Do not stop pipetting until you no longer see the pellet. PIPETTE CAREFULLY TO AVOID INTRODUCING BUBBLES INTO YOUR SAMPLE. Use a different pipette tip for each pellet.
9. Incubate for 30 minutes at room temperature in a microfuge tube rotator.
10.Centrifuge for 5 minutes at max speed in the large centrifuge.

11.Label two new standard sized (1.5 ml) microfuge tubes with your initials, group number, section number, and the following:
a. pGLO + Ara Lysate
b. pGLO – Ara Lysate
12.Transfer the supernatant (the cell lysate) to the corresponding new tubes. SAVE THE NEW TUBES! Throw away the old tubes (these pellets contain only cellular debris).

Part 2: Protein Quantification and Dilution

Materials:

- Cell lysates from the previous part
- dH2O
- 2 mg/ml BSA (do not discard this tube)
- Bradford reagent
- 96-well microplate
- Microplate reader
- Computer with Excel or graphing program

Procedure:

1. Make a standard curve using BSA. Dilute your lysates as you diluted your Lab 2 unknowns. Remember that you need to prepare dilutions for each lysate (50%, 20%, 10%), but avoid using too much of your lysates – make your dilutions in as small a volume as possible that's still reasonable to pipette (e.g., for the 50% dilution, use 3 µl dH2O and 3 µl lysate). See Lab 2 for more information about the Bradford assay.

	1	2	3	4	5	6	7	8	9	10	11	12
A			.5 pGLO/DH5α + Arabinose		.5 pGLO/DH5α - Arabinose		1 mg/ml BSA					
B			.2 pGLO/DH5α + Arabinose		.2 pGLO/DH5α - Arabinose		.5 mg/ml BSA					
C			.1 pGLO/DH5α + Arabinose		.1 pGLO/DH5α - Arabinose		.25 mg/ml BSA					
D							.125 mg/ml BSA					
E												
F												
G												
H												

2. SAVE THE LYSATES YOU JUST MADE! Make sure they are labeled with your initials, group number, and section number, and then place them in the storage box for your section.

Results:

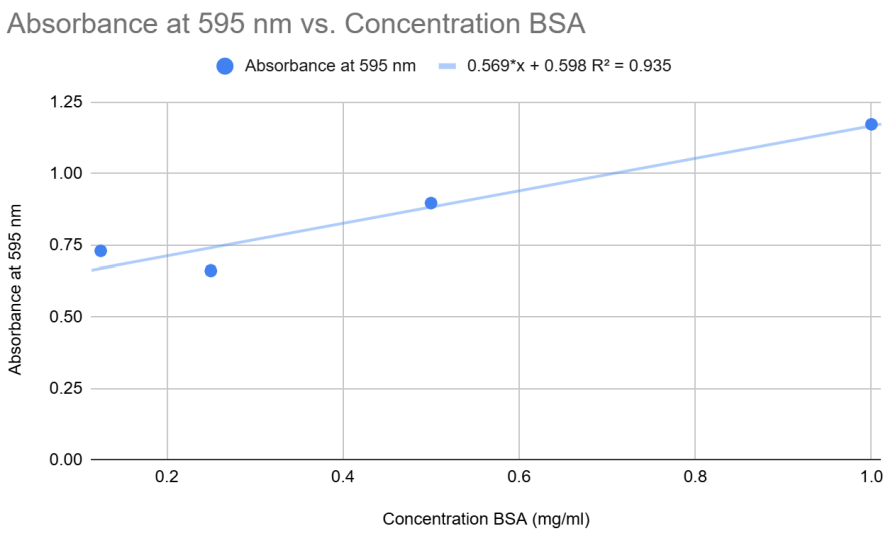


Figure 1: BSA Standard Curve

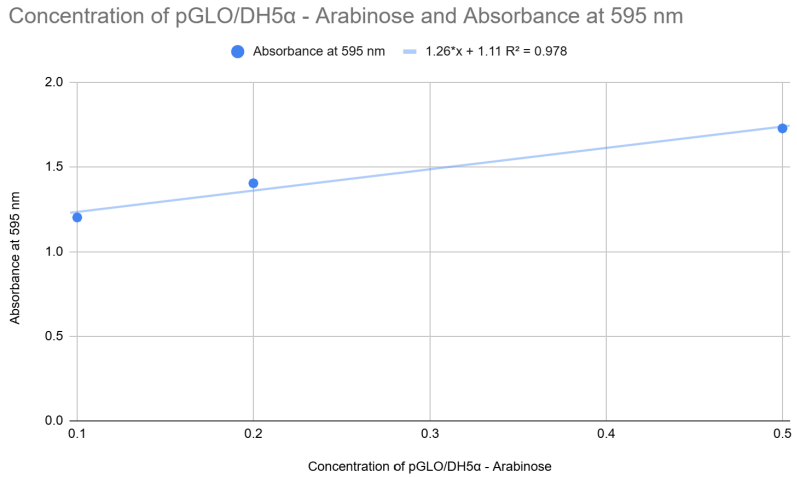


Figure 2: Concentration of pGLO/DH5α - Arabinose Versus Absorbance at 595 nm

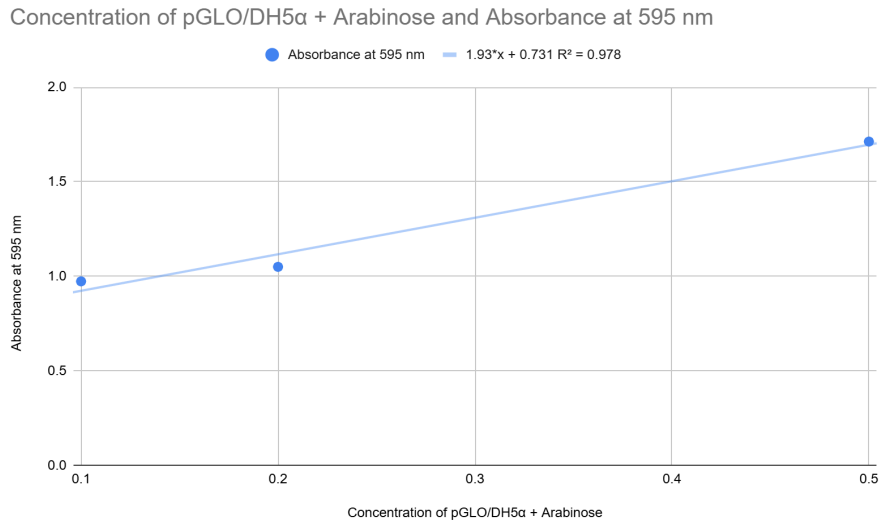
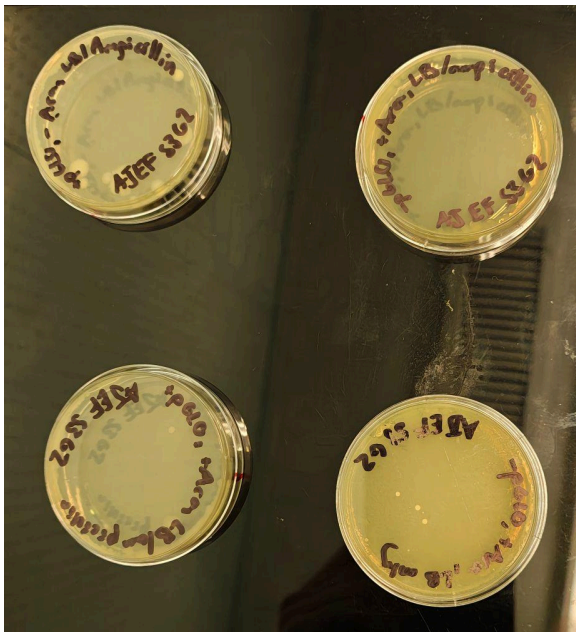


Figure 3: Concentration of pGLO/DH5α + Arabinose Versus Absorbance at 595 nm



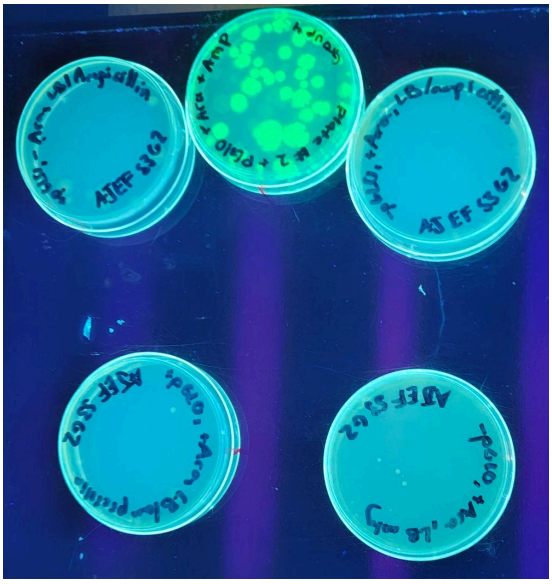


Figure 4: Samples without UV Illumination
Upper Left: + pGLO, – Ara (LB/ampicillin)

Upper Right: - pGLO, + Ara (LB/ampicillin)
Lower Left: + pGLO, + Ara (LB/ampicillin)
Lower Right: – pGLO, + Ara (LB only)

Figure 5: Samples with UV Illumination
Upper Left: + pGLO, – Ara (LB/ampicillin)
Upper Center: + pGLO, + Ara (LB/ampicillin) Example
Upper Right: - pGLO, + Ara (LB/ampicillin)
Lower Left: + pGLO, + Ara (LB/ampicillin)
Lower Right: – pGLO, + Ara (LB only)

Lab Questions:

Assignment 1:

A. View each plate under UV illumination and take pictures; make figures out of each picture. Describe and explain any differences between the plates. Are there any signs of contamination on the plates? You can tell by the appearance of colonies that are differently colored or look fuzzy (likely mold).

See figures 1 and 2 above.

Our plates all appeared to have low growth and no glowing. However, the example plate with + pGLO, + Ara (LB/ampicillin) had significant growth and glowing. There doesn't appear to be any contamination.

B. On which plate(s) did bacteria grow? Does the growth look like individual colonies, or Does it look more like a bacterial lawn? On which plate(s) did the bacteria glow green?

The bacteria only seem to have grown on the example plate with + pGLO, + Ara (LB/ampicillin). The growth appears to have many different colonies. Glowing was only seen on the example plate. We should have seen growth on the +pGLO plates and -ampicillin plate and glowing only on the +pGLO +Ara plate.

C. Count the number of colonies on the plates where colonies grew. If there are a lot, divide the plate into 2, 4, or 6 equal sections (based on how crowded the colonies are) and count the colonies in one section. If you divided the plate, multiply by 2, 4, or 6 to get an estimation of the total number of colonies on the plate.

There appear to be 6 colonies on the edge of the + pGLO, – Ara (LB/ampicillin) plate, 0 on the - pGLO, + Ara (LB/ampicillin) plate, 2 on the + pGLO, + Ara (LB/ampicillin) plate, and 3 on the – pGLO, + Ara (LB only) plate. Additionally, the example + pGLO, + Ara (LB/ampicillin) plate appears to have approximately 30 colonies.

D. Determine the transformation efficiency: Transformation efficiency is expressed as the number of transformants (transformed bacteria) per microgram of plasmid DNA. This can be calculated by counting the number of colonies on the LB/ampicillin + arabinose plate, but it is possible for a bacterial colony to acquire ampicillin resistance independently of the plasmid. Therefore, only those colonies that glow green should be counted on this plate as these are the true transformants that acquired the pGLO plasmid. Next, calculate how many total micrograms (µg) of plasmid you used for the transformation. Divide the number of green colonies on the plate by the amount of plasmid you added to the transformation tube (in micrograms). This is the transformation efficiency if the entire transformation mixture had been put onto the agar plate. But we only used 100 µl out of 500 µl total volume of the transformation. Therefore, the calculation above should be multiplied by the dilution factor of 5 to obtain the actual transformation efficiency.

For the example plate:

30 colonies

266.1 µg plasmid (concentration 2.661 µg/µl times 100 µl used)

(30/266.1)(5) = **.564 cfu/µg**

Assignment 2:

A. In Lab 6, for each lysate, you will be loading 20 µg of protein into 2 wells on an SDSPAGE gel. The volume you will be pipetting into these wells will be 20 µl, but remember that your lysates must be combined with equal amounts of SLB (sample loading buffer) beforehand, so to get 20 µg of protein into each well, your lysates must start out at with a concentration of 2 µg/µl protein. For each lysate, calculate the volumes of lysate and dH2O needed to make 25 µl of lysate at 2 µg/µl protein (use C1V1 = C2V2). You will use these calculations in the next lab; they must be completed before the lab starts.

Why 25 µl? You will be combining this with an equal amount of SLB, making a total of 50 µl (now at 1 µg/µl protein). Since you'll need to pipet 20 µl of this sample into 2 wells, you'll need at least 40 µl, leaving 10 µl extra left over.

18.8 µl lysate and 6.2 µl dH2O are needed for the pGLO/DH5α + Arabinose lysate and 21.1 µl lysate and 3.9 µl dH2O for the pGLO/DH5α - Arabinose lysate.

pGLO/DH5α + Arabinose lysate concentration: 2.661 µg/µl (From the equation of the absorbance versus concentration graph: 1.93(1)+.731)

(25(2))/2.661 = 18.8 µl Lysate 25-18.8 = 16.2 µl dH2O

Perform Same Calculations but with - Arabinose Graph (Concentration 2.37 µg/µl)

B. Explain which steps of today's protocol would significantly impact the amount of protein in your lysates. If your lysates are less than 2 µg/µl or the total amount of protein extracted was less than 50 µg, indicate this and hypothesize as to what you can do differently in the future to increase your protein yield.

