PRINCIPLES OF BIOLOGY

LABORATORY MANUAL -BIO11L

Fall 2018

Department of Biology

Yeshiva University

INDEX

Syllabus

Course: Principles of Biology Lab - BIO 1011L

INSTRUCTOR: **Danielle Wasserman**
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COURSE OBJECTIVES, SCOPE AND GOALS:
An introduction to the fascinating world of Biology. The principle aim of this laboratory course is to introduce the students to classical and modern techniques used to explore Biology. This course will provide the foundation that you will require for learning and understanding Biology.

STUDENT LEARNING OUTCOMES:
Upon completion of this course, the student will be able to:

1) Understand the basics of pipetting, microscopy, spectrophotometry and centrifugation techniques.
2) Learn the process of Respiration and Fermentation.
3) Understand the biochemistry pattern in enzymatic activity and Gene Expression.
4) Learn the basis of, Osmosis and Diffusion.
5) study the fascinating world of microbes and follow the gene regulation.
6) Understand Mitosis & Meiosis, Mendelian and Molecular Genetics.
7) Gain exposure to Animal Development.

PREPARATION FOR CLASS
It is expected that you complete assigned readings and review any posted sources before attending class. It is critical that you read the lab prior to coming into class to give you a complete understanding of what will be done during lab.

ATTENDANCE
Students are expected to attend class and arrive on time. Late arrival is disruptive to the class and absence from class will be detrimental to your final grade. **If late or absent, it is your responsibility to get information missed from your classmates. Make up quiz or exams are not permitted**, except under extreme circumstances such as serious illness or death in the immediate family. Please contact the instructor before the scheduled exam to request and schedule a make-up exam. Please be courteous and respectful of your classmates by being punctual, by turning off all noise-making devices (e.g. cell phones), and by remaining attentive in class. Disruptive behavior may result in lowering of grades or class dismissal.
CELL PHONES OR SIMILAR DEVICES ARE NOT TO BE USED DURING EXAMS

ACADEMIC INTEGRITY
All work must be your own. Copying from any source (e.g. classmates, published sources, and internet) for any assignment is plagiarism, a serious offense, and can result in a grade of 'F' for the course as well as disciplinary action from the college. You must read and understand the section of the *Student Handbook* entitled *Violations of Academic Integrity.*

OFFICE OF THE ACADEMIC SUPPORT

The Office of Academic Support provides services and resources designed to help students on Wilf Campus develop more efficient and effective study skills and strategies. Individual support is available in areas such as time management and organization, active reading, note-taking, exam preparation and test-taking skills. The office is located in Furst Hall, Suite 412. To schedule an appointment, call 646-592-4285 or email academicsupport.wilf@yu.edu.

STUDENTS WITH SPECIAL NEEDS

Students with documented special needs should notify the instructor as soon as possible (the first week of class) so that the necessary arrangements can be made. Policy Statement on Non-Discrimination on the basis of Disability (ADA). The Institution is an equal opportunity educational organization. Please read The Institutional Policy Statement on Nondiscrimination on the Basis of Disability Americans with Disability Act Compliance. "Students with disabilities who are enrolled in this course and who will be requesting documented disability-related accommodations should make an appointment with the Office of Disability Services, akelsen@yu.edu or 1-646-592-4280 during the **first week of class**. Once you have been approved for accommodations, contact me to ensure the successful implementation of those accommodations.

POLICY ON CHEATING AND PLAGIARISM

From the undergraduate catalog, Academic Regulations and Procedures:

"Cheating and plagiarism are contrary to the purpose of any educational institution and must be dealt with most severely if student's work is to have any validity. An instruction who determines that a student has cheated on a test or assignment will at a minimum give a zero for that item and may give a failure for the course. Normally the matter is handled between the instructor and the student, but the department chairperson may be consulted by either party to ensure fairness.

Plagiarism, which is the appropriation of words or ideas of another without recognition of the source, is another form of cheating. An instructor who determines that student has plagiarized will give a zero for the paper or project and may give a failure for the course. Both cheating and plagiarism are grounds for dismissal from the college."

Make sure that your lab report is your own. You may work together with a lab partner to understand the information, but your report should be written separately. You may not use reports from students from previous years. If a student is found to have violated this, the matter may be brought to the Dean's office and will likely be placed on your permanent record.

POLICY ON MAKEUP EXAM AND INC GRADE

Students are strongly encouraged not to miss any examination. If for valid reason a student misses an examination and has documentation to prove the predicament then he will be given an Incomplete (**INC**) grade and the student has to arrange with the Instructor to reschedule the examination such that the final grade can be submitted with 30 days of the end of the course semester.

COURSE REQUIREMENTS

(A) Prior Notice:

If a student finds that he must be absent on the day of a scheduled quiz, he must inform the instructor before the day of the quiz. At this time, a makeup time will be agreed upon.

(B) Unexpected absence:
If a student is unable to attend class on the day of the quiz he must call the school and either inform the instructor directly or leave a message. It then becomes the responsibility of the student to contact the instructor as soon as he returns to school. Failure to arrange a makeup time within **2** days of the student's return will result in a zero grade for the missed quiz.

QUIZ & FINAL EXAMINATION:
There is no provision for making up a missed quiz or final exam - except under exceptional and documented circumstances. Makeup of final exam is then possible on specified dates- the students should contact Dean's Office.

EVALUATION AND GRADING
Evaluation: Learning will be evaluated by written examinations as per schedule. The Lecture will account for **70%** of the total grade and the Laboratory section will be responsible for **30%** of the overall grade. Letter grades being determined from the total number of points earned in the semester. The values of the assessments are as follows:

Laboratory Grading

Quizzes	**10%**
Midterm	**30%**
Final	**30%**
Lab Reports	**25%**
Class Participation & Skills	**5%**
Laboratory Total	**100%**

Quizzes: At the start of each class, you will complete a short quiz based on the information from the previous experiment and that day's experiment. There will be a total of 11 quizzes and you will drop your lowest score.

Lab Reports: A typed lab report will be submitted by the start of each lab based off of the previous lab. A total of 12 reports will be submitted.

Lab Report Format

Goal – 2-3 sentences.
Answer the prelab questions formally by copying from manual.
Results: Tables & graphs, any data from experiments (including what didn't work)
Conclusion: What can we conclude from the results? 2-3 sentences.
Answer the post-lab questions precisely and concisely to the point.

Class Participation & Skills: Will be assessed by the instructor in each lab, based on participation in class discussions and overall skills in group procedural work.

A percentage will be calculated on your cumulative total score.

Tests: The tentative test schedule is given in the Course Syllabus.

Course Grade: Letter grades for the course will be computed from the total score as follows:

Highest	Lowest	Letter
100.00 %	93.00 %	A
92.99 %	90.00 %	A-
89.99 %	87.00 %	B+
86.99 %	83.00 %	B
82.99 %	80.00 %	B-
79.99 %	77.00 %	C+
76.99 %	73.00 %	C
72.99 %	70.00 %	C-
69.99 %	67.00 %	D+
66.99 %	60.00 %	D
59.99 %	0.00 %	F

Course Schedule

(Subject to change)

Lab	Topics	Monday	Tuesday	Wednesday	Thursday
1	Microscopes and Cells	**08/27**	**08/28**	**08/29**	**08/30**
2	Enzymes	**10/08**	**09/04**	**09/05**	**09/06**
3	Respiration and Fermentation	**10/15**	**10/09**	**10/10**	**10/04**
4	Biochemistry of Milk	**10/22**	**10/16**	**10/17**	**10/11**
5	Microbiology	**10/29**	**10/23**	**10/24**	**10/18**
6	Osmosis and Diffusion	**11/05**	**10/30**	**10/31**	**10/25**
	Midterm	**11/12**	**11/06**	**11/07**	**11/01**
7	Mitosis and Meiosis	**11/19**	**11/13**	**11/14**	**11/08**
8	Mendelian Genetics	**11/26**	**11/20**	**11/21**	**11/15**
9	Beta Galactosidase (Gene Expression) & Flies	**12/03**	**11/27**	**11/28**	**11/29**
10	Molecular Genetics	**12/10**	**12/04**	**12/05**	**12/06**
11	Animal Development	**12/17**	**12/11**	**12/12**	**12/13**
12	FINAL	**12/26**	**12/26**	**12/26**	**12/26**

YESHIVA UNIVERSITY
Department of Biology

LABORATORY SAFETY RULES

1. **EYE PROTECTION MUST BE WORN,** when you are working with **any** liquid, powder or granular materials googles must be worn even if you are wearing glasses. Contact lenses are forbidden when working with preserved materials or volatile solvents. If you must remove goggles for looking through the Microscope eye piece or any such work, replace them as soon as possible.

   All students are entitled to **one** warning about wearing safety glasses. A second offense will result in expulsion from the lab for the day. **ABSOLUTELY NO EXCEPTIONS TO THIS RULE WILL BE TOLERATED.**

   A single pair of goggles will be issued to you either in Biology or Chemistry for all of your courses at Yeshiva University. You are responsible for bringing them to each session. If you forget them you will have to go back to your room to get them. If you lose them you will have to replace them with a new pair certified to meet ANSI Z87.1 requirement for chemical splash protection, which may be available at the bookstore. Only the goggles certified to meet ANSI Z87.1 for chemical splash protection are allowed in the laboratory.

2. Material Safety Data Sheets (MSD Sheets) for each chemical used in each lab are available in a folder in the front of the room. These MSD sheets are also on reserve in the library and in the prep. room if needed.

3. NO SMOKING, EATING or DRINKING in the lab.

4. No open-toed shoes, sandals, or bare feet or shorts are allowed in the laboratory.

5. Keep your work area clean. Spills should be attended to immediately. Sodium bicarbonate should be applied to acid spills. Boric acid should be applied to alkaline spills. Both can be found in the laboratory.

6. ALL LABS ARE USUALLY KEPT LOCKED. ONLY STUDENTS REGISTERED FOR GENETICS HAVE PERMISSION TO ACCESS LABS DURING NON-CLASS HOURS. IN ORDER TO GAIN ENTRY, FOR ANY REASON, YOU MUST ASK ONE OF THE LAB PERSONNEL TO OPEN THE DOOR (9AM-5PM or during lab hours) Rm: BH1405.

7. Never perform unauthorized experiments. It is prohibited to perform experiments using hazardous chemicals, open flames, or high voltage electricity unless the laboratory instructor or his/her designee is present.

8. All injuries, however slight, must be reported to your instructor.

9. Any special medical conditions (for examples, diabetes, allergies) must be reported to your instructor at the beginning of the semester.

10. When heating glassware on a hot plate or in boiling water use safety equipment to handle.

11. Do not light burners in the vicinity of flammable liquids.

12. Always extinguish flames when NOT being used or when unattended.

13. Never deeply inhale any gas of unknown properties.

14. Never taste anything in the lab.

15. Never pipet anything with your mouth. Always use a rubber bulb or plastic pipetter.

16. Use the fume hood when toxic or flammable gases may be release or when instructed to do so.

17. Proper handling of equipment and chemicals used in an experiment is your responsibility. If you do not know how to operate equipment or handle chemicals, ask your instructor and read Materials Safety Data Sheets (MSD Sheets) provided for each experiment.

18. Use only the required chemicals in proper concentrations for the experiment. Reading labels is your responsibility. When in doubt ask your instructor.