The step where the supernant is removed would significantly impact the amount of protein in the lysates. If some of the pellet was accidentially removed, the cell lysate would have a decreased amount of protein. Additionally, pipetting the different amounts of substances (pGLO and LB broth) into the tube could significantly impact protein amounts if the pipetting was done incorrectly or if the pipette was pushed to the wrong stops.

Conclusion/Discussion:

The purpose of the lab was achieved because my partner and I practiced aseptic technique, know the functional components of a bacterial plasmid, performed a bacterial transformation, and understand the differences between genetic selections and screenings.

Our pGLO results were not as hypothesized. We should have seen growth on the +pGLO plates and -ampicillin plate and glowing only on the +pGLO +Ara plate. However, we saw minimal growth on all of the plates and no glowing on any of our plates. If I were to repeat this experiment, I would make sure that the pGLO and arabinose were added correctly to the plates.

Western Blot Part I

Date: 10/22/25

Pre-Lab Questions:

1. For each lysate, what volumes of lysate and dH2O are needed to make 25 µl of lysate at 2 µg/µl? (This is the same question as last week's notebook assignment.) Show your calculations.

18.8 µl lysate and 6.2 µl dH2O are needed for the pGLO/DH5α + Arabinose lysate and 21.1 µl lysate and 3.9 µl dH2O for the pGLO/DH5α - Arabinose lysate.

pGLO/DH5α + Arabinose lysate concentration: 2.661 µg/µl (From the equation of the absorbance versus concentration graph: 1.93(1)+.731)

(25(2))/2.661 = 18.8 µl Lysate 25-18.8 = 16.2 µl dH2O

Perform Same Calculations but with - Arabinose Graph (Concentration 2.37 µg/µl)

2. What is the total amount of protein you will be pipetting into each well of your gel this week?

We will be pipetting 20 µl of protein into wells 2-4 and 8-10 and 8 µl into wells 1 and 7. The proteins get pipetted twice each so that one side will get Coomassie Blue and the other proteins will be put onto nitrocellulose.

3. Compared to Lab 4, why do you think we are increasing the percentage of acrylamide for this SDS-PAGE gel?

We are increasing the percentage of acrylamide from 5% and 8% in lab 4 to 10% in lab 5 because GFP is a 27 kDa protein, meaning it is smaller and needs more acrylamide. A greater percentage of acrylamide makes the pores in the gel smaller and therefore better for our smaller protein.

4. Instead of transferring proteins onto a membrane for Western blot detection, why can't we just apply the antibodies directly to an SDS-PAGE gel?

We can not just apply the antibodies directly to an SDS-PAGE gel because the antibodies are unable to get into the gel because of their size. We need to move the proteins to a membrane where the antibodies will be able to reach them in order to bind and allow the protein to be seen.

Objectives:

- Prepare cell lysates for SDS-PAGE
- Transfer proteins from an SDS-PAGE gel to a nitrocellulose membrane for Western Blot
- Understand the purpose of a Western Blot

Hypothesis:

I predict that the + and - Arabinose lysates will have similar masses when viewing the results of the gel electrophoresis. I also think they will weigh more than GFP. Additionally, I think that the + Arabinose lysate will be slightly heavier than the - Arabinose lysate when viewing the Western Blot results.

Methods:

Materials:

- Pipets and tips
- Microcentrifuge
- Microfuge tubes
- Kimwipes
- Gel assembly apparatus
- Long and short glass plates
- Comb
- dH2O
- 1.5 M Tris pH 8.8
- 1.0 M Tris pH 6.8

- 40% Acrylamide
- 10% SDS
- 10% APS
- TEMED
- Gel wedge
- 95°C heating block
- Cell lysates from previous week
- Purified GFP (25 ng/µl)
- Kaleidoscope MW Marker
- Microfuge tube at least 150 µl SLB+
- 1X SDS-PAGE running buffer
- SDS-PAGE gel apparatus
- Power supply
- Bio-Rad SD Trans blot apparatus
- Large square (Ziploc®) container
- Nitrocellulose membrane
- Thick filter pads
- Transfer buffer
- Gel Code Blue
- Clear staining box
- Western blot box (black)
- 1X PBST (Phosphate-Buffered Saline, 0.1% Tween 20, pH 7.5)

Part 1: Pour a 10% acrylamide gel

Procedure:

1.Use the measurements from Lab 4 except adjust the amount of acrylamide and water accordingly for the resolving gel only. Write out the recipe.

Resolving Gel

Concentrated Stock	Final Concentration	Volume
1.5 M Tris pH 8.8	195 mM Tris pH 8.8	0.65 ml
40% Acrylamide	10% Acrylamide	1.25 ml
10% SDS	0.1% SDS	0.05 ml
10% APS	0.12% APS	0.06 ml
dH_2O (Q.S. to 5 ml)		2.99 ml

2. Pour the 10% resolving gel. Prepare and pour the stacking gel the same as in Lab 4.

Part 2: Prepare samples for loading the gel. You will need to load each sample onto the gel twice. This will allow us to cut the gel in half; one half will be used for Coomassie Blue staining (Part 3), the other half will be used in Part 4 – transfer to nitrocellulose membrane. Dilute and prepare your samples in SLB+ appropriately (refer at Lab 4), including the heating step. Write out the procedure in your lab notebook. Load your gel according to Table 1 shown below. Be sure to use only 8 µl of MW marker in wells 1 &2.

1) Put 20 µl SBL+ in the GFP, pGLO/DH5α - Arabinose, and pGLO/DH5α + Arabinose solutions.
2) Briefly run the tubes in the centrifuge.
3) Put all of the protein samples on the heater set to 95°C and put the mass on top of them. Wait 5 minutes.
4) Wait for the samples to cool down and spin them briefly.
5) Micropipette the correct volume of each solution into the gel according to the table below.

Table 1: Gel Loading Scheme

Well #	Volume	Contents
1	**8 µl**	Kaleidoscope MW Marker
2	20 µl	GFP
3	20 µl	pGLO/DH5α - Arabinose
4	20 µl	pGLO/DH5α + Arabinose
5		Empty
6		Empty
7	**8 µl**	Kaleidoscope MW Marker
8	20 µl	GFP
9	20 µl	pGLO/DH5α - Arabinose
10	20 µl	pGLO/DH5α + Arabinose

Part 3: Run your gel.

1. Run the gel as before (Lab 4). Lower voltage will make the gel look better but will take longer. DO NOT EXCEED 300V!
2. Stop electrophoresis when the dye front is close to the bottom of the gel; turn off the power supply, disassemble the apparatus, and pour the used running buffer back into the carboy.
3. Using a gel separator (the green wedge), carefully separate the glass plates.
4. With the gel flat on a glass plate, use the gel separator wedge to cut the gel in half, bisecting it along lanes 5 and 6 (Figure 2).
5. Stain the left half of the gel with Coomassie stain (Gel Code Blue) as in Lab 4; don't forget to wash the gel with dH2O before adding the stain.
6. Wash out the electrophoresis apparatus with water in the sink and set out to dry.

Part 4: Use right half of gel for transfer to nitrocellulose membrane.

Read the steps below about how to assemble the Trans-blotter "sandwich". Also see the BioRad manual for the Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell. When you are ready to proceed, check in with your instructor.

1. Place the gel half in a large square (Ziploc®) container and rinse the gel with dH2O.
2. Equilibrate the gel by immersing it in transfer buffer for 5 minutes with shaking. This removes SDS which can interfere with the transfer.
3. Submerge a nitrocellulose membrane and 2 thick filter pads in the transfer buffer container with the gel and equilibrate for at least 1 minute (do not shake).
4. Place a thick equilibrated filter pad onto the platinum anode (positive electrode) surface of the apparatus. It helps to add some transfer buffer on top of each part of the stack before adding the next part.

5. Roll a conical tube over the filters to remove air bubbles.
6. Place the equilibrated nitrocellulose membrane on the filter pad(s) and roll out bubbles.
7. Place the equilibrated gel onto the nitrocellulose membrane and roll out bubbles.
8. Place the other thick equilibrated filter pad onto the gel and roll out bubbles. Remove excess transfer buffer from the anode surface.
9. Carefully place the cathode (negative electrode) on the stack.
10.Place the protective cover on the transfer apparatus and connect the leads to the power supply.
11.Turn on the power supply; set the voltage to 20 V and transfer for 7 minutes.
12.Turn off the power supply and disconnect the leads from the transfer apparatus.

Part 5: Trim and store the nitrocellulose membrane.

1. Remove the protective cover from the transfer apparatus and carefully remove the cathode.
2. Open the stack and retrieve the nitrocellulose membrane. You can quickly check that the transfer has occurred by the presence of the Kaleidoscope MW marker bands on the nitrocellulose membrane.
3. If needed, using scissors to trim the membrane so it will fit flat in the back Western blot staining box. Do not let the membrane dry out!
4. Place the membrane face up in the black Western blot staining box. Add enough PBST to keep the membrane submerged and hydrated.
5. Using tape, label the Western blot staining box with your section number, group number, and initials. Place the box in the designated bin or basket for your section and store at 4°C until the next lab session.
6. Clean up: the rest of the stack (filter pads and gel) can be discarded in biohazard waste; wipe off the remaining transfer buffer from the cathode and anode plates of the transfer apparatus and wipe them down with a paper towel soaked in dH2O.

The membranes will be stored in 1X PBST at 4°C until next week. Next week before class, the instructor will remove the 1X PBST and replace it with 1X PBST + 5% milk and incubate it for >30 minutes with shaking at room temperature. The milk will help to prevent the antibodies used next week from sticking to the nitrocellulose membrane non-specifically. This is known as blocking

Results:

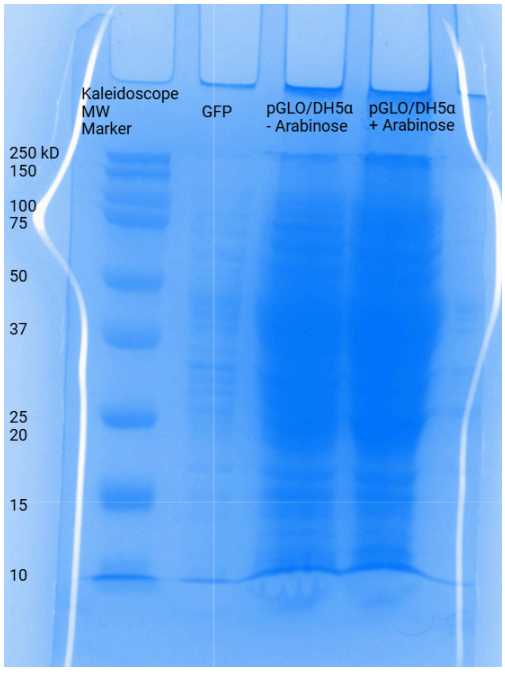


Figure 1: Labeled Coomassie-Stained SDS-PAGE Gel

Lab Questions:

Assignment: (next week)

A. Take a picture of the gel on the GelDoc; include a labeled image of the gel in your notebook. Each lane should have its contents indicated.

See Figure Above

B. Label each band of the MW marker and estimate the weight of GFP.

The weight of GFP appears to be about 32 kDa.

Conclusion/Discussion:

The purpose of the lab was achieved since my partner and I learned how to prepare cell lysates for SDS-PAGE, transferred proteins to a nitrocellulose membrane, and learned the purpose of a Western Blot.

Our gel was successful and our estimated weight of GFP is approximately 32 kDa, which is close to its actual weight of 27 kDa. My hypothesis was partially correct in that pGLO/DH5α - arabinose and pGLO/DH5α + arabinose have similar weights but was incorrect in believing that pGLO/DH5α + arabinose would weigh more than pGLO/DH5α - arabinose. It is impossible to determine the differences in the GFP and pGLO/DH5α - arabinose and pGLO/DH5α + arabinose weights.

Western Blot Part II

Date: 10/29/25

Pre-Lab Questions:

1. What is the purpose of adding milk to the membrane?

Adding milk to the membrane allows the many proteins found in milk to fill up the membrane's free spots and keep the antibodies from nonspecifically binding everywhere in the membrane. This needs to happen because otherwise the antibodies will bind wherever they can on the membrane and we won't be able to analyze the membrane.

2. What is the purpose of the primary antibodies and the secondary antibodies?

Using primary and secondary antibodies is less expensive and easier. Additionally, multiple secondary antibodies are able to bind on the primary antibody and amplify the signal given off. This makes it easier for us to observe the reaction. The secondary antibody we use in this reaction is able to be seen by a color change.

3. Which part of an antibody binds to antigens? What are the antigens of the primary antibody and the secondary antibody?

The ends of the "Y" of the antibody (binding sites, Fab) bind to the epitope of antigens. The primary antibody's antigen is GFP and the secondary antibody's antigen is mouse IgG.

4. For your membrane, in which lane(s) do you expect to see band(s)? Explain why.

I expect to see a band in the GFP and pGLO/DH5α + Arabinose lanes because the primary antibody we are using binds with GFP and the secondary antibody binds to the primary antibody to allow us to see the bands. GFP should be found in both the GFP and pGLO/DH5α + Arabinose lanes.

Objectives:

- Perform a Western Blot
- Understand the use of each component of a Western Blot including the purpose of the primary and secondary antibodies
- Interpret Western Blot and SDS-PAGE data and compare and contrast the lanes between the same samples with the two different techniques

Hypothesis:

I expect to see a band in the GFP and pGLO/DH5α + Arabinose lanes because the primary antibody we are using binds with GFP and the secondary antibody binds to the primary antibody to allow us to see the bands. GFP should be found in both the GFP and pGLO/DH5α + Arabinose lanes.

Methods:

Materials:

- Membranes from Lab 6
- PBST (PBS + 0.1% Tween-20)
- PBST + 5% (w/v) milk (50 mg/ml)
- 1° antibody, mouse anti-GFP (1:1500)
- 2° antibody, goat anti-mouse (1:1000)
- 4-Chloro-1-naphthol (3 mg/ml in methanol)
- 3% H_2O_2
- TBS, pH 7.5

Part 1: Probe the Nitrocellulose Membrane

1. Pour off the PBST + 5% milk blocking solution. This can go down the drain.
2. Add 2.5 ml of 1° antibody (anti-GFP) solution.

3. Incubate for 30 minutes with gentle shaking. Proceed to Part 2 during this incubation.
4. Pour off the 1° antibody into the 1° antibody collection container if provided.
5. Wash 3 times for 5 minutes each with 2.5 ml of PBST with gentle shaking. PBST washes can go down the drain.
6. Pour off the last wash and add 2.5 ml of 2° antibody (anti-mouse IgG).
7. Incubate for 30 minutes with gentle shaking.
8. Pour off the 2° antibody into the 2° antibody collection container if provided.
9. Wash 3 times for 5 minutes each with 2.5 ml of PBST with gentle shaking

Part 2: Examine the Coomassie-Stained Gel

1. Retrieve your Coomassie-stained SDS-PAGE gel from the previous lab.
2. Take pictures on the GelDoc, then dispose of the gel in biohazard waste

Part 3: Antibody Detection

1. Prepare detection reagent IMMEDIATELY BEFORE DETECTION: add 500 µl of 4-CN solution to 2.5 ml of TBS; then add 27 µl 3% hydrogen peroxide (H_2O_2), mix by inverting tube gently, don't make bubble.
2. Pour off the last PBST wash from your membrane.
3. Add the detection reagent to the membrane.
4. Incubate for up to 20 minutes (bands should appear between 5-10 minutes). DO NOT SHAKE! The membrane should be kept in its staining box and kept still on the benchtop.
5. Once prominent bands appear, take a picture of the membrane using your phone. If available, take a picture on the GelDoc and use this image for your notebook.
6. You can dispose of the membrane in biohazard waste after taking pictures.

Results:

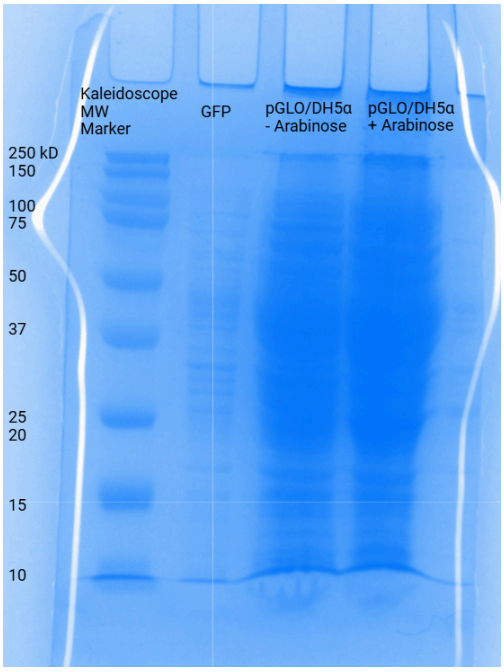


Figure 1: Labeled Coomassie-Stained SDS-PAGE Gel

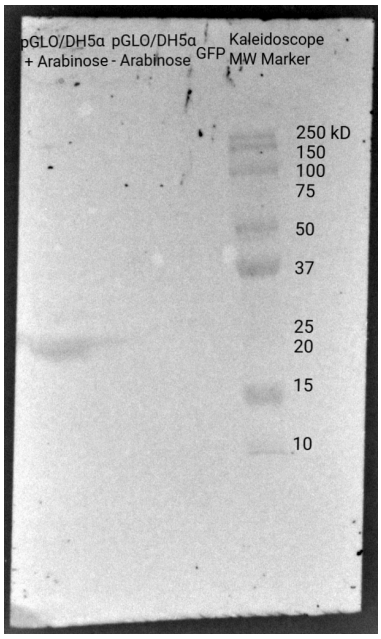


Figure 2: Labeled Western Blot

Lab Questions:

A. Prepare a labeled figure of the Western Blot as you did with the Coomassie-stained SDSPAGE gel from Lab 5. Label each band in the molecular marker lane and each lane within both the gel and membrane.

See Figures 1-2 Above

B. Discuss the differences in what can be observed for the SDS-PAGE and Western Blots. Include a discussion about the different (and similar) types of information each experiment provides.

SDS-PAGE allows us to observe the molecular weights of different proteins. Western Blots allow us to determine if proteins contain a specific antigen related to the specific antibody we use. Although both tests tell us the weight of proteins, a Western Blot tells us the weight only of the protein that the antibody binds to. In the experiment, we were able to see the molecular weights of all of the proteins in the pGLO/DH5α - arabinose and pGLO/DH5α + arabinose solutions as well as in the GFP when we did the SDS-PAGE. This SDS-PAGE yielded a gel with many bands in every lane of the gel (GFP should have had only one band). However, when we did the Western Blot we saw only one band in the lane for pGLO/DH5α + arabinose and no bands in the pGLO/DH5α - arabinose and GFP lanes (GFP should have had a band). This means that only pGLO/DH5α + arabinose had GFP in it because the antibodies bound to this and produced the band.

Conclusion/Discussion:

The purpose of the lab was achieved because my partner and I were able to perform a Western Blot and interpret Western Blot and SDS-PAGE results. Additionally, I now know the purpose of each component of a Western Blot.

My hypothesis was partially correct because the nitrocellulose membrane did yield a band in the pGLO/DH5α + arabinose lane. However, I did not see a band in the GFP lane like I should have. These results imply that there was GFP in the pGLO/DH5α + arabinose lane but none in the GFP lane. If I were to do this experiment again, I would make sure that the GFP was put into the gel correctly since the GFP lanes were incorrect in both the SDS-PAGE and Western Blot. GFP should have had one band in both but instead had many bands in the SDS-PAGE and no band in the Western Blot.

PCR and Motility Assay

Date: 11/5/25

Pre-Lab Questions:

1. What is Taq polymerase? What is a primer? What is a transposon?

Taq polymerase is a specific type of DNA polymerase (helps to grow the DNA by adding nucleotides) that is very heat stable. A primer is a DNA sequence found on the ends of the strand that tells the polymerase where to begin. A transposon is a DNA segment that changes location within DNA molecules. A transposon has a palindromic sequence (inverted repeats) on each side that permits it to be put into DNA.

2. Name the three steps of a PCR cycle and describe what happens during each step.

The three steps of PCR are denaturing, annealing, and extension. In denaturing, the DNA is heated to approximately 95℃ and hydrogen bonds are broken. During annealing, the DNA is cooled to approximately 55℃. At 55℃, Taq polymerase is not operating but primers are being added. Lastly, in extension, temperature is at 72℃ and Taq polymerase is growing the primers so that the DNA can eventually be complementary compared to the template strands.

3. How will a smaller PCR product appear on an agarose gel after electrophoresis compared to a larger PCR product? Explain why they appear differently.

A smaller PCR product will appear farther from its original location on the agarose gel compared to the largerPCR product. This is because smaller molecules have less mass and are able to migrate faster on the gel.

4. Do you expect motile bacteria or non-motile bacteria to have a larger PCR product? Explain why.

I expect motile bacteria to have a larger PCR product because they are able to move farther along the gel whereas non-motile bacteria will be stuck in one place.

Objectives:

- Perform a PCR
- Perform and interpret an agarose gel electrophoresis of PCR products
- Perform and interpret a microbiological motility assay plate
- Understand the link between genotype and phenotype

Hypothesis:

I expect the non-motile A53 E. coli to travel a shorter distance than the motile A24 E.coli since the increased motility will lead to increased movement. On the motility plate, this will mean that there is no longer a dot of A24 E.coli since it has moved. The A53 E.coli should have a dot in the same spot. On the agarose gel, the A24 E.coli should appear farther down the gel compared to the A53 E.coli.

Methods:

Step 1: Setup colony PCR

Materials:

- Nuclease-free H2O
- Two PCR tubes
- 10 µM Forward Primer (5'-TGTGCGGTAAGTGTCTGTTT-3') in tube labeled “Fwd”
- 10 µM Reverse Primer (5'-TCCCACCCAGAATAACCA-3') in tube labeled “Rev”
- 2X PCR Mix in tube labeled “PCR” (keep on ice!)

- Motile E. coli strain on plate labeled “A24”
- Non-motile E. coli strain on plate labeled “A53”

Procedure:

1. Put the following components into two PCR tubes in this order:

a. 6 µl nuclease-free H2O

b. 2 µl forward primer

c. 2 µl reverse primer

d. 10 µl 2X PCR mix

2. Label the sides of the PCR tubes with your group number. On one tube write “A24” and write “A53” on the other tube. Avoid labeling the tops of the tubes since this usually gets wiped off in the thermocycler.

3. Very lightly, touch a colony from the appropriate plate with a sterile pipette tip and swirl the tip in the corresponding PCR tube. You don’t need to see any cells on the tip, and do not try to pipette anything off the plate - we only want to transfer a few cells from each plate. Too many cells can inhibit the PCR.

4. Place your PCR tubes in the thermocycler. Avoid placing your tubes in the outer ring of positions or crowding them together with the tubes from other groups.

5. Wait for everyone in the class to place their tubes in the thermocycler. Your undergraduate mentor will prepare a no-DNA control PCR tube; wait for them to add this tube to the thermal cycler; they will then close the lid and start the PCR run.

6. If not already selected, choose the “BIOL2125” program and press run.

a. BIOL2125 Cycle:

1. 95°C, 5 minutes
2. 95°C, 30 seconds
3. 55°C, 30 seconds — 25 cycles (2-4)
4. 72°C, 1 minute
5. 72°C, 5 minutes

b. The program should take about 90 minutes to run. Get the gel ready (Part 2) and perform the motility assay (Part 3) during this time.

Part 2: Pour a 1% w/v agarose gel

Materials:

- 1X TAE buffer (Tris, acetic acid, EDTA)
- 50 ml tube containing 0.35 g agarose in 35 ml TAE buffer, labeled “1% Agarose”
- Horizontal gel electrophoresis apparatus
- Buffer dams or lab tape

Procedure:

1.One group of students will prepare the 1% agarose solution while the other group will set up the electrophoresis apparatus with gel casting tray:

a. Group A: Uncap the “1% Agarose” tube and microwave it until it starts to boil, usually between 1-2 minutes. Watch it carefully while it microwaves; be careful not to let the solution boil over the top of the tube. Recap the tube and gently swirl the agarose solution to verify that all the agarose has dissolved.

b. Group B: Setup up the electrophoresis apparatus with gel casting tray. Your instructor/TA will demonstrate the method you will use. If buffer dams are not available, use a casting tray clamp. If this isn’t available either, you can use the tape method: be sure to tape entirely around the casting tray to avoid leaks.

2. Insert the comb into the casting tray before pouring in your agarose solution. Pour enough agarose solution so it reaches halfway up the comb; 30 ml should be appropriate for your casting tray. Use a pipette tip to move any bubbles away from the wells before the gel sets.
3. Let the gel solidify; agarose will solidify around 40°C, so this will take some time, about 15 minutes. You can work on the motility assay (Part 3) while you wait.
4. Once the gel is set, remove the dams (or tape). Do not remove the gel from the casting tray; the tray with the gel still in it will be used directly in the electrophoresis apparatus.
5. Assemble the horizontal gel electrophoresis apparatus according to the instructor/TA's directions. Add enough 1X TAE buffer to cover your gel completely. The comb should be removed from the wells after the gel is submerged in 1X TAE

Part 3: Motility Assay

Materials:

- Motility plate (0.3% agar, 0.5% NaCl, 1% tryptone)
- Motile E. coli strain A24 broth culture in microfuge tube labeled "A24"
- Non-motile E. coli strain A53 broth culture in microfuge tube labeled "A53"
- Sterile tips for P-20 pipette

Procedure:

Note: Do not turn these plates upside down. The media is very soft and might drip down.
1. On the bottom of the plate, draw a line through the center and a dot on either side of the line about halfway towards the plate's edge. Label one dot "A24" and the other "A53" (Figure 3).
2. Along the edge of the plate's bottom, write your section number, group number, and initials.
3. Turn the plate over and open the lid. Carefully pipette 5 µl of each broth culture onto the agar using sterile pipette tips; aim for the corresponding dots you drew on the bottom of the plate for each strain.
4. Close the plate lid and let it sit without moving for at least 10 minutes.
5. Move the plates to the collection area or the room temperature incubator. Remember that the agar is very soft and could drip down so do NOT invert your plates!
6. Your instructor/TA will incubate your plate at room temperature for ~24 hours and then place it in the refrigerator until you can view it next week.

Part 4: Run PCR samples on the agarose gel

Materials:

- PCR tubes from Part 2
- No-DNA PCR control (if available)
- 6X GelRed prestain/loading-buffer in tube labeled "GelRed" (Biotium)
- 1 kb+ DNA Ladder with GelRed in tube labeled "1 kb+" (Thermo Scientific)
- Ziploc container with paper towels
- Bio-Rad Gel Doc imaging system and computer

Procedure:

1. Add 4 µl of prestain/loading-buffer (from the tube labeled "GelRed") to your PCR tubes and mix.
a. Remember, the prestain/loading-buffer contains GelRed, which is toxic, so handle it and anything else that come in contact with GelRed with care.
b. Note that GelRed stain will look BLUE to your eyes (it fluoresces orange/red) c. Return the "stain" tube to the TA/instructor.

2. Briefly spin down the PCR tubes in your small centrifuge using tube adaptors so the tiny PCR tubes fit properly. If adaptors are not available, a special centrifuge will be set up that accepts PCR tubes.
3. Load 20 µl from each PCR tube onto the gel according to the layout in Table 1. Make note if you are Group A or B so you'll remember where your samples were loaded.

We are Group A.