19. Dispose of waste chemicals as instructed.

20. No inappropriate behavior or playing with equipment will be tolerated.

EMERGENCY FACILITIES

Your lab instructor will point out the following safety features:

1. **Shower**: If a large amount of a substance in spilled on you, stand under the shower and rinse thoroughly. It may be wise to remove some clothing.
2. **Eye Wash**: Should any substance be splashed in your eyes, hold your eyes open with your fingers and rinse for 15 minutes.
3. **Fire Extinguisher**
4. **First Aid Kit:** Located in Front of Room and Prep Room
5. **Fire Blanket**

WASTE DISPOSAL

The following regulations for waste disposal are **mandatory** for all Biology Labs

1. Regular garbage pails:
a. Unused paper and plastic
b. Used paper and plastic from water only
2. Red Medical Waste Containers:
   All used solid materials, including pipets, test tubes, slides and coverslips, gloves, paper towels used for spills, all microbiological waste.
3. Black Waste Containers:
   Solid Toxic Wastes, e.g., Ethidium bromide gels (Located in 1407)
4. Liquid Waste Containers:
a. Solvents: Alcohols, Acetone
b. Chlorinated Liquids: Chloroform, Buffers with Chloroform
c. Non-Chlorinated Liquids: Stains, dyes or other liquids, e.g., Copper Sulfate
d. Acids
e. Bases
5. Broken Glass Containers: For unused Broken Glass Only

   The appropriate containers will be available in each lab at places which will be designated at the beginning of the semester. You are responsible for disposing of all of your waste materials before you leave the lab. Any violation of the regulations will be recorded and acted upon.

6. Animal waste containers for sacrificed or preserved specimens. (Fresh animal carcasses must be frozen in appropriate disposal bags and placed in Morgue Freezer in prep. room).

NOTE: The only material you may pour down the drain is aqueous, non-toxic solutions, e.g., salts, buffers (pH 3-8), sugars, proteins or derivatives, food products, e.g., milk.
Students with disabilities who are enrolled in this course and who will be requesting disability-related accomodations should make an appointment with the Office of Disability Services, akelsen@yu.edu, (646) 592-4280 during the first week of class. Once you have been approved for accommodations, please contact me to ensure the successful implementation of those accommodations.

I _____ have been advised for the safety features and procedures of the biology laboratory. I agree to abide by all the above safety rules, and in particular to wear eye protection at all times when required.

DATE_____/_____/_____ NAME_____

SIGNATURE_____

Continuum Health Partners

Beth Israel

Yeshiva University Student Health

Lab Animal Exposure Questionnaire

Name_____ Date_____

Date of Birth _____ YU ID #_____

Gender: (Circle one) Male Female Phone _____ Email_____

Course_____ Campus_____

Category of animal contact:

1. Rodent ☐ Live ☐ Dead 2. Cat ☐ Live ☐ Dead 3. Other._____ ☐ Live ☐ Dead

Have you ever been told by a physician that you have:

1.	Asthma	Yes_____	No_____
2.	Allergic rhinitis (runny nose due to allergy)	Yes_____	No_____
3.	Allergic conjunctivitis (itchy watery eyes from allergy)	Yes_____	No_____
4.	A history of hay fever?	Yes_____	No_____
5.	A history of skin rashes?	Yes_____	No_____
6.	A natural parent or natural sibling with allergies to animals or their substances?	Yes_____	No_____
7.	Have you ever had a positive allergy skin test performed by a physician?	Yes_____	No_____

8. If yes to 7, how many positive skin tests to <u>non-animal</u> antigens (such as grasses, pollen, house dust) have you had?
   Circle the appropriate number: 0 1 2 3 4 5 or more

9. If yes to 7, how many positive skin tests to <u>animal</u> antigens (such as dog, cat, mice) have you had?
   Circle the appropriate number: 0 1 2 3 4 5 or more
   If known, which animal allergies:_____

10.	Do you have an allergy to medications/foods/chemicals?	Yes_____	No_____
11.	If yes to 10, what causes it? _____		
12.	Do you smoke?	Yes_____	No_____
13.	Do you work with any chemicals or fumes outside of school?	Yes_____	No_____
14.	When did you last receive a tetanus shot?	Date_____	

Do you have any of the following symptoms that you feel are caused by, or made worse, because of exposure to [laboratory] animals?

15.	Watery, burning or itchy eyes	Yes_____	No_____
16.	Runny nose	Yes_____	No_____
17.	Sneezing	Yes_____	No_____
18.	Wheezing	Yes_____	No_____
19.	Cough	Yes_____	No_____
20.	Shortness of breathe	Yes_____	No_____
21.	Chest tightness	Yes_____	No_____
22.	Hives	Yes_____	No_____
23.	Rash	Yes_____	No_____
24.	Other (specify)	_____	
25.	Would you like to talk to the health care professional who will review the questionnaire about your answers?	Yes_____	No_____

Please return this form to your professor who will forward it to the Health office on campus.

DD.Mv-07.06

LAB 1

MICROSCOPE & CELLS

Goal: Make a calculated prediction using what you know about microscopes and light. Enhance sample contrast with chemical staining. Witness cell diversity.

Pre-Lab Question:

How does magnification and resolution differ?

_____

_____

Microscopes

The microscope is a central tool in biology. The unaided eye cannot detect anything smaller than 0.1 millimeters (mm) in diameter. A light microscope enables us to see objects as small as 0.1 micrometers (μm) in diameter. The electron microscope further extends our viewing capability down to 1 nanometer (nm). At this level, it is actually possible to see the outlines of individual protein or nucleic acid molecules. Needless to say, microscopy has greatly improved our understanding of the normal and pathological functions of organisms.

Good **magnification** and resolution are needed for clear **visualization**. The distinction between resolution and magnification can best be illustrated by an analogy: If a photograph of a newspaper is taken from across a room, the resulting photograph would be small, and the print is too small to read. Dramatically enlarge that photograph and the print is still illegible because it's pixelated. To read the print, you'd have to enlarge the photo until it is more or less the size of a billboard (impractical).

The theoretical limit for the resolving power of a microscope depends on; 1) light wavelength and, 2) numerical aperture of the lens, multiplied by a constant (0.61). Aperture is determined using an equation that measures the light cone hitting the specimen. If all other factors are equal, we can increase resolving power by reducing wavelength. Microscopes are often equipped with blue filters because blue light has the shortest wavelength in the visible spectrum.

Minimum distance at which a specimen can be resolved =wavelength numerical aperture x 0.61
Example: if green light with a wavelength of 500 nm is used and the numerical aperture is 2.0, then the theoretical resolving power is 153 nm (or 0.15 μm).

Even with sufficient magnification and resolution, a specimen can only be seen if there is sufficient **contrast** between the structures that are within the specimen. Contrast the differential absorption of light within the specimen. Chemical staining can improve contrast. We will do some today and you will use staining many times in advanced classes. A microscopist may improve contrast by the use of stains that

bind to cellular structures and absorb light to provide contrast. Some stains are specific for certain chemicals and only bind to structures composed of those chemical. Others are nonspecific and stain all structures. The study of staining is called cytochemistry or histochemistry.

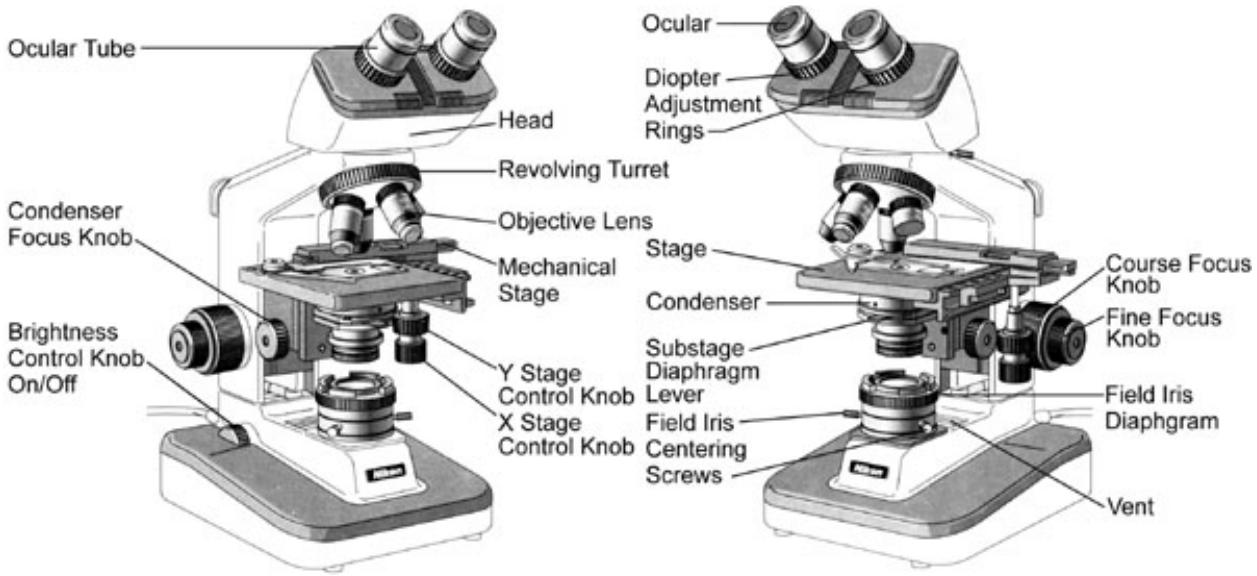
To summarize, microscopy is dependent on three variables: resolution, magnification and contrast.

Handling

1. Carry microscope in upright position using both hands, as shown. One hand supporting the base and the other on the neck.
2. If cord is not secure, wrap it before removing it from cabinet.
3. Set down gently. Keep scope on a cotton pad if one is provided (both in cabinet, and on lab bench). Keep away from counter edge.
4. Clean scopes when done. Do not leave a wet, stained or watermarked scope. Clean with the Kimwipes and ethanol from spray bottle unless otherwise instructed. Do not use paper towels on glass surfaces. Remember to turn the scope off. If instructed, you must return scope to storage in an orderly fashion. Wrap the cord, tucking in the loose end. Replace the soft cover. It should have space around it in the cabinet (not crowded).
5. Acquire a pre-made practice slide.

The Microscope

You will be using a Nikon AlphaPhot 2. There are hard copies of AlphaPhot 2 manuals in the lab and good instruction is published online. Use the terms below to communicate with each other, with instructors and in your write up if you find yourself describing processes in detail.



The *coarse adjustment* knob raises and lowers either the body tube or stage (depends on model) to focus the optics on the specimen. This rack and gear mechanism facilitates a relatively large movement with only a partial revolution of the adjustment knob. Use the coarse adjustment only with the scanning (4X) and low (10X) power objectives. If you are nearsighted or farsighted, there is no need to wear your glasses. If you have a stigmatism, you should wear your glasses because microscope lenses do not correct for this problem.

Microscopy Exercises

Image is shaped by orientation, magnification, field of view, brightness, focal plane, and contrast. The following activities are designed to familiarize you with your microscope. Use the prepared letter "e" slide in your slide box. Experiment with orientation. The total magnification of a microscope is the product of the magnification of the objective and the ocular. If the objective lens has a magnification of 5X and the ocular 12X, then the image produced by these two lenses is 60 times larger than the specimen.