Table 1: Gel Loading Scheme

Well #	Contents
1	(empty)
2	20 µl PCR tube "A24" from Group A
3	20 µl PCR tube "A53" from Group A
4	6 µl 1 kb+ DNA ladder
5	20 µl PCR tube "A24" from Group B
6	20 µl PCR tube "A53" from Group B
7	5 µl no-DNA PCR control
8	(empty)

4. Add 6 µl of 1 kb+ DNA ladder to lane 4. The ladder already contains prestain/loadingbuffer, so you do not need to add anything to it before loading the ladder onto the gel. Do not through away this tube when you're done with it; return it to the TA/instructor.
5. The no-DNA PCR control should already have prestain/loading-buffer in it too.
6. Close the lid of the electrophoresis apparatus; make sure it closes all the way or the electrodes might not connect properly.
7. Plug the leads from the electrophoresis apparatus into the power supply; match the colors: black-to-black, red-to-red.
8. Turn on the main power switch of the powers supply, set the voltage to 175 V, and press the run button. Watch for bubbles being released from the electrodes in the electrophoresis apparatus.
9. Monitor the progress of the gel until the dye front has moved about 2/3 the way down the gel, then turn off the power supply. This should take about 12 minutes if you're running at 175 V.
10. Disassemble the electrophoresis apparatus. Be careful with this since the buffer now likely contains GelRed (toxic).
11. Place a few paper towels in a square Ziploc container and place the casting tray with the gel still in it on top of these towels. Do not place the gel directly on the towels! This step is to contain the buffer that would otherwise drip off of the casting tray (GelRed hazard).
12. If present, remove the white transilluminator plate from the GelDoc's UV light source. Slide the gel out from the casting tray onto the UV light source. Change your gloves before handling anything else (like the computer).
13. Image your gel using ethidium bromide settings; the program will alert you to flip a lever to change to the correct filter. Make sure to save the gel image.
14. Assess the image of your gel. If the bands have not sufficiently resolved, place your gel back in the casting tray, reassemble the electrophoresis apparatus, and run the gel for a bit longer, then take another picture. If

there are extraneous patches of stain on the gel, bring the gel over to the sink and rinse it a few times with dH2O and re-image.

15.Discard the gel in the biohazard waste container. Clean off the GelDoc's surface with 70% EtOH.

16.Clean up your gel electrophoresis apparatus: pour the used 1X TAE back into the bottle/carboy to be reused, rinse the gel apparatus (including the lid and tray) with water, and set it out to dry.

Results:

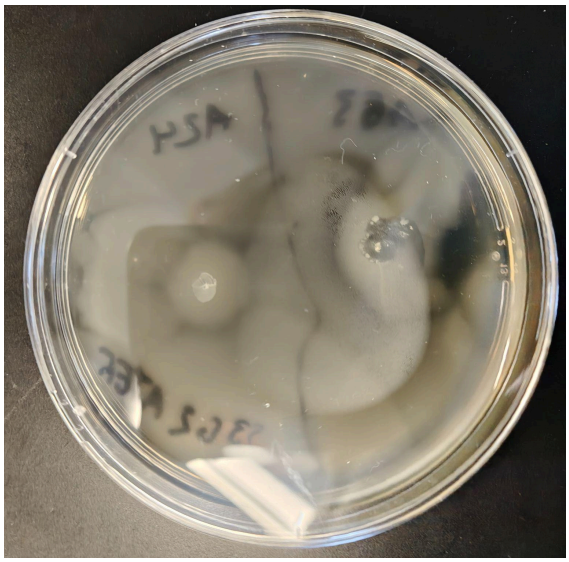


Figure 1: Motility Plate Without UV Light
Left: A24 E.coli (Motile)
Right: A53 E.coli (Non-Motile)

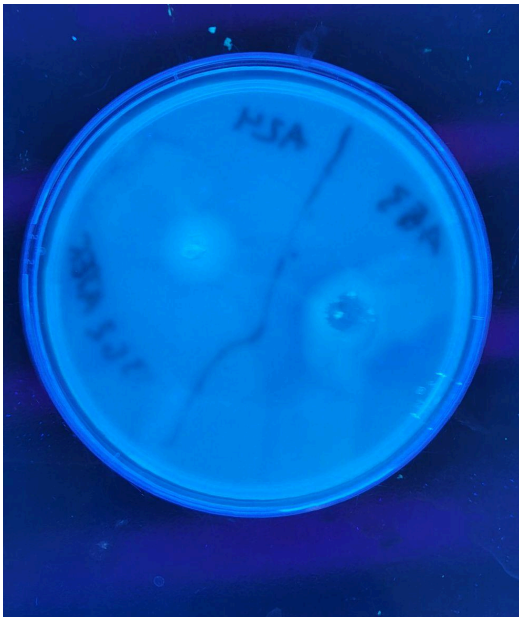


Figure 2: Motility Plate With UV Light
Left: A24 E.coli (Motile)
Right: A53 E.coli (Non-Motile)



Figure 3: Labeled Agarose Gel

Lab Questions:

Assignment 1:

A. Next week, inspect your motility plate and take a picture. Prepare a labeled figure and indicate which side of the plate contained the motile A24 strain and the non-motile A53 strain.

See Figures 1-2 Above

B. You might notice some differences/lobes for the non-motile strain; what do you think happened here? What are the lobes? Hint: it has something to do with the transposon.

The lobes could have appeared on the non-mobile strain due to the transposon found in the A24 strain migrating to the other side of the plate and infecting the A53 strain. This would cause some of the A53 strain to gain mobility and create lobes.

C. Make a hypothesis about what you expect to get if you perform another PCR using one of the lobes of the non-motile A53 strain. What would the PCR product look like on an agarose gel?
If I were to perform another PCR using the lobes of the non-motile A53 strain, I would expect the band to appear in between the band of the A24 and A53 strains from this lab. I think this because the lobe should contain slightly more motile A53 E.coli.

Assignment 2:
A. Prepare a labeled figure of the agarose gel. Label each lane and the size of each band in the ladder. Note that some of the ladder's bands are brighter than others (i.e., contains more DNA), this should help you identify bands more easily. Indicate the sizes of the PCR products.
See Figure 3 Above.
The PCR products for my group (Group A) appear to be 40,000 kb in size. The PCR products for group B appear to be 0 kb in size.
B. Do the sizes of the PCR products correspond to your hypothesis regarding which strain should have the larger product? Describe anything anomalous about the gel and propose explanations for these.
The sizes of the PCR products do not correspond to my hypothesis regarding which strain should have the larger product because both groups had both bands in the same spot for the motile and non-motile strands. This should not have occurred. There should have been a band farther down the gel for the A24 motile strain and a band higher up on the gel for the A53 non-motile strain. This could have occurred because my group put too many cells in our solutions, which would cause the solutions to be too heavy to move. The other group could have not put enough cells in so that the differences in motility were unobservable.

Conclusion/Discussion:
The purpose of the lab was achieved since my partner and I performed a PCR, created and interpreted an agarose gel of the PCR products, created and interpreted a motility assay plate, and learned the link between genotype and phenotype.

My hypothesis for the motility plate was correct since the non-motile A53 E.coli stayed by the dot whereas the motile A24 E.coli moved away from the dot where they were placed. However, my hypothesis for the gel electrophoresis was incorrect since the bands for the non-motile and motile strands were at the same size. This could have occurred due to our group putting too many cells in our solutions, which would cause the solutions to be too heavy to move. If I were to repeat this experiment, I would redo the gel electrophoresis with a solution with a smaller amount of cells included in order to view the differences in molecular size between the motile and non-motile strands.

Contrast Microscopy and Eukaryotes

Date: 11/12/25

Pre-Lab Questions:

1. What are the benefits of using phase-contrast instead of bright-field microscopy?

Phase-contrast microscopy differs from bright-field microscopy in that staining (which can kill cells) is not needed and images are shown in higher contrast and detail. This increased contrast is due to phase-contrast microscopes having condenser annuluses and phase plates.

2. What is the difference between the terms “protozoa” and “protist”?

Protozoa are a unicellular type of protist that seem to act in a way that is typically characteristic of animal cells. These characteristics are acting in a predator-prey way, motility, and metabolisms that are heterotrophic. Protist is a more general term than protozoa that means that an organism is not a fungus, plant, or animal but is still a eukaryote.

3. What are heterotrophs, autotrophs, and mixotrophs? Assign each protist in this lab to these categories.

Heterotrophs are usually motile cells and eat food via cilia sweeping it into their mouths or by engulfing food particles with pseudopods. Autotrophs are usually not motile and get food via photosynthesis. Mixotrophs are less common and are sometimes motile, they also use both heterotrophic and autotrophic processes to obtain food.

Heterotrophs	Autotrophs	Mixotrophs
Chaos carolinensis	*Spirogyra*	*Euglena*
Amoeba proteus	*Volvox globator*	*Chlamydomonas*
Stentor coeruleus		
Blepharisma		
Spirostomum		
Paramecium		

4. In Exercise 3 you are going to pit protists against each other in Protist Hunger Games! Pick protists to compete against each other. Make a table of their approximate sizes, feeding strategy and motility mechanism. Provide a rationale for your hypothesis. This information will be used in the assignment under Exercise 3.

	Size	Feeding Strategy	Motility Mechanism
Blepharisma	50 µm - 1 mm Long Ovular	Cilia Heterotroph Cannibalism at Times	Cilia Spinning
Spirogyra	10 - 150 µm Wide Multiple cm long	Autotroph Chloroplasts Photosynthesis	Filaments Roll Up Group Together Align

			Move Toward Blue Light

I believe that *Blepharisma* will beat *Spirogyra* in the Protist Hunger Games. I think this because *pirogyra* makes its food via photosynthesis and is therefore unlikely to be aggressive compared to *Blepharisma*, which is a heterotroph and needs to be more competitive for food (sometimes it is even competitive to the point of cannibalism).

Objectives:

- Understand the difference between bright-field and phase-contract microscopy
- Make simple wet mounts of specimens
- Make and document their observations in an informative manner
- Understand the diversity of eukaryotic cell types

Hypothesis:

I believe that *Blepharisma* will beat *Spirogyra* in the Protist Hunger Games. I think this because *Blepharisma* is more likely to be the larger protist. Additionally, *Spirogyra* makes its food via photosynthesis and is therefore unlikely to be aggressive compared to *Blepharisma*, which is a heterotroph and needs to be more competitive for food.

Methods:

Exercise 1: Phase-Contrast Microscope Setup

Before you can make any observations, you will need to familiarize yourself with the Zeiss phase-contrast microscope. Identify the following components of the microscope and make sure you understand their function:

- Power Switch
- Brightness Adjustment Knob
- Ocular Lenses
- Objective Lenses
- Course and Fine Focus Knocks
- Condenser Lens
- Iris/Diaphragm
- Condenser Annulus
- Stage and Stage Positioning Knobs
- Stage Slide Holder
- Camera
- Ocular-to-Camera Port Switch

The instructor/TA will go over how to use both of these microscopes. Since you will be using these microscopes again next week, write a protocol for each microscope in your notebook (see assignment below).

Important reminders: The condenser annulus must be matched with the correct objective lens to achieve a phase-contrast image. The iris/diaphragm must be completely open when using a condenser annulus. The 10X objective does not have a corresponding condenser annulus on our microscopes; you can adjust the iris/diaphragm to help enhance contrast, but be sure to open it back up when switching to phase-contrast mode. If no image appears through the ocular lenses, switch the ocular-to-camera port knob to the ocular position.

Download the "Tutorial for AmScope Cameras on Phase-Contrast Microscopes.pdf" from this link: https://drive.google.com/file/d/1xGxHwFdXrquTo6Fw2D2QNxQLzyzufI_A

Like in Lab 1, we will be using AmScope software to capture images with an AmScope camera, but this time we will be using a built-in camera on the phase-contrast microscopes – ocular cameras should NOT be used on phase-contrast microscopes. If needed, you can find links to the installation files in the tutorial. The calibration

settings you established for each objective power in Lab 1 will only be valid for the bright-filed microscope you used in that lab – you will need to establish new calibrations for the phase-contrast microscope. Do NOT clear the calibration settings from Lab 1 – you will need them when you use a bright-field microscope again (see Exercise 4). So that you don't get confused between settings for the two different microscopes, append "Zeiss" to the name you set for each objective power on the phase-contrast microscope; e.g., instead of simply "10X" name the objective "10X Zeiss".

Exercise 2: Survey of Protists

Materials:

- Depression microscope slides
- Cover slips (as needed)
- Micropipettes and tips
- Dissecting microscope (for picking protists)
- Protoslo methyl cellulose
- Lens paper
- Lens cleaner or 95% EtOH

Protists:

Spirogyra
Chlamydomonas
Chaos carolinensis
Amoeba proteus
Euglena
Stentor coeruleus
Blepharisma
Spirostomum
Paramecium
Volvox globator

Procedure:

1. Use a dissecting microscope to pick out a protist from their container using a micropipette. You can use P200 pipettes for the smaller protists, but use a P1000 for larger ones like Amoeba proteus, Chaos, Stentor, and Spirostomum. For your first protist, chose one of the protists you'll use in Exercise 3, the Protist Hunger Games.
2. Place a coverslip over the depression to prevent drying. Your TA or instructor will show you how to prepare this wet mount without catching too many bubbles under the coverslip.
3. Place the slide onto the phase-contrast microscope so that the sample is centered in the light path.
4. Observe under the 10X objective; since the objective does not have a corresponding condenser annulus, set the condenser annulus slider to the middle position and use the iris slider to close the iris/diaphragm to help enhance contrast – you will need to increase the brightness to compensate.
5. Once you find something, switch to the 20X objective, set the condenser annulus for the 20X objective (left position), and open the iris/diaphragm completely.
6. Capture images under the 20X and 40X. The 40X objective will require the condenser annulus in the right position. Note different features of the cells and which magnification best allows observation. Remember to change the objective power in AmScope when you switch between objectives!!!
7. Using a fresh slide, repeat steps 3-7 for the second protist you will use in the Protist Hunger Games.