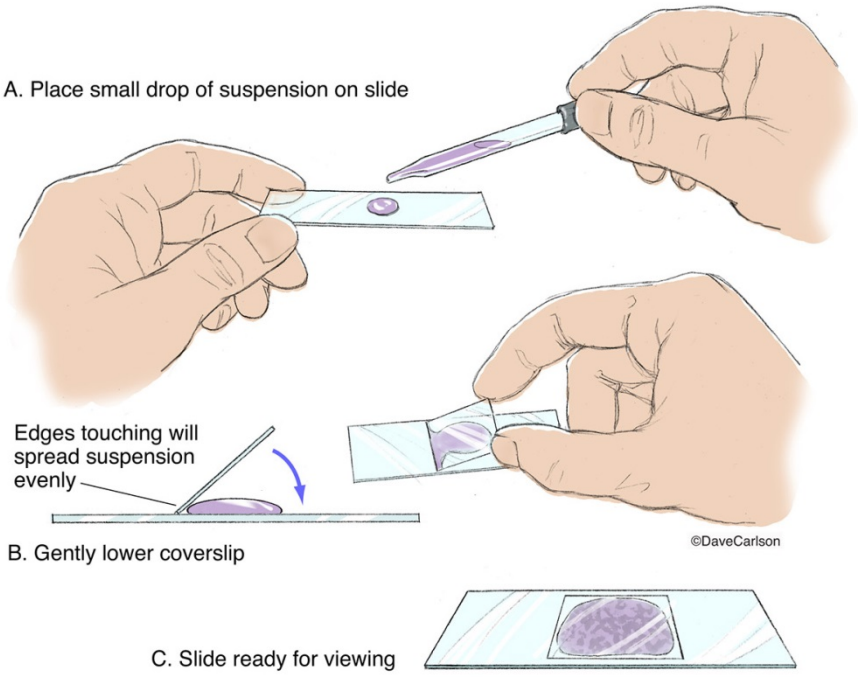
Procedure:

Learn to make wet mount slides and practice bringing samples into focus. The secondary objective is to gain exposure to the diversity of life forms that are around us.

You will collect water from three aquariums and study drops of it under the microscope. Each tank contains at least two species that are exclusive to that tank. You will succeed at this exercise by recognizing the exclusive species in a live sample using the biodiversity profiles below.

The exclusive species are abundant enough for you to be able to find them quickly, but prepare to make more than one slide for each sample as one may not be enough.

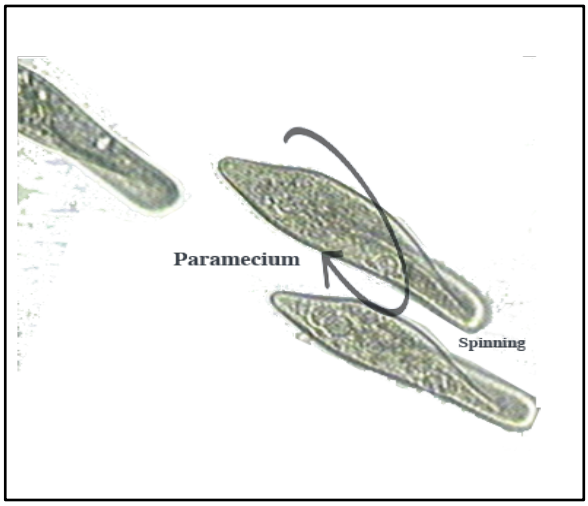
In your lab report, you will identify the water sources based on the profiles here. You will also compare and contrast sample biota by description and illustration. You must illustrate at least three species. They may be from any sample.

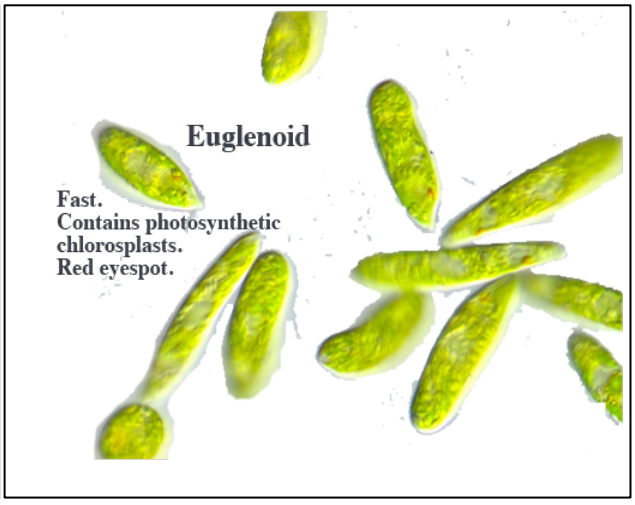


Procedure For Mounting a Wet Slide.

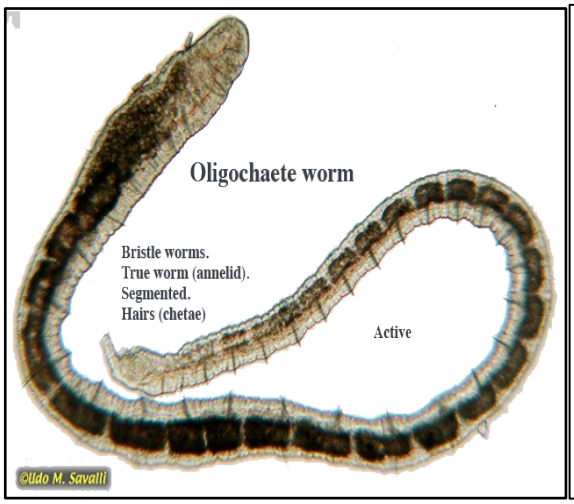
a) Place a drop of water on a clean slide
b) Place specimen in water
c) Place one edge of coverslip against the water drop and lower it onto slide. Try to avoid trapping air bubbles.

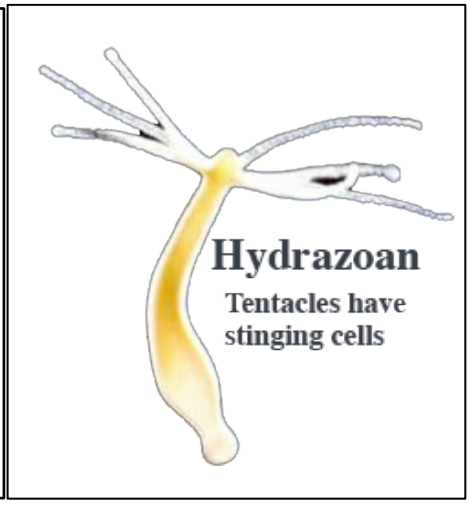
<u>Exclusive To Water Source 1:</u>

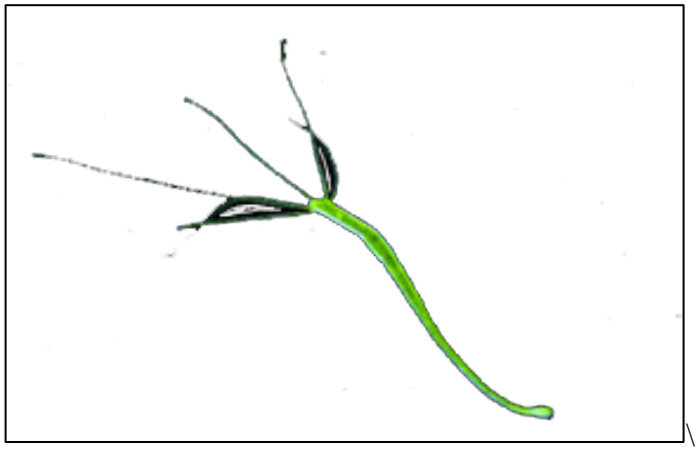




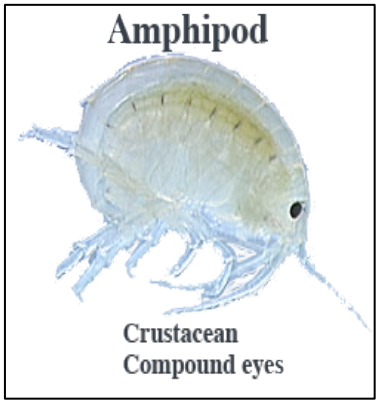
Exclusive To Water Source 2:

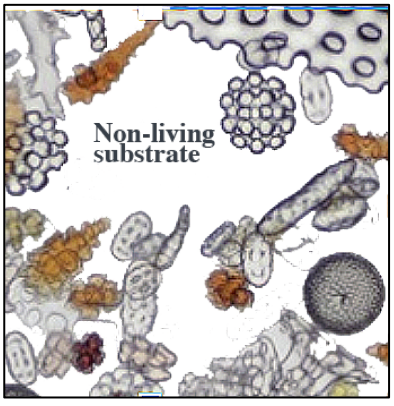




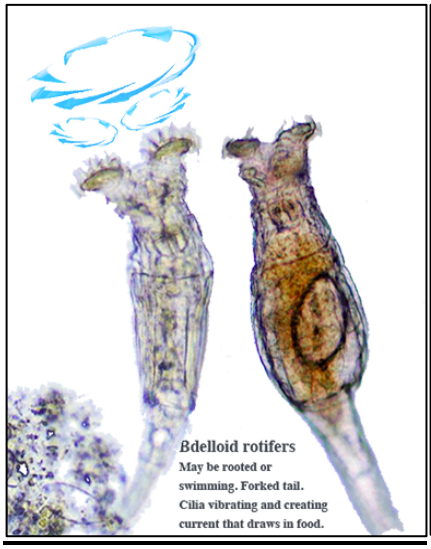


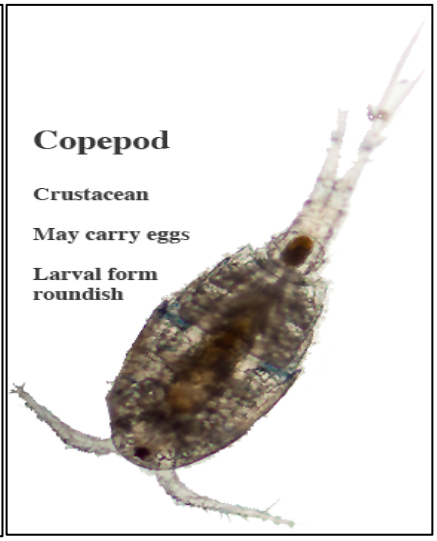
Exclusive To Water Source 3:

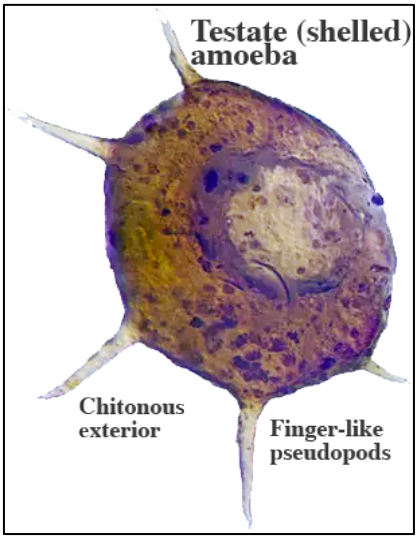


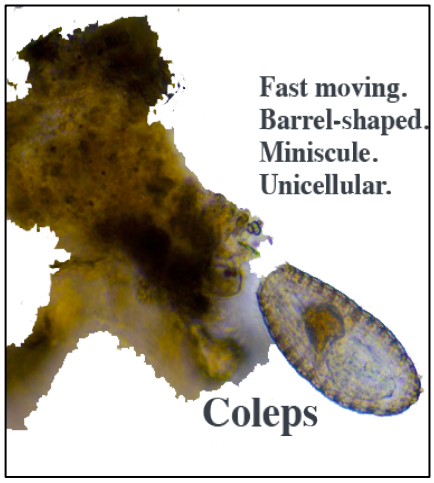


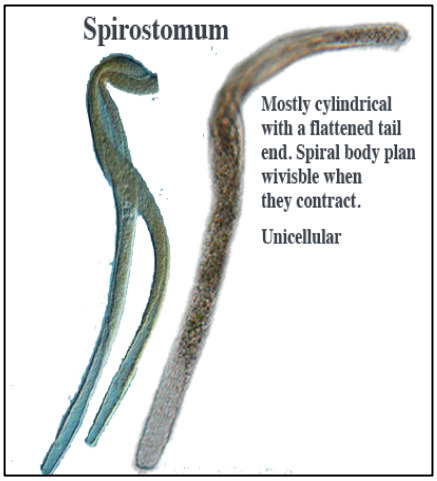
Present In Multiple Sources:

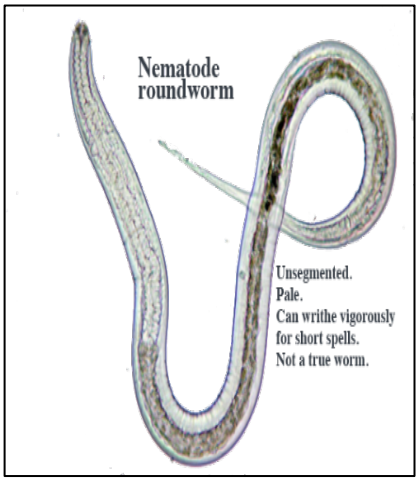












LAB 2

PROPERTIES OF ENZYMES

Goal: To determine what affects the reaction rates of enzymes

Pre-Lab Questions: (*1-3 sentences each. Have answers written before class*).

1. What category of biochemicals do most enzymes belong to? _____
2. What is the general function of an enzyme?

   _____

   _____

   _____
3. What physical aspect of an enzyme molecule determines what substrates it helps react?

   _____
4. What are two things that can speed up or slow down the reaction rate of a chemical reaction being assisted by an enzyme (use your own logic)?

   1._____

   2._____
5. Do enzymes get used up or not when they perform their function? _____
6. Look up and define the following terms:

a. Substrate_____

b. Active site_____

c. Cofactor_____

In today's lab we will measure the reaction rate of the following chemical reaction catalyzed by the enzyme **peroxidase**:

$2H_2O_{2\,(l)} \rightarrow 2H_2O_{\,(l)} + 2O_{2\,(g)}$

Since there is no visual difference between hydrogen peroxide and water, it would not be possible to visually assess the progress of the chemical reaction without a second reaction. We will use a dye called **guiacol** that changes color (from clear to brown) in the presence of oxygen. This is called a **dye-coupled reaction.**

Hypothesis: Using the dye coupled reaction, how would you compare the reaction rates of the decomposition of hydrogen peroxide using peroxidase under different conditions? What would you be looking for_____

_____

_____

Tools and Techniques:

Pipette and Pipettor: These are instruments for measuring precise volumes of liquid. Notice the order of the numbers on the side of the pipette. We will be using a manual pipettor, which works by forming a vacuum. To draw up liquid, use the thumbwheel. To dispense a particular volume of liquid, use the thumbwheel in the opposite direction. To dispense all the liquid, squeeze the trigger on the side to break the vacuum. Then push down on the top platform to get any residual liquid out. Your instructor will demonstrate this.

Spectrophotometer: This device allows for the measurement of light absorption and light transmission. It contains a filter that lets you control the precise wavelength (color) of light that will pass through the sample. Useful for determining the concentration of a solution.

Before continuing with our experiment to determine what affects the enzymatic activity of peroxidase, we will do a short experiment to practice using the spectrophotometer to determine the concentration of a solution.

Experiment #1: Determining the concentration of a solution using a spectrophotometer

Question: What do you think the relationship between light transmission through a sample and light absorbance by a sample?

_____
_____
_____

Hypothesis: If one has two solutions of the same solutes and solvents but at different concentrations (one is more concentrated than the other), which will have a) higher transmission of light and which will have b) higher absorbance of light?

_____
_____
_____
_____

Your instructor will play a short video at this point which demonstrates the way a spectrophotometer works.

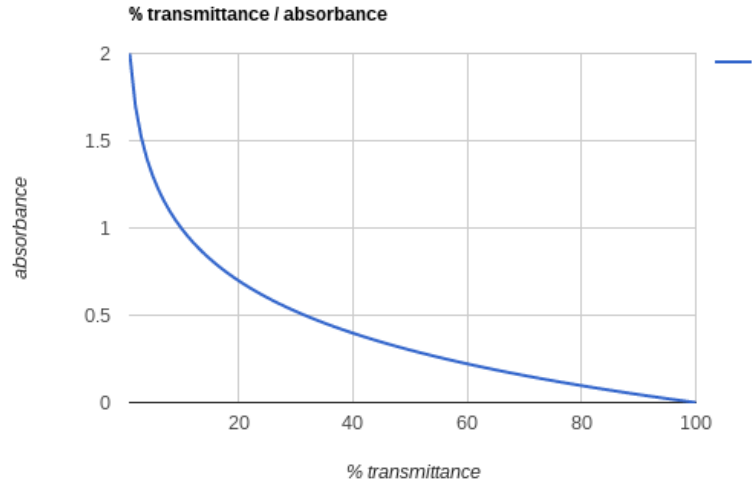
% Transmittance= % of intensity of light that's getting transmitted through sample compared to that of pure solvent

Absorbance= Amount of light being absorbed. It is calculated as

Absorbance = -log (%transmittance/100)

thus 100% transmittance = -log 1= 0 absorbance and 1% transmittance = -log 0.01 = -(-2) = 2

Although you can have a dark solution that transmits less than 1% of the light shining on it and thus have an absorbance higher than 2, our machines cannot accurately measure that high an absorbance. We therefore need to make sure the absorbance values we use are 2 or lower. See graph below for relationship between % transmittance and absorbance



Our spectrophotometers will do the calculation for you automatically.

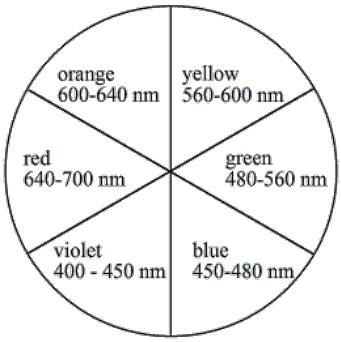
Beer's Law:

Light absorbance by a solution is directly proportional to its concentration.

Which would be darker: a teaspoon of KoolAid dissolved in 500 liters of water or 2 teaspoons of KoolAid dissolved in 1 liter of water? Note that darkness=light absorbance

<u>Pre-Activity Questions</u>:

1. **Picking The Correct Wavelength Of Light To Use**



Look at the color wheel. If colors on opposite sides absorb each other highest, which color light and wavelength range would be best for solutions of the following colors?:

a. Red: color_____ wavelength_____nm
b. Yellow: color_____ wavelength_____nm
c. Green: color_____ wavelength_____nm
d. Blue: color_____ wavelength_____nm

2. We will be using blue dye today. Based on your answers to question 1, which of the following wavelengths of light would work best for measuring absorbance? *Circle one*

a. 580 nm
b. 410 nm
c. 625 nm
d. 700 nm

Procedure:
Part A: Making Dilutions

1. Label the test tubes provided on the **top** of the test tube (towards the opening), **not the bottom.**

B , 1 , 2 , 3 , 4 , U

2. Use a pipette to measure out the volumes of **water** listed in the table below:
Note: leave "**U**" empty for now

3. Use a pipette to measure out the volumes of **1x solution (stock solution)** listed in the table below:
Note: leave "**U**" empty for now

4. Use the formula $c_1v_1=c_2v_2$ to calculate the new concentrations (c_2) of each dilution in in the table below.
C_1= 1x
V_1= Volume of 1x solution
V_2= Total volume of the new solution

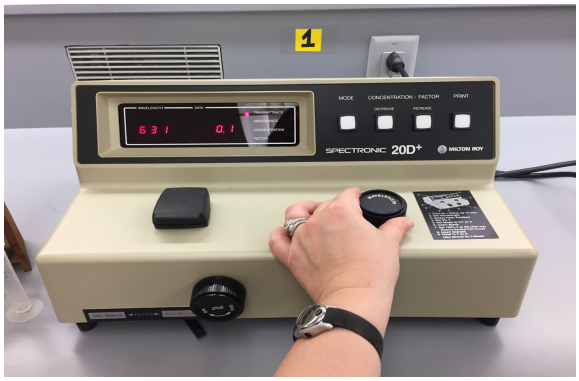
Calculate and fill in concentrations in table below

	Volume of water	Volume of 1x solution (v_1)	Total volume of new solution (v_2) Volume of water + volume of 1x solution	**Concentration of new solution (c_2) in terms of x (eg 0.1 X)** **$c_1v_1=c_2v_2$**
B (blank)	5 ml	0 ml	5 ml	
1	4 ml	1 ml	5 ml	
2	3 ml	2 ml	5 ml	
3	2 ml	3 ml	5 ml	
4	1 ml	4 ml	5 ml	

5. Fill tube "**U**" with two pipets full from "**unknown**" solution. This solution is the same food dye as used in the other tubes, but we don't know the concentration.

Part B: Setting Up and Calibrating the Spectrophotometer (*older model*)

1. Set the wavelength to what you chose in pre-lab question #2 using the upper dial as pictured below: (*the leftmost display indicates the wavelength in nm*)



2. Push mode button until "*percent transmittance*" is selected

3. With no sample inside, a metal shutter completely blocks all light from shining on the detector. If all light is completely blocked, what percent of the light is being transmitted? _____ %

4. Use the lower **left** dial to adjust the percent transmission reading until you get the % transmittance you answered in question #3. This is **calibrating** the spectrophotometer.

5. Insert your **blank** (test tube "B") into the sample holder.

6. Since the blank is pure solvent, the full amount of light will shine through compared to any of the solutions, which will be darker and absorbing light. What percent of light is being transmitted through the blank if all the light gets through?

_____ %

7. Set transmittance reading to the % transmittance you answered for question #6 using the **right** lower dial. The spectrophotometer is now calibrated for your wavelength.

8. Press the "mode" button once to put it in Absorbance mode.

Part C: Measuring Absorbance of dilutions and unknown

1. With blank still in holder, record the absorbance of pure water in table below
2. Remove blank and return to test tube rack. Insert test tubes 1, 2, 3, 4 and U in sequentially and record the absorbance for each. **DO NOT ADJUST DIALS OR YOU WILL NEED TO RECALIBRATE.** Simply insert the test tube, record the absorbance, remove the test tube, and insert the next one.

Test tube	Concentration (*copy from table above*)	Absorbance
B (*blank*)		
1		
2		
3		
4		
U (*unknown*)	?	

Cleanup: Leave spectroscope on. Remove last tube from spectroscope. Pour all contents of test tubes into sink, rinse out test tubes in sink, shake out water and dry off outside of test tube with paper towel.