Proceed to Exercise 3 now; you will return to finish Exercise 2 after setting up the Protist Hunger Games slide.

8. Repeat steps 3-7 for 3 more protists of your choice. You will have captured 10 images total for the exercise: 5 different protists at 20X and 40X.

Exercise 3: Protist Hunger Games

What happens when two or more protists meet? Not sure? Let's make a hypothesis and test it! To do this, you must first gather a bit of information about each protist (see assignment below). The best place to do that is Google. You will mix a small amount of protists in a depression slide early during the class (during Exercise 2) so that we can observe them throughout the period.

Materials:

Same as in Exercise 2

Procedure:

1. Using the same techniques as in Exercise 2, combine the two protists from the first two depression slides in Exercise 2. This will be the “Coliseum” in which these protists will compete. Try to get as little liquid as possible onto the slide; this will help the protists find each other faster.

2. Observe for a few minutes with phase-contrast microscope. Label the slide and set it off to the side in a safe place. Do NOT leave the slide on the stage with the light on – this will dry out the slide and kill photo-sensitive and heat-sensitive cells.

Go back and complete Exercise 2 now, but every so often, check on your Protist Hunger Games slide to see how the participants are doing.

Process to Exercise 4 if you need to wait longer for the protists to interact.

3. When you observe interactions between the protists on your Hunger Games slide, take pictures. It might help to take a video so you can capture the dynamics of their interactions. You can pull frames from the video to make figures later. You might need to wait a while (30+ minutes) for your protists to interact.

4. After completing your observations, remove the camera from the microscope and replace the ocular lens. Make sure you didn’t leave any slides on the microscope stage. If you've finished Exercise 4 already, ensure the light is off and place the dust cover on the microscope.

5. Wash off the depression slides with water, dry with paper towels or kimwipes, and place them back in slide boxes.

Exercise 4: Diversity of Eukaryotic Cell Types

Materials:

- Stage micrometer
- Lens paper
- Lens cleaner or 95% EtOH
- Immersion oil (only for 100X objective)
- Prepared slides of eukaryotic cells

Procedure:

1. Set up the bright-field microscope as you did in Lab 1. If the lenses are dirty, use lens paper and some 95% EtOH to clean them. Insert the camera into one of the ocular lens positions and place the ocular lens in the camera box for safe keeping.

2. Animal cells: Two slides of your choice

a. Pick out two slides of stained animal cells. These slides can be anything that interests you (e.g., cancerous cells, blood cells, tissue sections).

b. Note different features of the cells and which magnification best allows observation. Take pictures at this magnification.

c. Use arrows to indicate the nucleus of at least one cell and any other interesting features you find.

d. Add a descriptive figure legend; indicate the animal species and tissue/cell type.

3. Plant cells: Two slides, one of a leaf and another of your choice

a. Pick out a slide showing a cross-section of a leaf.

b. Observe the cells at a magnification where you can clearly see chloroplasts.

c. Take pictures and use arrows to point out chloroplasts and the cell wall of the plant cells. If there is a central vacuole present, indicate this as well. Don’t forget to add scale bars to this and all following images you take.

d. Add a descriptive figure legend and indicate the plant species.

e. Pick out a second plant slide of your choice; examine it, capture an image of it, and make a figure as described above.

4. Fungal cells: One stained mold slide

a. Pick out a stained mold slide. Molds are multicellular fungi and grow tube-like structures called hyphae and generate spores that are usually highly pigmented which are contained within a sack. Observe the mold slide at a magnification where you can clearly see the cellular structure of the hyphae and any spore sacs present.
b. Capture an image of these structures, indicate the hyphae and spores with arrows, and make a figure as described above. Include the mold species in the legend.
5. Remove the camera from the microscope and replace the ocular lens. Make sure you didn't leave any slides on the microscope stage. Ensure the light is off and place the dust cover on the microscope.

Results:

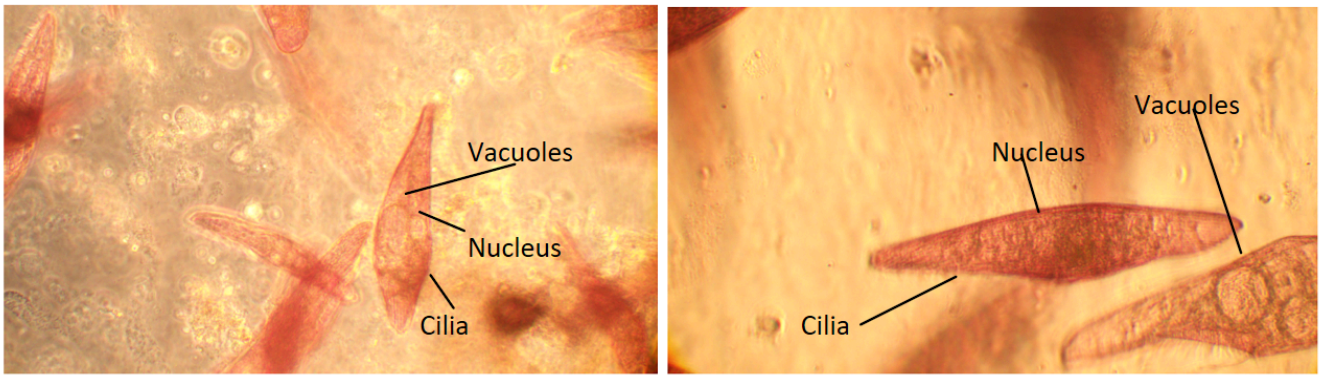


Figure 1
Left: *Blepharisma* 20X Objective **Right: *Blepharisma* 40X Objective**

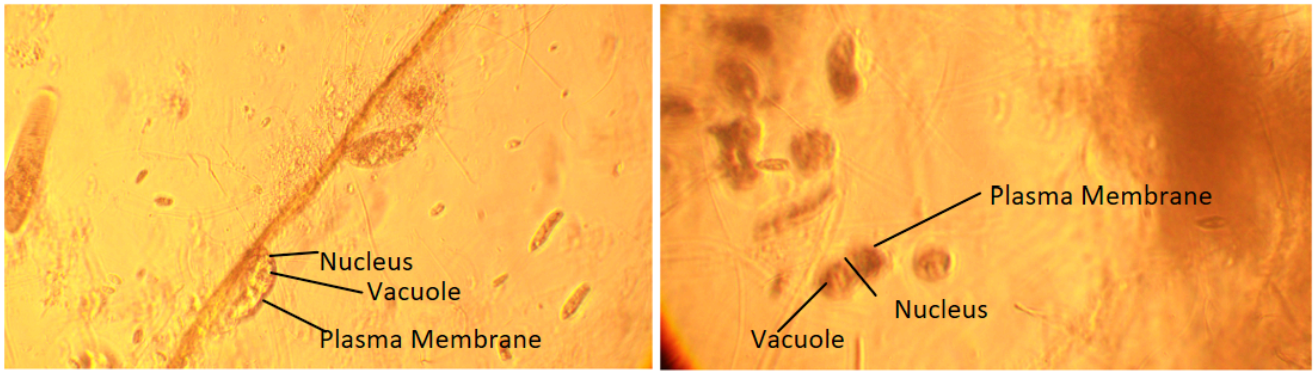


Figure 2
Left: *Chaos carolinensis* 20X Objective **Right: *Chaos carolinensis* 40X Objective**

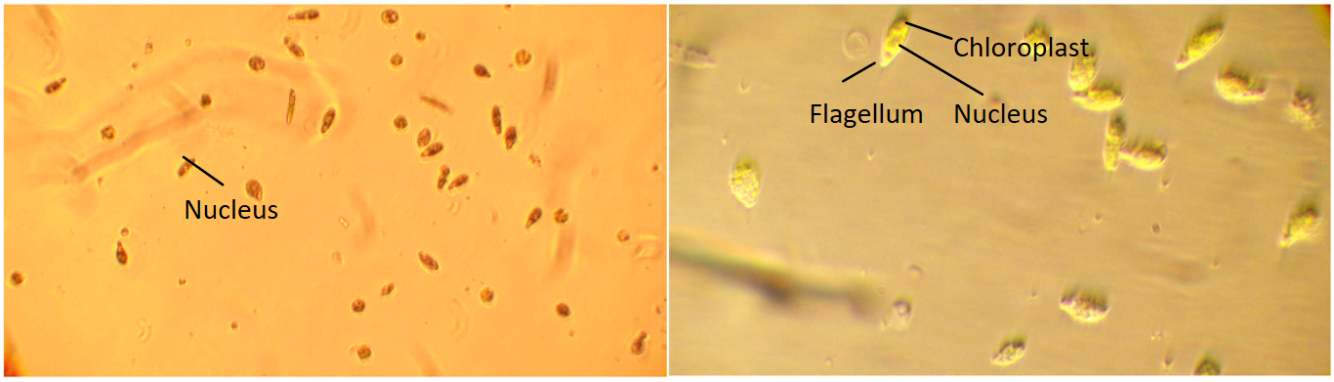
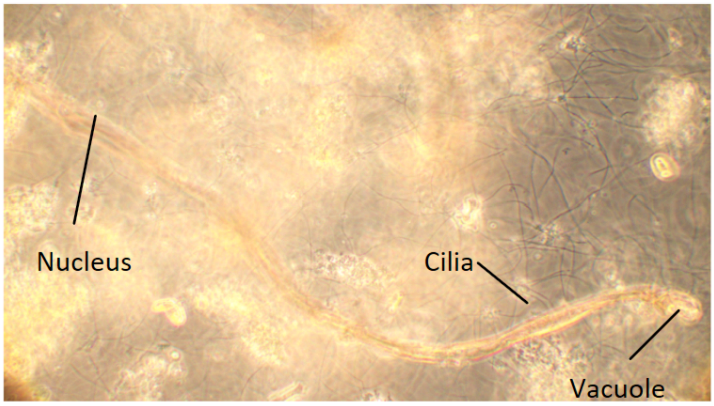


Figure 3
Left: *Euglena* 20X Objective **Right: *Euglena* 40X Objective**



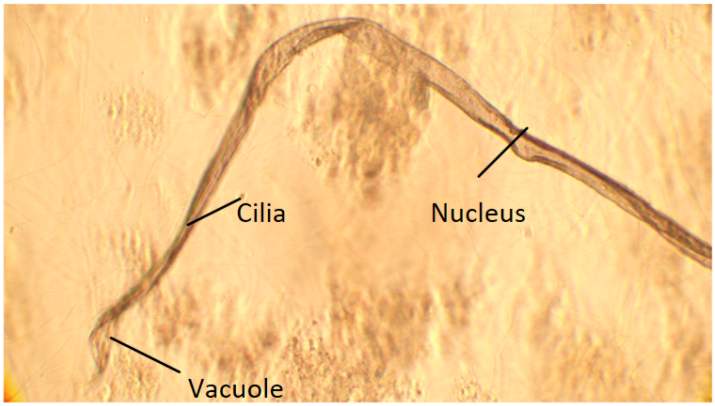
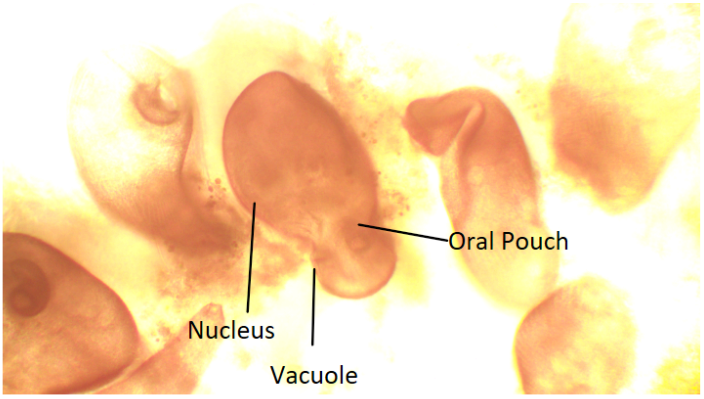


Figure 4

Left: *Spirostomum* 20X Objective **Right: *Spirostomum* 40X Objective**



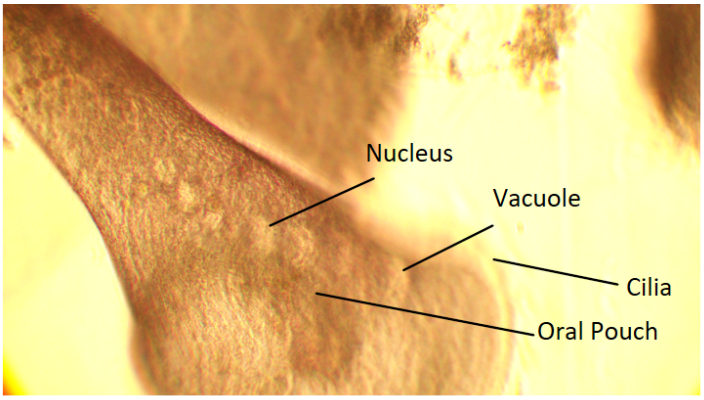


Figure 5:

Left: *Stentor Coeruleus* 20X Objective **Right: *Stentor Coeruleus* 40X Objective**