Analysis:

1. Graphing concentration vs absorbance

On google sheets or excel, enter the data for concentration and absorbance and graph as scatter plot (concentration should be x axis, absorption should be y axis).

2. Make a graph (for chart types, choose "scatter") and insert a linear trendline with formula.

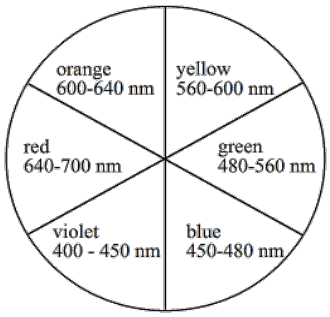
3. Title the x axis "concentration in x" and the y axis "absorbance". Title the graph "Concentration vs Absorbance"

4. Locate on the y axis the absorbance value you got for the unknown (U) and determine, using the trendline and the x axis, what the concentration of the unknown solution was.

Based on the x intercept, what is the concentration of the unknown? _____X

Application:

1. If we had been measuring concentrations of chlorophyll (green) instead of blue food dye, what would have been a good wavelength of light to use?



_____nm

2. How could we use a spectrophotometer to determine reaction rates in a dye-coupled reaction?

_____
_____
_____
_____

Experiment #2 Determining optimal volume of enzyme extract to use

Now that we have learned how to use the spectrophotometer, the instructor will make an extract of peroxidase for the class to use using turnip and buffer to stabilize the pH.

Preparing an Extract Containing Peroxidase (instructor only)

1. Weigh 1g of peeled turnip, horseradish, or potato tissue on a balance. Cut into pieces.
2. Homogenize the tissue by adding it to 100ml of cold (4^{o}C) 0.1M phosphate buffer at pH 7. Grind the mixture in a cold mortar and pestle with sand or blend it for 15 seconds at high speed in a cold blender. The extract will keep for about ten hours in a refrigerator.

The extract that was prepared contains hundreds of different types of enzymes, including peroxidase. The activity of each enzyme will vary, depending on the size and age of the turnip, horseradish, or potato; the extent of the tissue homogenization; and the age of the extract. Only peroxidase, however, will react with H_2O_2.

Activity: We will now determine the correct amount of extract to use in future experiments by performing a trial run in which the only variable is the amount of enzyme added.

1. Obtain the following solutions from supply area: peroxidase extract made by instructor; buffer (pH 5); 10mM H_2O_2 (the substrate); and 25mM guaiacol.

2. Number seven test tubes from 1 to 7.

Note: Pairs 2 and 3, 4 and 5 or 6 and 7, will be quickly mixed together when it is time to measure a reaction. Mix a pair only when you are ready to measure that reaction in the spectrophotometer. The exact quantities to be added to each tube are listed in the table.

1. Add stock solutions to each tube using the corresponding graduated 5ml pipette. **Do not reuse the same pipette for different reagents. This will contaminate the reagents and ruin them.**
   Mixing table for trial run to determine extract concentration (all values in ml)

	pH 5 buffer	H_2O_2	Peroxidase extract	Guaiacol (dye)	Total volume
Tube #1 (control)	5.0 ml	2.0 ml	0 ml	1.0 ml	8 ml
Tube #2	0	2.0	0	1.0	3
Tube #3	4.5	0	0.5	0	5
Tube #4	0	2.0	0	1.0	3
Tube #5	4.0	0	1.0	0	5
Tube #6	0	2.0	0	1.0	3
Tube #7	3.0	0	2.0	0	5

2. Set the wavelength of light on the spectrophotometer 500nm (this is optimal for guaiacol). Use test tube #1 to "blank" the spectrophotometer, so that any color caused by contaminants in the reagents will not influence subsequent measurements.
3. Prepare a stopwatch on your phone or another device. Have lab partner operate it while experiment is being performed.
4. Start stop watch as partner mixes the contents of tubes 2 and 3 by pouring them back and forth twice. Mixing should be completed within 10 seconds.
5. Quickly wipe the outside of the tube and place it in the spectrophotometer. Read the absorbance at 20 second intervals from the start of mixing. If you are a little late in reading the meter, record the absorbance and the actual time. Record your measurements in table below. After two minutes (six readings) remove the tube from the spectrophotometer and visually note the color change. Discard the solution.
6. Mix the contents of Tubes 4 and 5, and repeat your measurements for two minutes at 20-second intervals. Record the results in your table
7. Mix the contents of Tubes 6 and 7, and record the absorbance measurements

Record Absorbance On This Table:

	Tubes 2+3	Tubes 4+5	Tubes 6+7
20 sec			
40 sec			
60 sec			
80 sec			
100 sec			
120 sec			

Now plot the values from the table on graph paper. The x axis should be the independent variable (time) and the y axis the dependent variable (absorbance). Plot all three tests on the same graph using google sheets or excel.

Which volume of enzyme extract gave a linear absorbance change from 0 to 1 in approximately 120 seconds? Use this amount in all subsequent experiments in this exercise.

Volume that gave linear absorbance change from 0 to 1 in approximately 120 seconds: _____ml

Experiment #3 Factors Affecting Enzyme Activity

Now that we have learned how to use a spectrophotometer and have determined the optimal concentration of enzyme extract to use in our experiment, we can perform the main activity of this week's lab and determine what factor affect enzyme activity and how.

If the scheduled laboratory period does not allow enough time, your instructor may divide you into teams, each of which will tackle one of more of the following experimental variables. The results will be shared at the end of the lab period and must be included in your report.

A. Temperature Effects

Four temperatures are available for you

1. In ice at approximately 4°C
2. At room temperature (about 23°C)
3. At 32°C
4. At 48°C

DV= determined volume of enzyme extract (from Experiment #2)

Mixing Table for trial Run to Determine Extract Concentration (all values in ml)						
Temperature	Tube	Buffer (pH 5)	H2O2	Extract	Guaiacol (dye)	Total volume
-	1 Control	7-DV	0	DV	1.0	8
4°C	#2 #3	0 5-DV	2.0 0	0 DV	1.0 0	3 5
23°C	#4 #5	0 5-DV	2.0 0	0 DV	1.0 0	3 5
32°C	#6 #7	0 5-DV	2.0 0	0 DV	1.0 0	3 5
48°C	#8 #9	0 5-DV	2.0 0	0 DV	1.0 0	3 5

Number nine test tubes in sequence 1 through 9. Refer to the table reagents to be added to each tube.

Pre-incubate all the solutions at the appropriate temperatures for at least 15 minutes before mixing. After reaching temperature equilibrium and adjusting the spectrophotometer with the contents of test tube 1,

mix pairs of tubes (2 and 3, 4 and 5, 6 and 7, 8 and 9) one pair at a time and measure changes in absorbance for two minutes at 20-second intervals for each temperature. The temperatures will not remain exact, but the effects of cooling or warming can be overlooked. (Keep your tubes at the appropriate temperature until before mixing and reading)

Record changes in absorbance for each temperature in a table

These results should be graphed at the end of the laboratory period. The slopes of the linear portions of these curves are a measure of enzyme activity. Does activity vary with temperature? Your room temperature values should be consistent with the values in the previous experiment using the same concentration of enzyme.

B. Effect of Boiling on Peroxidase Activity

Most proteins denature at temperatures above 70˚C. Denaturation is a non-reversible change in a protein's three-dimensional structure, leading to a loss of catalytic ability.

To show that the color changes you observed in the other experiments are due to an enzyme, you should add 3ml of extract to a test tube and place it in a boiling water bath. After five minutes, remove the tube and let it cool to room temperature. Number three test tubes and add reagents as called for in the mixing table.

Use the contents of tube 1 to blank the spectrophotometer. Mix the contents of Tubes 2 and 3, and read the absorbance at 20-second intervals for two minutes. Record the results in a table.

Describe the effect of boiling on the activity of peroxidase.

DV= determined volume of enzyme extract (from Experiment #2)

Mixing table for boiling extract (all values in ml)					
Tubes #	Buffer (pH 5)	H2O2	Boiled extract	Guaiacol	Total volume
#1 (control)	7-DV	0	DV	1.0	8
#2	0	2.0	0	1.0	3
#3	5-DV	0	DV	0	5

C. Effect Of Different pH Levels

Your instructor will supply you with buffers at pH's 3, 5, 7 and 9. Number nine test tubes in sequence and label 1 through 9. Set up pH-effects by adding the reagents described in table.

After adjusting the spectrophotometer with the contents of test tube 1, mix pairs of tubes one at a time (2 and 3, 4 and 5, 6 and 7, 8 and 9) and measure absorbance changes at 20-second intervals for two times. Record the results

The values in this table should be graphed at the end of the laboratory period. The slopes of the linear portions of these curves are a measure of enzyme activity. Does activity vary with pH? Describe the relationship.

DV= determined volume of enzyme extract (from Experiment #2)

Mixing Table for pH experiment (all values in ml)						
pH	Tube	Buffer	H_2O_2	Extract	Guaiacol (dye)	Total volume
5	#1 (control)	7-DV	0	DV	1.0	8
3	#2	0	2.0	0	1.0	3
	#3	5-DV	0	DV	0	5
5	#4	0	2.0	0	1.0	3
	#5	5-DV	0	DV	0	5
7	#6	0	2.0	0	1.0	3
	#7	5-DV	0	DV	0	5
9	#8	0	2.0	0	1.0	3
	#9	5-DV	0	DV	0	5

D. The Effects of Inhibitors

Hydroxylamine (NH_2OH) has a structure similar to hydrogen peroxide (HOOH, or H_2O_2). This molecule binds with the iron atom at the active site or peroxidase and inhibits enzyme activity.

To test this effect, mix five drops of 1% hydroxylamine (neutralized to pH 7) and 2ml of enzyme extract, letting the mixture stand for at least five minutes. Then measure the peroxidase activity, comparing the

activity in this created enzyme preparation to that of the enzyme without the inhibitor. The table lists the proportions of each solution to be used for the tests.

After adjusting the spectrophotometer with the contents of test tube 1, mix parts one at a time (2 and 3, 4 and 5) and measure the changes at 20-second intervals for two minutes. Record your measurements

Mixing table for inhibitor experiment (all values in ml)						
Tube 1 Control	Buffer (pH 5) 6.0	H2O2 0	Extract 1.0	Guaiacol 1.0	Hydroxylamine and extract 0	Total volume 8
2 3	0 4.0	2.0 0	0 1.0	1.0 0	0 0	3 5
4 5	0 4.0	2.0 0	0 0	1.0 0	0 1.0	3 5

The data should be graphed at the end of the laboratory period. The slopes of the linear portions of the curves are a measure of enzyme activity. Explain why slopes differ.

Additional Questions

1. In all these experiments, tube 1 has been listed as a control. What is it controlling for? No reaction happens in this tube. Why was it included?
2. Why did the boiling reduce the activity of peroxidase?
3. Explain the basis of the dye-coupled assay used in this experiment.

LAB 3

RESPIRATION & FERMENTATION

Goal: To perform experiments that demonstrates aerobic and anaerobic respiration. This lab activity will be divided into two sections:
Part 1 will analyze the waste product of aerobic respiration and respiration rate changes in response to exercise.
Part 2 will assess the ability of yeast to ferment different materials.