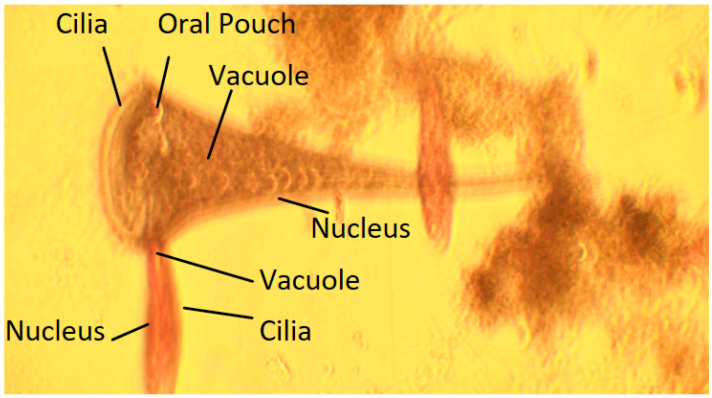




Figure 6:

Left: *Blepharisma (Pink)* and *Stentor coeruleus (Green)* 20X Objective **Right: *Blepharisma* and *Stentor coeruleus* 40X Objective**

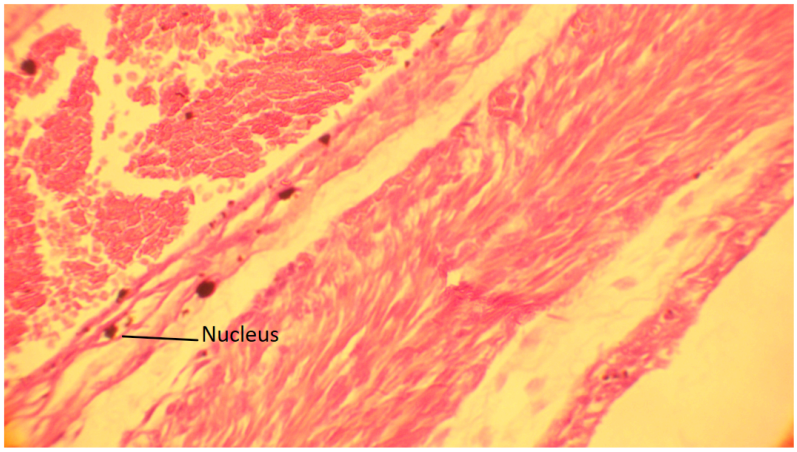


Figure 7: Human Fetus Bone Cartilage, 40X Objective

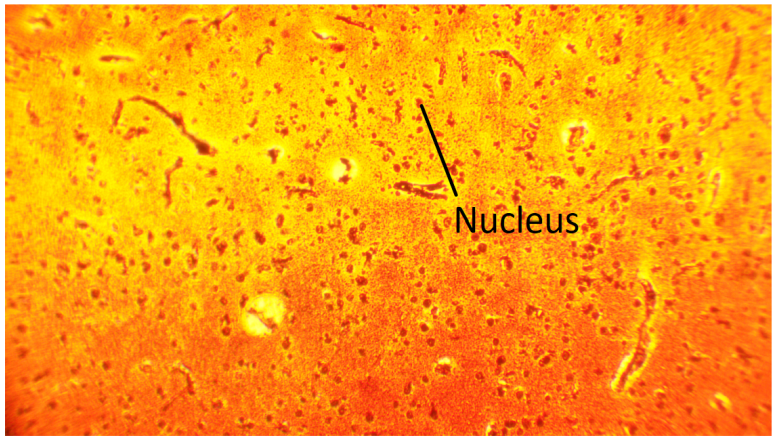


Figure 8: Human Cerebral Cortex, 20X Objective

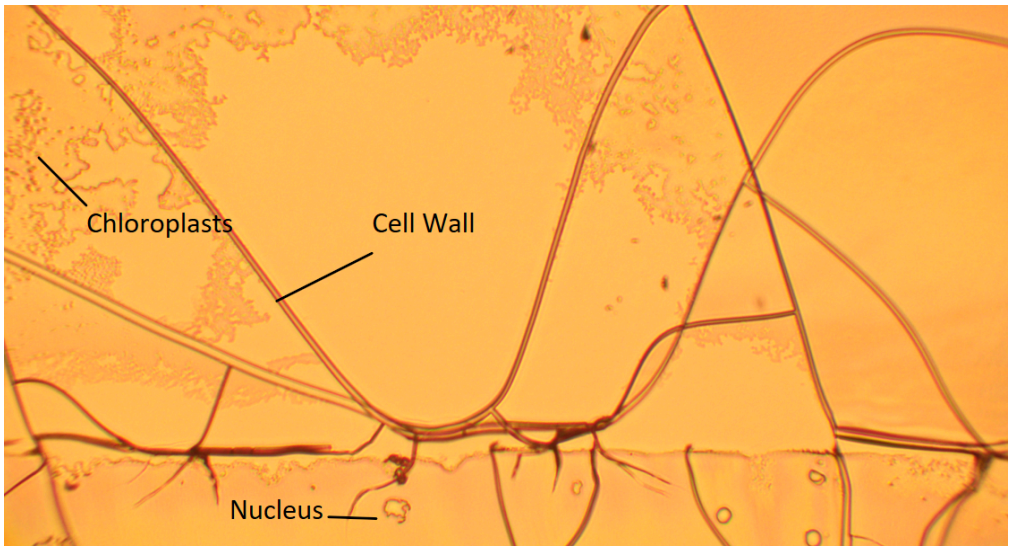


Figure 9: Elodea Leaf, 40X Objective

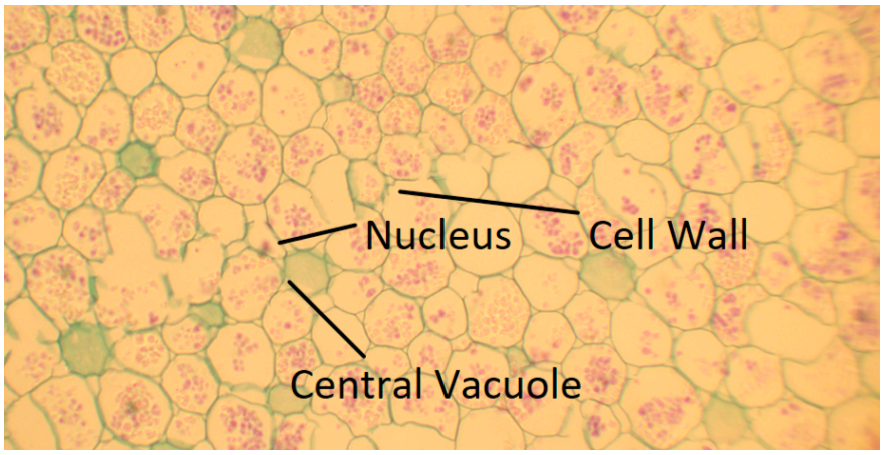


Figure 10: Monocot and Dicot Roots, 20X Objective

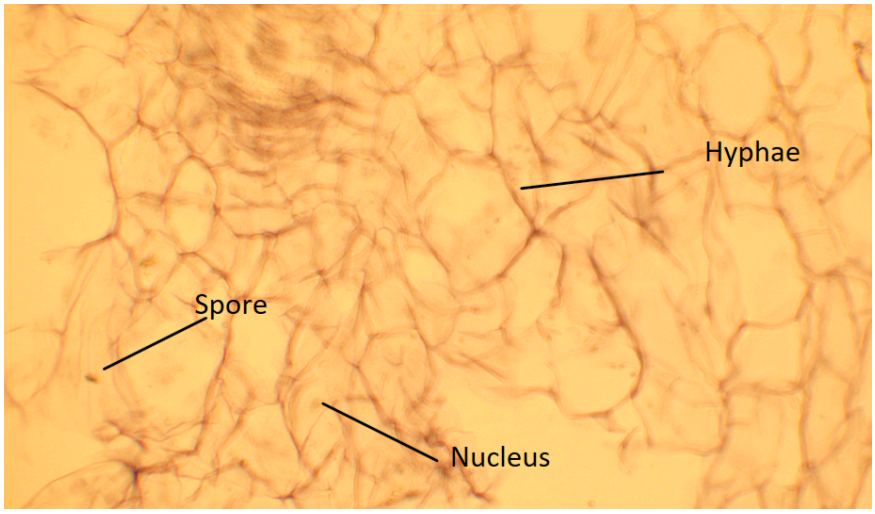


Figure 11: Exoascus Deformans, 40X Objective

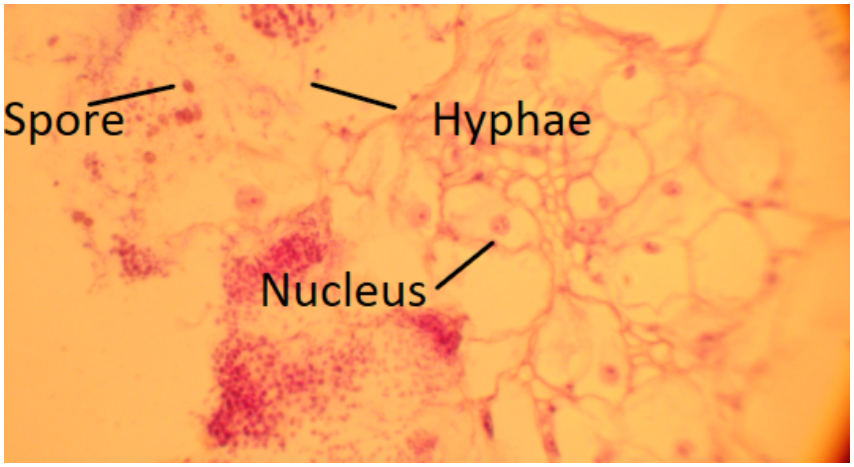


Figure 12: Ustila Gozeae, 40X Objective

Lab Questions:

Assignment 1:

A. Write a protocol for using the phase-contrast microscope in your notebook. Use the protocol for bright-field microscopes in Lab 1 as a guide for the protocol you write.

1. Plug the phase-contrast microscope in, making sure the cord isn't tangled. Use the knob on the bottom right of the microscope to make sure the microscope is not set to the camera setting.

2. Turn the light to medium brightness using the dial at the front right side of the microscope.
3. Look through the microscope, using both eyes, and use the adjustment knobs until the light appears as one circle.
4. Put the phase plate on the center setting and lock the 10X objective into place under the stage.
5. Open the stage slide holder and put the slide in, closing the stage slide holder after. It should be locked into place.
6. Use the knobs on the left side of the stage plate to move the slide to the center of the light.
7. Use the coarse adjustment knob to focus on the specimen. Also alter the light intensity if needed to better view the specimen.
8. Put the 20X objective in place and move the phase plate setting to the left one.
9. Use the fine adjustment knob to refocus the specimen. Increase the light if needed.
10. To take photos, plug the cord that is not plugged into the wall into your laptop and switch the knob on the bottom right to the camera setting.
11. Open Amscope, click on the camera, and then click snap. Switch the knob off of camera mode.
12. Put the 40X objective in place and move the phase plate setting to the right one.
13. Use the fine adjustment knob to refocus the specimen. Increase the light if needed.
14. Put the knob on camera mode again and take pictures using this objective.
15. Repeat again with the other specimen.

Assignment 2:

A. Create figures for the three micrometer images you captured (one for each objective power – 10X, 20X, and 40X). Add a scale bars to each so you can verify that you accurately calibrated the resolution of the image.
We were told not to take the stage micrometer slide photos.

B. Include a figure description that indicates the objective power and the actual distances the delineations (tick marks) represent in the image (hint: the distance between the smallest tick marks on our stage micrometers is NOT 1 μm).
We were told not to take the stage micrometer slide photos.

Assignment 3:

A. Make labeled figures of each protist: there will be 5 figures (one per protist), each figure should contain two images, each taken at different magnifications. Include scale bars and objective power.
See figures above. We were told not to use scale bars.

B. Indicate and label the nucleus/nuclei and other prominent cellular structures that are visible in your images.
See Figure Above

C. Discuss the structures that each protist uses for feeding and motility. Include labels on figures if relevant.
Blepharisma use cilia to help sweep food into their mouths. They also use those cilia to help with movement.
Chaos carolinensis participate in phagocytosis and use vacuoles to help engulf their food. They move via a flexible plasma membrane.
Euglena utilize chloroplasts to make food via photosynthesis. They also use flagellum to move.
Spirostomum have vacuoles that help to digest food. They also move via cilia.
Lastly, *Stentor* eat by having their cilia sweep food into their oral pouch. They also use these cilia to help with motility.

Assignment 4:

A. Copy over the information from the answer you gave to Question 4 of the pre-lab. Based on this information, formulate a hypothesis (predict a “winner”) and explain your rationale.

	Size	Feeding Strategy	Motility Mechanism
Blepharisma	50 µm - 1 mm Long Ovular	Cilia Heterotroph Cannibalism at Times	Cilia Spinning
Spirogyra	10 - 150 µm Wide Multiple cm long	Autotroph Chloroplasts Photosynthesis	Filaments Roll Up Group Together Align Move Toward Blue Light

I believe that *Blepharisma* will beat *Spirogyra* in the Protist Hunger Games. I think this because *pirogyra* makes its food via photosynthesis and is therefore unlikely to be aggressive compared to *Blepharisma*, which is a heterotroph and needs to be more competitive for food (sometimes it is even competitive to the point of cannibalism).

There was not a *Spriogyra* sample available. Below is a modified chart for the protists we actually used for the protist hunger games.

	Size	Feeding Strategy	Motility Mechanism
Blepharisma	50 µm - 1 mm Long Ovular	Cilia Heterotroph Cannibalism at Times	Cilia Spinning
Stentor coeruleus	1-2 mm Long Trumpet Shape	Heterotroph Cilia Oral Pouch	Cilia Very Mobile

Based on this updated chart, I would expect that *Stentor coeruleus* will beat *Blepharisma* in the Protist Hunger Games. I think this because *Stentor coeruleus* are large and more motile, which will make it much easier for them to attach and eat the *Blepharisma*.

B. Take pictures and/or videos of the competition. At least one image should be captured showing the interaction (or non-interaction) of the protists. Construct a figure from the image as you did in Exercise 2.