Pre-Lab Questions:

1. What is the difference between anaerobic and aerobic respiration?

_____
_____
_____
_____

2. How many ATP molecules are produced in total by anaerobic respiration? Aerobic?
a. Anaerobic:____ATP molecules
b. Aerobic:____ATP molecules

3. Where in the cell does anaerobic respiration take place? Aerobic?
a. anaerobic: _____
b. Aerobic: _____

4. Balance the following overall chemical reaction for fermentation in yeast:
$C_6H_{12}O_6 \rightarrow$___C_2H_5OH + ___CO_2

5. Identify the compounds in the fermentation reaction by name:
a. $C_6H_{12}O_6$ _____
b. C_2H_5OH_____
c. CO_2_____

6. Balance the following overall chemical reaction for aerobic respiration:
$C_6H_{12}O_6$ +__$O_2 \rightarrow$___H_2O + ___CO_2

7. How does CO_2 affect the pH of water when it is dissolved in it? Does it make the water more acidic or more basic?

_____
_____

8. What is one way we could measure the rate of fermentation of a sugar by yeast? Be creative!

_____

_____

_____

Experiment #1: Aerobic Respiration

CO_2 reacts with water to form a weak acid known as carbonic acid in the following reaction:
$CO_2 + H_2O \rightarrow H_2CO_3$

In fact, this reaction is how your brain knows whether you need to breath. Your medulla detects a change in pH based on the buildup of CO_2 in your blood. It cannot detect oxygen deprivation.

We will be looking at the following comparative amounts of CO_2:

a. in atmospheric air
b. in resting exhaled breath
c. exhaled breath following exercise

Hypothesis: What will the order be of concentration of CO_2, from lowest to highest, in the above scenarios? Why?

_____

_____

_____

_____

_____

Comprehension: Why are we measuring the level of CO_2 in atmospheric air? What stage does it represent in aerobic respiration?

_____

_____

_____

_____

To measure CO_2 levels, we will use a pH indicator called bromothymol blue (BTB). We will perform a quick experiment to understand how to use a pH indicator and how to determine its range.

Experiment 1a: pH Indicator experiment

Procedure:

1. Dispense, using a 5 ml pipette, 1 ml of BTB into 3 different wells (1 ml each).
2. In well #1, add 1 drop of distilled water (pH=7). Record color below
3. In well #2, add one drop of hydrochloric acid (HCl, pH=~1). Record color below
4. In well #3, add one drop of sodium hydroxide (NaOH, pH=~10). Record color below

BTB in presence of...	Color
Distilled water	
HCl	
NaOH	

Analysis: Based on your observations:

1. When the ph is___ is it an acid, base, or neutral:
a. ph<7_____
b. pH=_____
c. pH>7_____
2. BTB will be the following colors:
a. In the presence of an acid: _____
b. In the presence of a base: _____
c. When neutral: _____

Experiment 1b: Carbon Dioxide levels in aerobic respiration

Now that you have seen how BTB can measure the pH of a solution, we will use it to measure CO_2. As seen above, it will produce carbonic acid when dissolved in water.

Hypothesis:

1. If CO_2 is bubbled through BTB, what color do you expect it to change to? _____

2. It would be very hard to determine the quantity of CO_2 passed through the BTB by just looking at it, as the color change tells you it is either above or below pH=7, but not by how much. We have NaOH, a base, available. How might you use NaOH to **quantify** how much CO_2 was bubbled through the BTB?_____
_____
_____
_____

Safety: In this activity, you may be blowing through a straw into chemicals. DO NOT INHALE THROUGH THE STRAW! Breathe in through your nose and exhale through your mouth. If you accidentally swallow liquid, rinse your mouth thoroughly and drink plenty of water. Be sure to tell your professor.

Procedure:

Part 1: Using BTB to Test for Carbon Dioxide

1. Add 5ml water to each of the five larger cups (A-E) of a SEPUP tray.

2. Add 2 drops of BTB to each cup and stir
3. Record the initial color of each solution in Table 1, "Testing for Carbon Dioxide."
4. Use the dropper to bubble air into Cup B. Place the dropper into the solution and press the air out of the bulb. Before releasing the bulb, remove the tip from the solution. This will prevent uptake of solution into the dropper. (If you accidentally get solution into the dropper, simply squirt it back into Cup B). Repeat this for 15 seconds.
5. Record the final color of the solution in Cup B in Table 1
6. Have one person from your group unwrap a straw and place one end in Cup C. this person should take a deep breath and then gently blow through the straw for 15 seconds. (Remember not to inhale through the straw!). record the final color of the solution in Cup C in Table 1
7. Have another person from your group blow through a clean straw into Cup D for 15 seconds. (Remember not to inhale through the straw!) Record the final color in Table 1.
8. Add 3 drops of sodium hydroxide to Cups C and D. record any changes that you observe in Table 1.

Table 1: testing for Carbon Dioxide

Cup	Initial BTB Color	Final BTB Color	Observations after adding sodium hydroxide
A (control)			
B (air)			
C (person 1: exhaled breath)			
D (person 2: exhaled breath)			

9. Work with your group to answer the following questions:

a. What was the purpose of the solution in Cup A?

_____

b. Which of the solutions in Table 1 contained carbon dioxide? Support your answer with evidence from your experimental results.

---

c. What can you conclude about the amount of carbon dioxide in the inhaled vs. exhaled breath of human beings? Hint: Think about which solution in Table 1 was similar to a person's inhaled breath, and compare the results. _____

_____

Part 2: Using BTB to measure carbon dioxide in exhaled breath, resting vs exercise

1. Work with your group to set up a control
a. Measure 10 ml of water using the 30ml graduated cup
b. Add 3 drops of BTB to the graduated cup and stir
c. Pour the BTB solution into a beaker. This solution will be the control for each pair in your group
2. set up a bag of BTB solution:
a. Measure 10 ml of water using the 30 ml graduated cup
b. Add 3 drops of BTB to the graduated cup and stir
c. Pour the BTB solution into a 1-gallon plastic bag.
3. Remove the air from the plastic bag by slowly flattening it. Be careful not to spill any of the BTB solution out of the bag. While keeping the air out of the bag, place a straw in the mouth of the bag. Make an air-tight seal by holding the mouth of the bag tightly around the straw.
4. Decide which person in your pair will be blowing into the bag. He should sit down and then fill the bag with air from his lungs by blowing through the straw until the bag is fully inflated. (Remember not to inhale through the straw!) When the person has finished blowing, pull out the straw while making sure to squeeze the bag tightly shut so that no air escapes
5. Holding the bag closed, shake the bag vigorously 25 times
6. Pour the BTB solution from the bag into a beaker.
7. How much carbon dioxide is in exhaled breath? You can find out by counting how many drops of sodium hydroxide are needed to bring the color of the BTB solution to that of the control

a. Add 1 drop of sodium hydroxide to your beaker
b. Gently stir the solution and wait at least 10 seconds
c. Record that you added 1 drop: _____

d. Compare the color of your solution to the control. Is it the same color as the control for at least 30 seconds? If your answer is no, repeat steps 7 a –d. be sure to keep track of the total number of drops inline 7c. If your answer is yes, go on to step 8.
8. Record the total number of drops it took to change your solution back to the same color as the control: _____ then record your total on the class data table.
9. Repeat steps 2-7, this time exercising (do 20 jumping jacks in the hall) before breathing into the bag.
10. Record the total number of drops it took to change your solution back to the same color as the control: _____ then record your total on the class data table.

Class Data Table

Student	# of drops of NaOH for resting exhalation	# of drops of NaOH for post-exercise exhalation
#1		
#2		
#3		
#4		
#5		
#6		
#7		
#8		
#9		

Questions

1. Review the class data table.
a. What was the **average** of carbon dioxide in exhaled breath (as measured by drops of sodium hydroxide) for resting? Post-exercise?

Resting_____ Post-exercise_____

b. What was the ***range*** of carbon dioxide in exhaled breath (as measured by drops of sodium hydroxide) for resting? Post-exercise?

Resting_____ Post-exercise_____

2. a. Were the data collected in Part One qualitative or quantitative?

Explain_____

_____

b. Were the data collected in Part Two qualitative or quantitative?

Explain_____

_____

3. Look back at your hypothesis regarding CO_2 concentrations in atmospheric, resting breath, and post-exercise breath. Based on your averages, was your hypothesis correct?

Explain_____

_____

_____

_____

Experiment #2: Yeast Fermentation

In this experiment you will test to see what types of sugar substitutes yeast ferments.

Fermentation Experiment

1. Obtain six test tubes and six 5ml pipettes without cotton plugs. Label your tubes water, sucrose, fructose, lactose, lactose + lactase, and sweet n' low.
2. Place a plug of clay into the pointed tip of each pipette by jabbing the pointed tip into clay.
3. Turn the pipettes upside down (pointed tip up) and place each into a test tube. Set them aside.
4. Mix one package of yeast with 10 ml of distilled water in a plastic bottle. (The yeast will not dissolve) keep the yeast suspended by swirling.
5. Make 10% concentration by mass solutions (remember, water has a density of 1 g/ml) of the following: sucrose (tablet sugar), Splenda, fructose, aspartame, (Equal), and saccharin (Sweet n Low). You will need 10 ml of each solution.

a. **Calculate**: How many grams of each solute will you need to make 10 ml of 10% solution? ____grams

Use a balance and weigh boat/weigh paper to measure mass. Pour each powder into its labeled test tube. Note: for the lactose+lactase tube, add the calculated amount of lactose as in the lactose-only tube. Then take a "Lactaid" tablet, crush it into a powder, and add that powder to the tube as well.

6. Remove the 5ml pipette (stopped with clay) from the tube labeled "water." In the test tube, mix 5ml of yeast solution with 5ml of distilled water. Mix and fill the 5ml pipette to the brim with this solution using a Pasteur pipette. (How much solution will be left in the test tube?)
7. Put your finger over the top of the 5ml pipette, invert it so that the clay tip is pointing upward, and place into the solution in the same test tube. Try not to let any air bubbles get into the pipette. (as this procedure is a little tricky, make sure you have your instructor demonstrate it for the class)
8. Repeat the above with the 5 other test tubes, except first fill each tube with 5ml of distilled water and mix by putting your thumb over the top and inverting repeatedly. Then add 5 ml of yeast solution and mix. Fill the 5ml pipette from each test tube to the brim with the corresponding sugar and yeast solution using a clean Pasteur pipette for each solution. Put your finger over the top of the 5ml pipette, invert it so the clay tip is pointing upward, and place it into the solution in the same test tube.
9. Place the 6 test tubes with their inverted pipettes into a 37^{o}C water bath or incubator. What do you think will happen? Record what happens after 5 minutes. Make readings every 5 minutes and record in a table in your lab notebook until one of your pipettes is empty. (Read the amount of fluid in the pipettes using the graduations on the side.)

In Your Lab Report:

Discuss fermentation of various sugars and sugar substitutes in your lab report. How can sugars of different compositions be fermented by the same organism? What did the lactase do to the lactose? Were any results surprising?

LAB 4

THE BIOCHEMISTRY OF MILK

Goal: Separate out the biological components of milk and test the physical properties. Heat, agitation, centrifugation, filtration, vaporization and desiccation are used to isolate casein (protein) and lactose (a carbohydrate).