See Figure 6 Above.

C. Discuss in your notebook the results and if they support your hypothesis

Blepharisma and *Stentor coeruleus* did not initially seem to interact. However, when checked on at the end of the lab, it appeared that all of the protists had died. This does not support my hypothesis since I believed that *Stentor coeruleus* would win. If I were to repeat this lab, I would make sure that the slide was far away from the light (which could kill them) and I would watch the entire time the protists are both on the slide in order to see what is actually happening.

Assignment 5:

A. Make the figures indicated above (two animal slides, two plant slides, and one fungal slide).

See Figures Above.

B. Discuss how the cellular structures of these multicellular organisms compare to those of the protists, particularly the single-cell protozoa, from the previous exercises.

The cells viewed today all showed a wide variety of cell shapes. Additionally, a protist cell is typically much larger than the cells of multicellular organisms. However, despite size differences, they still share many of the

same structures. Many plant cells have chloroplasts just like many autotrophic and mixotrophic protists. Furthermore, all of the cells have a nucleus. The nuclei of protists seem to have many different shapes and structures whereas the nuclei of multicellular organisms seem to be very similar.

Conclusion/Discussion:
The purpose of the lab was achieved since my lab partner and I learned the difference between bright-field and phase-contrast microscopy, made wet mounts of specimens, documented our observations, and learned the diversity of eukaryotic cell types.

My hypothesis about the Protist Hunger Games was not correct since all of the protists died and there was no clear winner. This does not support my hypothesis since I believed that *Stentor coeruleus* would win. If I were to repeat this lab, I would make sure that the slide was far away from the light (which could kill them) and I would watch the entire time the protists are both on the slide in order to see what is actually happening.

Additionally, this lab has shown the wide variety of cells that can be found in eukaryotes. The different cells viewed today had a wide variety of shapes, structures, and sizes. All of these differences contribute to their differing functions and help to create a wide variety of different organisms.

Fluorescence Microscopy

Date: 11/19/25

Pre-Lab Questions:

1. What are Stokes shift and anti-Stokes shift? Which of these will GFP exhibit under our microscopes?
The Stokes shift is the wavelength difference from the highest value in the emission spectrum to the highest value in the excitation spectrum. This occurs when the absorbed photon energy is greater than the released photon energy. The anit-Stokes shift is when the opposite is true (emitted photon energy is greater). GFP will exhibit the Stokes shift under our microscopes.
2. How does a fluorescence microscope differ from a bright-field microscope?
A fluorescence microscope differs from a bright-field microscope in that it has a filter set containing an excitation filter, dichroic mirror, and emission filter. The excitation filter makes it so that only a subset of light wavelengths are able to be seen in the microscope due to a targeted beam of light being let through. The dichroic mirrors are used to help the specimen emit light by targeting the light to the correct location. The dichroic mirror also allows light reflected from the objective to go through the microscope's emission filter. The emission filter works very similarly to the excitation filter but for emitted light.
3. What does the promoter region of a gene do?
The promoter region of a gene is the DNA sequence of the gene where proteins can bind in order to start transcription.
4. How many cells does a hermaphroditic C. elegans have? What types of cells do you expect to see fluorescence in each of the C. elegans strains used in this lab?
A hermaphroditic C. elegans has 959 cells. I expect to see fluorescent neurons in the IM324 and UA57 since IM324 has the nervous system express GFP and UA57 has dopaminergic neurons express GFP. I expect MH1317 samples to show fluorescent muscle cells since the cell wall muscles express GFP and I expect SD1241 to show all cells since most of the nuclei will show fluorescence.

Objectives:

- Use fluorescence microscopy as a tool to examine cellular protein expression and localization
- Understand the difference between excitation and emission spectra
- Describe the function of the dichroic filter
- Understand the types of natural and artificial fluorescence organisms can exhibit
- Pick *C. elegans* and transfer them to an agar pad using platinum wire
- Explain why GFP is localized to that area or cells using wormbase.org

Hypothesis:

I believe that all of the bacteria will glow green since they are all cyanobacteria and contain chlorophyll. I also expect to see fluorescent neurons in the IM324 and UA57 since IM324 has the nervous system express GFP and UA57 has dopaminergic neurons express GFP. I expect MH1317 samples to show fluorescent muscle cells since the cell wall muscles express GFP and I expect SD1241 to show all cells since most of the nuclei will show fluorescence.

Methods:

Exercise 1: Fluorescence Imaging of Bacteria

Materials:

- Phase-contrast/fluorescence microscope
- Glass slides with coverslips
- GFP E. coli culture on agar plates
- mCherry E. coli culture on agar plates
- Inoculation loops
- P100 micropipette and tips
- 1X PBS
- Microfuge tubes

Cyanobacteria Liquid Cultures:

Anabaena
Eucapsis
Fischerella musicola
Merismopedia
Spirulina
Tolypothrix distorta

Procedure:

1. Prepare a protocol for how to use the fluorescence capabilities of your microscope and the AmScope software based on the instructions given by your TA/instructor.

1. Plug the microscope in, making sure the cord isn't tangled. Use the knob on the bottom right of the microscope to make sure the microscope is not set to the camera setting.
2. Turn the light to medium brightness using the dial at the front right side of the microscope.
3. Look through the microscope, using both eyes, and use the adjustment knobs until the light appears as one circle.
4. Put the phase plate on the center setting and lock the 10X objective into place under the stage.
5. Put the fluorescence filter (bottom box under the microscope stage) onto either the red or green setting, depending on what image you are creating.
6. Open the stage slide holder and put the slide in, closing the stage slide holder after. It should be locked into place.
7. Use the knobs on the left side of the stage plate to move the slide to the center of the light.
8. Use the coarse adjustment knob to focus on the specimen. Also alter the light intensity if needed to better view the specimen.
9. Put the 20X objective in place, leaving the phase plate on the middle setting.
10. Use the fine adjustment knob to refocus the specimen. Increase the light if needed.
11. Put the 40X objective in place, again leaving the phase plate on the middle setting.
12. Use the fine adjustment knob to refocus the specimen. Increase the light if needed.
13. To take photos, plug the cord that is not plugged into the wall into your laptop and switch the knob on the bottom right to the camera setting.
14. Open Amscope, click on the camera, and then click snap. Switch the knob off of camera mode. You may need to adjust the exposure time and gain to account for the fluorescent light (turn off auto exposure to do this).
15. Repeat again with the other specimen and fluorescent color.

2. Mix together 1 colony of E. coli expressing GFP and 1 colony of E.coli expressing mCherry in 10 µl of 1X PBS. Transfer all 10 µl to a glass slide to make a for the wet mount.
3. On separate slides, prepare 3 additional wet mounts: one for Anabaena and two other cyanobacteria of your choice. Use 10 µl for each wet mount.
4. You should have 4 wet mounts total; capture fluorescence images of each wet mount in both the green and red channels – you might not see any fluorescence in one of these channels, but capture the image anyway – you'll be documenting that the specimen does not fluoresce in those channels. For Anabaena, capture a phase-contrast image as well; try not to move the stage between switching from phase-contrast and

fluorescence modes. Bacteria are very small, so you will need to use the highest power objective (40X) in order to see them properly, but even then, you might have difficulty finding them.

Exercise 2: Fluorescence Imaging of *C. Elegans*

Materials:

Microscope slides and cover slips
Dissecting microscope
1X PBS
60°C water bath
Fluorescent *C. Elegans*:
IM324 – the entire nervous system is expressing GFP
UA57 – only dopaminergic neurons are expressing GFP
MH1317 – body cell wall muscles are expressing GFP
SD1241 – nearly every nucleus glows green due to GFP localization
2% agar at 60°C
P100 or P200 micropipette and tips
Platinum wire worm picks

Part 1: Observe each strain of *C. elegans*

Procedure:

1. Practice picking one type of worm and transferring it to a fresh small plate under the dissecting microscope. During the course of this experiment, make notes about how each type of worm moves when viewing them in the dissecting microscope. You may need to tap the plate to induce movement.
2. Make a 2% agar pad by pipetting 50 µl of molten agar on a slide and immediately placing a second slide on top of it making an X. Gently glide the slides apart leaving the agar pad on one of the slides. Add 10 µl of PBS onto the pad.
3. From just one agar plate, pick one or two worms using the platinum wire hook and float the worms off the wire and into the PBS on your agar pad. Do NOT move the worm plates to different locations or use the same worm hook to pick different strains of worm – we do not want to cross-contaminate the strains.
4. Gently place a coverslip on top of the agar pad.
5. Confirm you have some worms under the coverslip using a dissecting scope.
6. Visualize under phase-contrast and fluorescence green channel modes and capture pictures of each. To make it easier to capture images, you might want to take videos of the worms and then grab a frame from the video to make into an image. The worms are usually fast moving, so to get clear images, you should turn OFF auto-exposure, decrease the exposure time to ~30 msec, and increase the gain as needed – this will give you a high frame rate and reduce motions blur.
7. Proceed to Part 2 where you will feed mCherry E. coli to the worms on this slide. You only need to feed one set of worms.
8. Repeat steps 2-6 for the other three strains of worms

Part 2: Map *C. elegans digestive tract*

Procedure:

1. Prepare more mCherry E. coli in PBS (do NOT use GFP E. coli).
2. Add mCherry E. coli to your first worm slide and incubate for about 30 minutes.
3. Return to Part 1 to finish visualizing the other strains of worms.
4. After 30 minutes have passed, view the worms under the fluorescence microscope and take pictures.

Results:

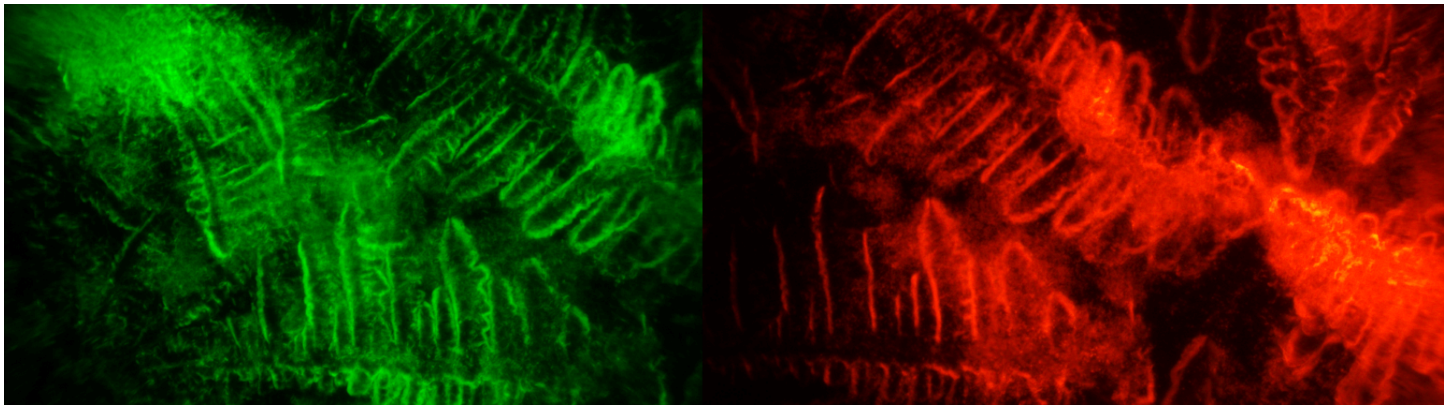


Figure 1: GFP and mCherry *E. coli* Bacteria Mixture, 40X Objective
Left: Green Fluorescence **Right: Red Fluorescence**

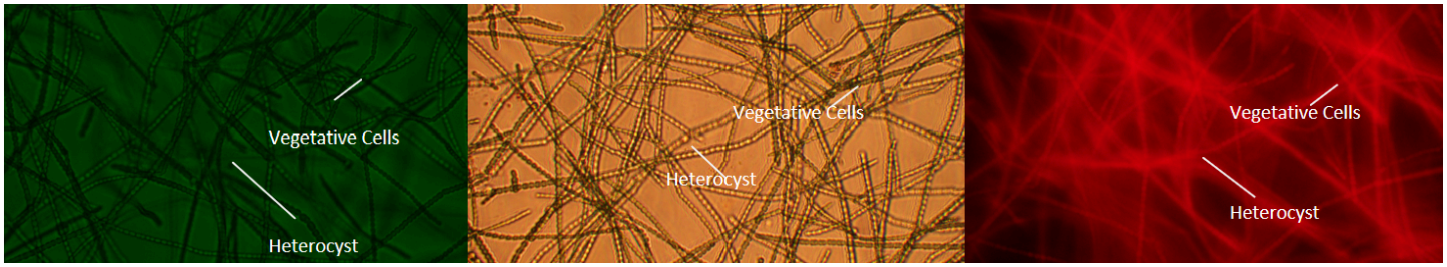


Figure 2: *Anabaena* Cyanobacteria, 20X Objective
Left: Green Fluorescence **Middle: Phase Contrast** **Right: Red Fluorescence**