Pre-Lab Questions:

1. What is a protein? What is it made of?

_____

_____

2. What are some properties of proteins?

_____

_____

3. What is a carbohydrate? What is it made of?

_____

_____

4. What are some properties of carbohydrates?

_____

_____

5. What is a lipid? What is it made of?

_____

_____

6. What are some properties of lipids?

_____

_____

7. List all of the solids you'll handle during the course of the days lab.

_____

_____

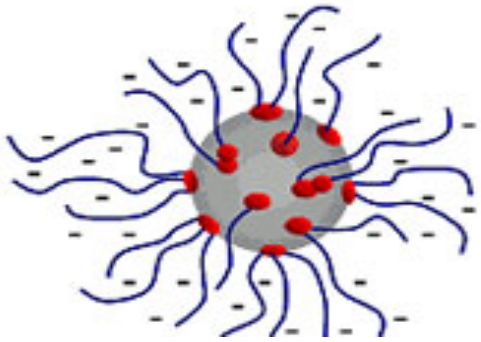
8. What labels should you write?

_____

_____

Milk is a mixture composed of protein, fats and carbohydrates. Proteins are made up of submits called amino acids. Fats (or lipids) are made up of submits called fatty acids and glycerol. Carbohydrates are single five or six carbon sugars, usually bound together in chains.

You will isolate protein and lactose from skim milk. First, you will isolate casein, a protein, from milk by adding acid to skim milk. This separates curds from whey. The solid curds are composed of mainly casein, and the liquid whey contains other proteins and the carbohydrate lactose, a six-carbon sugar. You will hydrolyze the casein by pouring on pancreatic enzymes. This speeds up the digestion process. You will then do colorimetric tests that definitively identify that protein and sugars are present in milk.

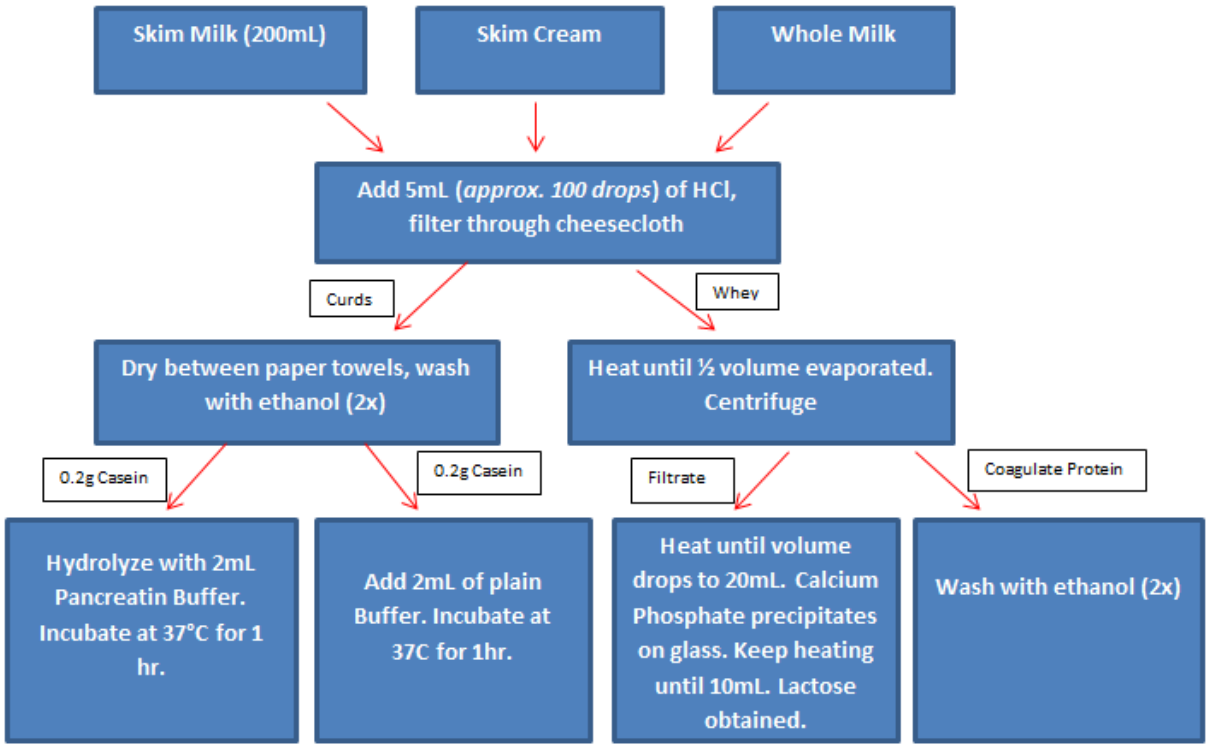


Question: What would adding an acid, which gives off H^+ ions, to a solution of casein do to the casein molecular structure? What macroscopic effect might this have?

_____

_____

Workflow of Biochemistry Experiment



<u>The Experiment</u>

1. Using a graduated cylinder, measure out 200ml of skimmed milk. Pour it into a 400ml beaker. Add 100 drops (*approximately 5 ml*) of Hydrochloric Acid (**HCl**) one drop at a time. Stir slowly with a glass rod until you see curds (*chunks*) appear. Curds are a precipitate of casein. Let the precipitate settle for five (5) minutes.
2. Lay two layers of cheesecloth over a fresh beaker. Make a cup-like depression in the middle of the cloth. Begin filtration by slowly pouring mixture into depression. Then draw the ends of the cheesecloth together, making a pouch. Gently squeeze the excess liquid into the beaker.
3. Place a marble boiling-chip into the beaker with the filtrate and mark the level with a wax pencil. Gently boil until the volume is reduced by just over one-half. This heating process will coagulate proteins that are left in the filtrate and irreversibly denature them. **Denaturing is the process of irreversibly disrupting the chemical structure of a protein.** These coagulated proteins have chains of amino acids that have rearranged into an insoluble mass.
4. Return to the casein in cheesecloth. Remove casein, place it between a sandwich of four (4) paper towels and press out the excess liquid. Place the dried casein in a beaker and cover it with 95% Ethanol (**<u>flammable, keep away from burner</u>**).

a. The Ethanol flushes out impurities. The casein remains intact because Ethanol doesn't dissolve it.
b. Agitate casein with glass stir rod and pour off the excess liquid into an appropriate waste container.
c. Rinse again with Ethanol
d. Lay the casein out on a paper towel to dry. When dry, scrape/shake it into a labelled container.

5. Once volume is sufficiently reduced, pour contents into a 50ml centrifuge tube.

a. Centrifuge for five (5) minutes at 3000 rpm. Make sure to balance machine.
b. Coagulated proteins will pellet at the bottom of the tube and the liquid supernatant will contain the lactose portion of milk.
c. Pour off the liquid into a small beaker with boiling-chips and label, "*Supernatant from Coagulated Proteins*". Set this aside.

6. Wash the coagulated proteins in the bottom of the centrifuge tube by pouring 95% Ethanol over the pellet. Stir the semi-solid with a glass rod. Let it settle. If it stays suspended, centrifuge again for one minute. Pour off Ethanol. Repeat.
7. Scrape out the coagulated protein left in the tube using a metal spatula onto a paper towel and blot dry. Place into a jar and label it, "*Coagulated Protein*".
8. Rinse out your glassware and discard all waste material properly.
9. Return the beaker labelled "*Supernatant from Coagulated Proteins*" to the burner set at low. Stir continuously with glass rod until 20 remains. At this point, you may notice a slight whitish precipitate of calcium phosphate on the sides of the beaker. Be very careful not to boil to dryness and do not lean over the beaker.
10. Further reduce the volume of the liquid to about 15 ml. It should be brown and syrupy. Remove from the burner, turn of burner. Add the product (**Lactose**) to a test tube.
11. Weigh out 0.2g of washed/dried casein and add it to a test tube. Add 2 ml of 0.1% Pancreatin Solution in phosphate buffer solution. Label "*Hydrolyzed Protein*". Re-suspend casein by tapping tube.
12. Weigh out another 0.2 g of casein and add to a second test tube. Add 2.0 ml of plain phosphate buffer. Label, "*Un-hydrolyzed Protein*".
13. Incubate both protein tubes at 37˚C for one hour.

Introducing Colorimetric Tests

You will now carry out a series of tests that detect proteins and sugars in different liquid blends. The results will inform you about the properties of the milk components you have isolated. Blue copper sulphate (*Biuret*) solution becomes purple where is comes into contact with proteins. The blue Benedict's

solution reacts with the aldehyde groups of sugar. If sugar concentration is low in a sample, the sample will turn green. The reaction can only occur in a hot environment so you'll place the tubes in a water bath that's between 65-70℃.

Procedure:

1. Assemble two sets of test tubes, 6 in one 7 in the other set. Each set will be used to test milk and its components for the presence of proteins and simple sugars separately. Make sure you label all tubes carefully. For example, you might label the first set of tubes "P1"- "P6". Make a chart listing the abbreviation of label for each test tube, and indicate which milk component is in each one. Leave a space in each square so that you can enter a (+) or (-) for each reaction, which is indicative of whether the particular macromolecule is present of absent.
2. Add 5 ml of skim milk to the first tube in each series. Add 5 ml whole milk to the second tube in each series. Add 0.2 g (or a pea sized piece of casein) to the third set of tubes in each series. Add 0.2 g or a pea-sized portion of coagulated protein to the fourth set of tubes in each series. Add 2 ml of your purified lactose to the Tube 5's. Tube 6's will have no milk components added and will serve as the control. (What should you add to the control tubes?) Add 2 ml of pre-prepared pure lactose to Tube 7's.
3. Add 5 ml of Biuret's to all 6 test tubes in the first series (marked "protein") and add 5 ml of benedict's solution to all 7 test tubes in the second series (marked "sugar"). The tubes containing the Benedict's solution should now be placed in a hot water bath for 8 minutes.
4. Record all reactions and colors in our chart. Make sure in your "conclusions" section that you mention what the normal (or control) color is for each test, and state what a positive reaction is.

Begin Chromatography:

5. Go back to your digested casein tubes in the 37°C incubator. You will use paper chromatography to separate the amino acids of your hydrolyzed protein. The color reagent you will dip your paper into after the amino acids have migrated is ninhydrin. It will turn purple if the reaction is positive (indicative of amino acids). Un-hydrolyzed proteins should appear colorless. Avoid touching the paper. Label four capillary tubes: "*Un-hydrolyzed Protein*", "*Hydrolyzed Protein*", and two with the names of amino acids. *There is an assortment available*.
6. Take a square of chromatography paper and draw a horizontal line 3 cm from the bottom (must use a pencil). Place four x's along the line, evenly spaced apart and at least 2cm from each edge. Label the x with a small pencil mark e.g. "**U**" for *un-hydrolyzed*. Place a drop of the appropriate solution on each spot. Let dry and then apply another drop. It's important to let the drops dry in between each application.

Repeat five times for each **x**. (You may use a hair dryer to speed the process of drying.) make a cylinder out of your paper with the spots outside and staple or clip the ends together.

7. Place the piece of paper in the chromatography jar into which a solvent Formic acid; Isopropanol. Water has been poured to a level of 2cm, cover it and do not disturb it. This procedure must be done under a hood. It will take about an hour for the solvent front to rise to about 6 cm from the top of the paper. At this point take the paper out of the jar. Mark the position of the solvent lightly with a pencil and let the paper dry for a few minutes. Dip in ninhydrin solution using forceps to hold the paper. Dry again. Place the paper in an 80℃ oven for 3-5 minutes. Observe the purple spots and make notes.

   (Your instructor may do the dipping for you since it must be done under a hood.)