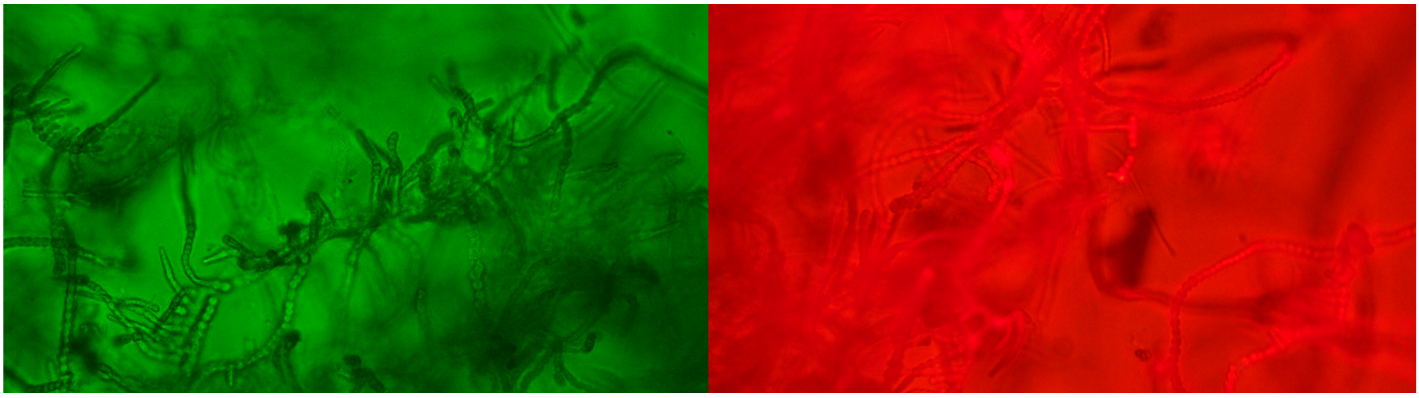


Figure 3: *Fischerella musicola* Cyanobacteria, 40X Objective
Left: Green Fluorescence **Right: Red Fluorescence**

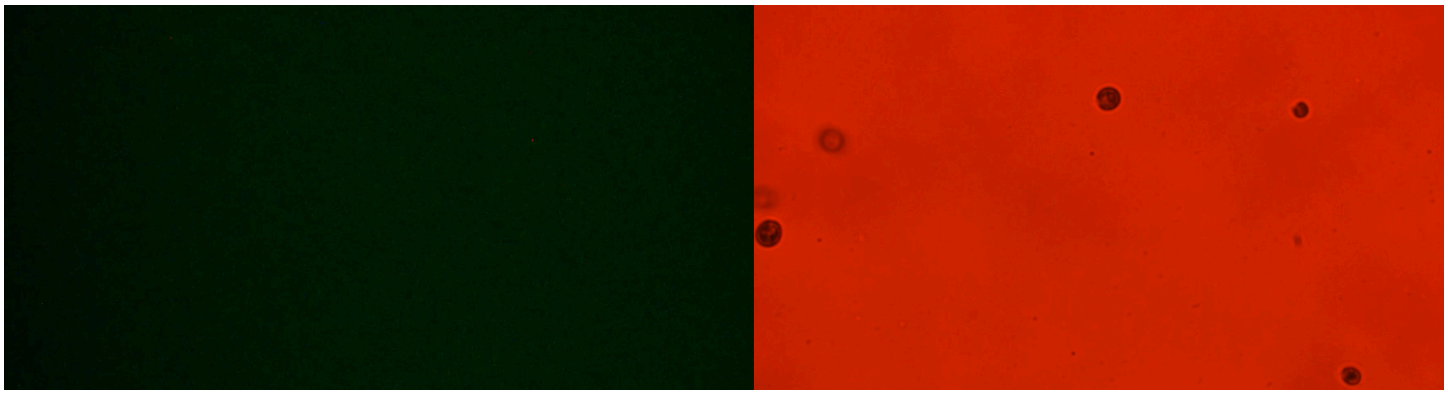


Figure 4: *Chlamydomonas* Cyanobacteria, 40X Objective
Left: Green Fluorescence **Right: Red Fluorescence**

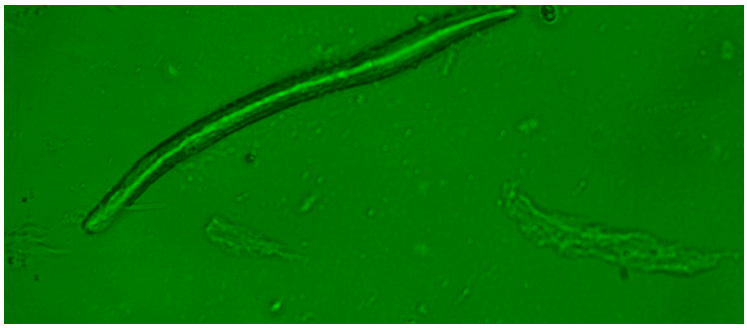


Figure 5: IM324 *C. Elegans*, 40X Objective

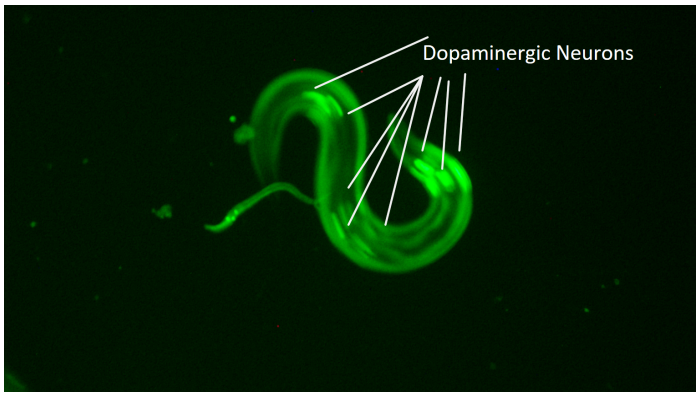


Figure 6: UA57 *C. Elegans*, 10X Objective (Borrowed From Another Group)

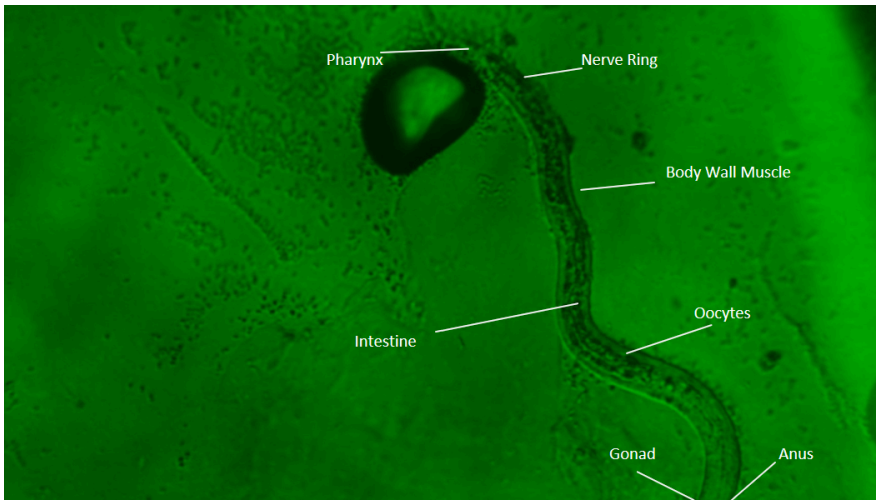


Figure 7: MH1317 *C. Elegans*, 40X Objective

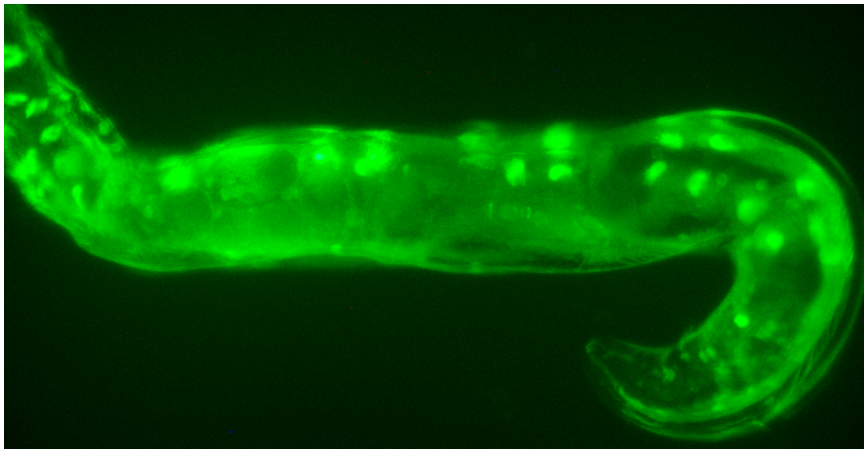


Figure 8: SD1241 *C. Elegans,* 40X Objective (Borrowed From Another Group)

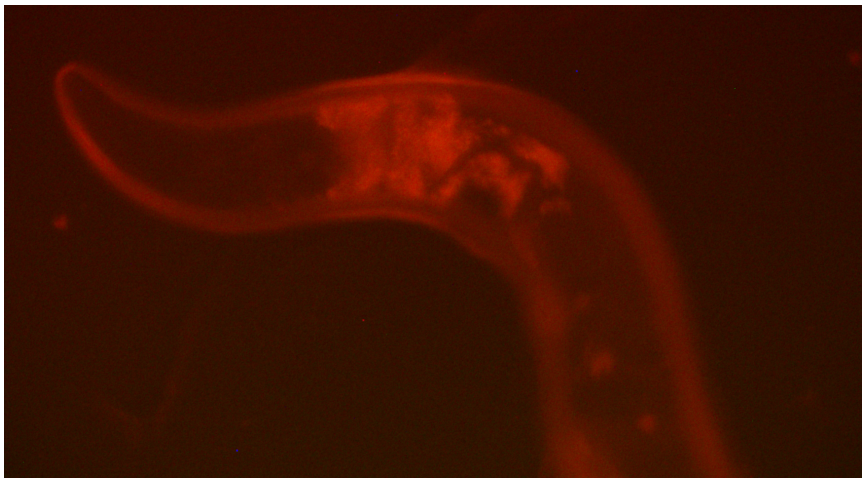


Figure 9: MH1317 and *E.coli*, 40X Objective (Borrowed from Another Group)

Lab Questions:

Assignment 1:

A. Create labeled figures for each wet mount. Each figure should contain all the images you captured for that wet mount (e.g., for Anabaena you'll have a phase-contrast image, a fluorescence green channel image, and a fluorescence red channel image). Make sure to include a scale bar, the organism type, and objective power. You should use the calibrations you established using stage micrometers in AmScope last week to make scale bars.

See Figures 1-4 Above. We were told not to use scale bars.

B. For the mixed E. coli culture, what is the approximate ratio of green to red bacteria?

The approximate ratio of green to red bacteria for *E.coli* is 1:1.

C. For Anabaena, some cells are fluorescent, and some are not. Label them and explain what these cells are and why they aren't fluorescent. Why was it important to have a phase-contrast image of Anabaena?

The fluorescent cells are called vegetative cells and the non-fluorescent cells are called heterocysts (ours are not super visible). Heterocysts aren't fluorescent because GFP is not expressed in these cells. It is important to have a phase-contrast image of *Anabaena* in order to be able to locate the heterocysts.

Assignment 2:

A. Create labeled figures for each sample from Parts 1 and 2. Make sure to include a scale bar, strain type, and objective power. You can also embed any videos you took of the worms.

See Figures 5-9 Above. We were told not to use scale bars and did not have phase contrast images of the *C. elegans*.

B. Label the anatomy of the C. elegans in at least one phase-contrast image.

See Figure 7 Above.

C. Which worm moves differently from the others on the plates?

MH1317 *C. Elegans* move differently due to the expression of GFP in the body cell wall muscles. I expect this because muscles help with movement and this strain of *C. Elegans* has a different gene being expressed in their body cell wall muscle cells.

D. Determine the gene promoter region in each fluorescent worm strain that is used to drive the GFP expression. You should do the following:

1. Go to https://wormbase.org/.
2. In the upper right side of the web page, click on "strain" from the pull-down menu and type in the strain name to search.
3. In the "Overview" box, click to expand the Curatorial remarks section.

4. The promoter is adjacent to GFP and is marked in something resembling the following way: dat-1p::GFP = the promoter region (P) of the dat-1 gene is used to drive expression of GFP. The WormBase is not uniform throughout, and you might observe differences between strain annotations.
E. For each gene promoter, report a description of the gene that this promoter typically regulates and what type of function this gene has in the worm. You can click the gene's link from the Curatorial remarks section as above (if this isn't available, type it in and search for the gene in WormBase).

Strain	Promoter	Description
IM324	F25B3.3	This promoter regulates a gene that helps with Ras signals and guanyl nucleotide exchange factor. Its function is mostly exchanging GTP for GDP, which helps with cell operations.
UA57	dat-1p	This promoter regulates a gene that helps with the movement of amines from one side of the membrane to the other. This also helps with dopamine and chloride channels. Its function is mostly transport for the cell.
MH1317	egl-13p	This promoter regulates a gene having to do with egg laying. This mostly causes muscles associated with egg laying to become defective in moving backwards.
SD1241	sur-5	This promoter regulates a gene that helps with acetoacetate-CoA ligases. This helps with the breakdown of fatty acids.

F. How many dopamine neurons are there in UA57? (Count the number of cell bodies you see and label them in your pictures). How many do you expect?
There are 8 dopamine neurons in UA57. I also expect there to be around 8 from the picture.

Conclusion/Discussion:
The purpose of the lab was achieved since my partner and I were able to use fluorescence microscopy to see proteins that are expressed and localized, understand the differences in excitation and emission spectra, know the purpose of a dichroic filter, know the types of natural and artificial fluorescence possible in organisms, learned to pick *C. elegans* and transfer to an agar plate, and know why GFP is localized to a specific cell or location through wormbase.com.

My hypothesis was only partially correct. We saw fluorescent neurons in the IM324 and UA57 *C. elegans* and fluorescent muscle cells in the MH1317 sample. We also saw many cell neurons in the SD1241 sample, like I predicted. However, all of the cyanobacteria glowed not only under green fluorescence but also under red fluorescence, aside from *Chlamydomonas*, which only glowed under red fluorescence. Additionally, the heterocysts of the *Anabaena* did not glow under the green or red fluorescence.

If I were to repeat this lab, I would work on picking the *C. elegans* since some of our photos had to be borrowed from another group. Additionally, I would try to redo the *Chlamydomonas* image under green fluorescence to make sure that it is truly not supposed to glow.