LAB 5

MICROBIOLOGY

Goals:

1. Learn the basics of working with bacterial cultures.
2. Learn about bacteria cell structure
3. Test the effects of three variables on bacterial growth
a. Effect of dilution (concentration) of a soil sample
b. Find out the temperature for *Serratia marscens* survival and growth
c. Experiment that tests the inhibitory effectiveness of different antibiotics on *Escherichia. coli.*
d. Compare the efficacy of soap, alcohol and UV lighting on bacteria eradication. In doing so, you will see that there is flora on your skin.
4. Learn about the system that biologists use to classify life.

Pre-Lab Questions:

1. List three:
a. Kingdom_____
b. Phylum_____
c. Class_____
d. Order_____
e. Family_____
f. Genus_____
g. Species_____

2. What do you think the outcome of the thumb test will be?
_____
_____
_____

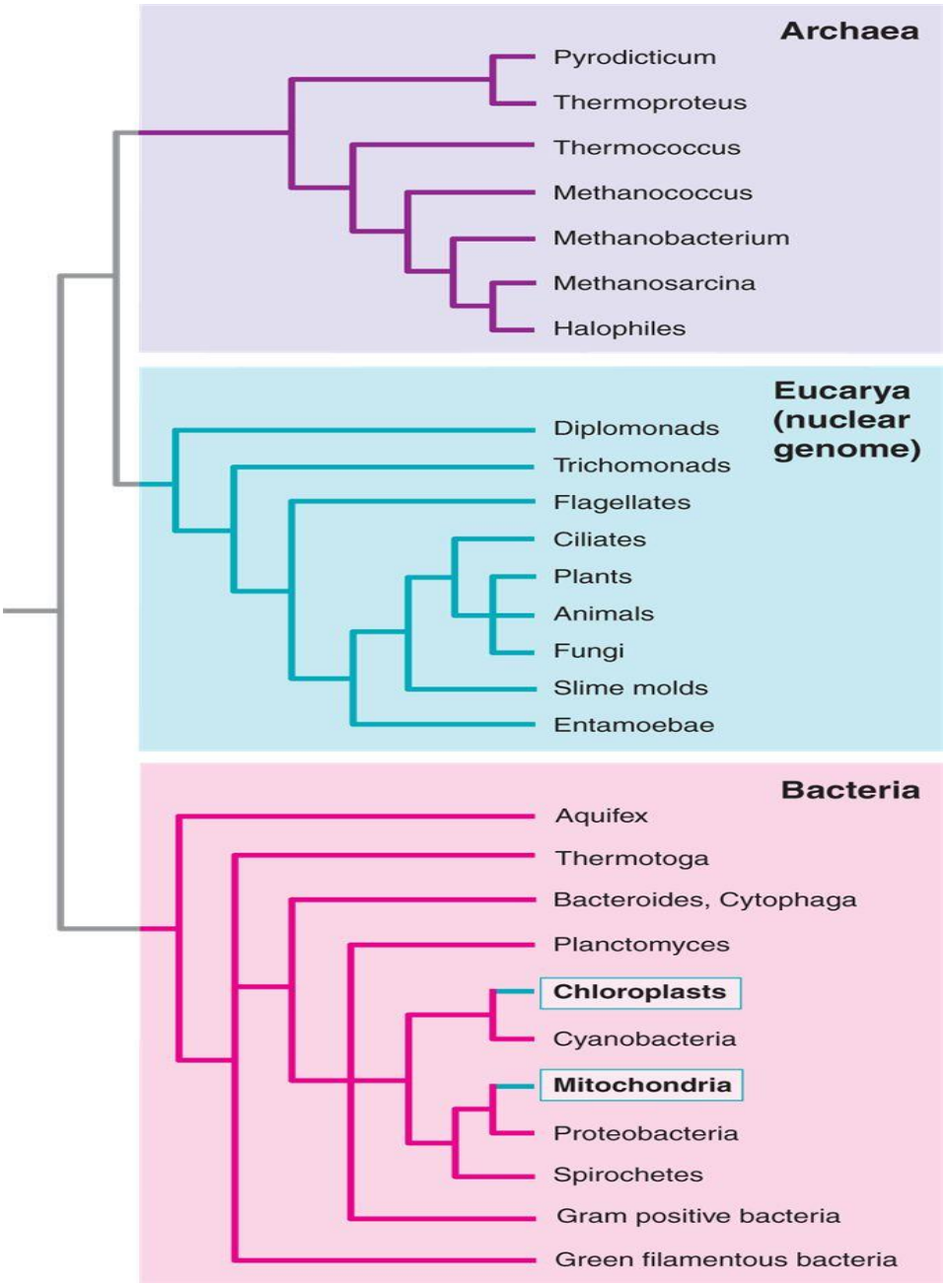
3. What makes a prokaryotic cell different from a eukaryotic cell in terms of :

a. Structure_____
_____
_____
b. History_____
_____
_____

Prokaryotic Cell

Prokaryotic cells are intrinsic to all members of the kingdoms Archaea and Bacteria. In order to organize information about the living world, biologists group organisms taxonomically, creating categories that contain related organisms. The largest of the categories is the **kingdom**. Within each kingdom are phyla

("phylum" if singular). Within a phylum are **classes**, each class multiple orders. Within each order, families are composed of genera that are further divided into species. Living organisms are divided into Archaea, Bacteria, and Eukarya. Archaea and Bacteria are prokaryotic. The phylogenies in the figure below are hypotheses of relatedness.



Members of the domain *Bacteria* are thought to have diverged early from the evolutionary line that gave rise to the archaea, bacteria and eukaryotes. Each type of organism is ultimately identified in this hierarchical classification system by two names, a binomial that includes the genus and a species name, both written in Latin and italicized. All binomials are unique: the same name is never given to two different species if genus is the same.

About 30 years ago, it was common to think of all organisms as belonging either to the plant or to the animal kingdoms. Bacteria, blue green algae, and fungi were placed in the plant kingdom, and protozoa

were considered animals. The primary difference between the kingdoms was how nutrients were procured. Plants were *autotrophs*, making their own food in photosynthesis, except for degenerate forms, such as bacteria and fungi. Animals were heterotrophs, acquiring their food by ingestion of plants to other animals. However, this system was too simplistic and taxonomically grouped organisms that were not necessarily closely related. In recent years, the six kingdom classification scheme has been widely accepted.

In it, living organisms are grouped into one of the following kingdoms:

1 & 2. **Archaea and Bacteria**: Prokaryotic organisms such as bacteria and blue-green algae.
3. **Protists**: Eukaryotic organisms that exist as single cells or as colonies of single cells. Food is obtained by ingestion, absorption or photosynthesis.

4. **Fungi**: Non-motile, multinucleate eukaryotic organisms with absorptive nutrition and mycelial organization.

5. **Plantae**: Non-motile, multinucleate eukaryotic organisms with photosynthetic nutrition, walled cells, and chloroplasts.

6. **Animalia**: Motile, eukaryotic multicellular organisms having cells without walls and ingestive nutrition.

All bacteria are prokaryotes; they lack a nucleus and membranous cytoplasmic organelles, such as vacuoles, mitochondria, and chloroplasts. All groups have cell walls composed of peptidoglycans, as opposed to cellulose in plant cells, and are often surrounded by a gelatinous sheath. Their DNA is arranged in circular molecules (*plasmids*) and is not complexed with histones to form chromosomes as in four of the other kingdoms.

Simple Stain Procedure:

1. Use a sterile cotton swab. Place it in a tube of bacteria, close the tube and rub the swab in a circular motion on a clean slide. Discard the swab in a disinfectant container.
2. Heat fix by running the slide over the flame back and forth **three** times.
3. Place enough stain, either carbol fuchsin, methylene blue, or safranin to cover the bacterial smear. Leave for 30 seconds.
4. Wash by dripping tap water gently onto the side. Drain and bolt with absorbent paper.
5. Look at the slide under the microscope (without a cover slip), first under the lowest power. You should see a colored smear. Have your instructor help you to use the immersion oil lens so you may focus on the individual bacterium. Draw the shape you see in your notebook.

Slant Inoculation Procedure:

1. Inoculate or streak four nutrient agar slants with a loop full of *Serratia. marcescens* bacteria. (*You instructor will show you how to streak a slant.*)

2. Incubate each tube at a different temperature

   a. 4°C: Refrigerator

   b. 25°C: Room temperature

   c. 37°C: Body temperature

   d. 55°C

Next week you will examine the slants of *S. marcescens* for growth and pigment production. Record the temperature at which each occurs. Keep in mind that enzymes reactions are involved. What can be said about the enzyme involved in pigment production?

Bacterial Population Counts:

Biologists frequently need to estimate the number of individual bacteria in a complex mixture of materials, such as in soil, in food powder, or in a body fluid sample. In this case, the goal is to obtain population size estimates, not to identify individual organisms to the species level. In such cases, the sample is mixed with a known amount of water, and then a small amount of dilution is spread on the surface of nutrient agar in a petri dish. For example, if 1g of soil is mixed with 99 ml of sterile water, and 0.1 ml of the mixture is placed on the agar, then 1/1,000 of the soil sample has been "plated". Any bacteria in the sample will be spread on the agar surface. They will draw nutrients from the agar medium and each single bacterium will repeatedly divide to form visible colonies. The colonies can be counted easily, and by multiplying by 1,000 the number of bacteria in the original gram of soil can be determined.

Make 4 Plates with The Following Dilutions:

1:10,000 1:100,000 1:1,000,000 1:10,000,000

1. Decide how you will achieve these dilutions. Also, learn to convert dilutions to exponential notation.

$10^{-1} = 0.1$ $10^{4} = 10{,}000$ $10^{-3} = .001$ $10^{6} = 1{,}000{,}000$

2. A total of **7** plates should be poured by each pair of students for the entire lab exercise. The instructor will assign the student heated or unheated samples for the soil experiment. If you are assigned heated samples, place your suspension in an 80°C water bath for 30 minutes before making dilutions.

3. Once the plates are inoculated, they should be incubated for three to seven days at room temperature. (If growth is too rapid, the plate can be placed in a refrigerator after three days and held until the next lab.) At the end of that time, count the colonies. Best results will be obtained when the number of colonies is in the range of 30 to 300. Some plates will simply have too many colonies to count and such results should be recorded as 'too many to count". Record the results in a table.

4. To arrive at the number of bacteria in the original soil sample, what must you do? Compare heated and unheated samples with other groups in your class. Put all data in your lab notebook.

5. Since bacteria multiply very rapidly, mutations can become evident in a population that is resistant to antibiotics. We will test several antibiotics to assess their effectiveness on the bacteria *Escherichia. coli.*

Antibiotics Experiment:

1. Streak out a swab of *Escherichia. coli* across an agar plate. Cover entire surface.

2. Place 3-5 different types of antibiotic discs on the plate. Make sure you record the name of the antibiotic in your notebook.

3. Next week you will measure the diameter of the clear area around each disc. What will this clear area represent? Make comparisons of the different antibiotics

Ultraviolet (UV) Radiation Experiment:

1. Make sure that you use **UV Safety Goggles** for this part of the experiment. Take a plate and divide the bottom of it into 4 quadrants with a grease pencil or permanent marker.

2. Streak out a swab of *Serratia marcescens*, a red pigmented bacterium, across the plate so that the plate is covered.

3. Using the white index cards, crisscross them and cover every quadrant except **#1**. Before exposing the plates to UV, make sure you take covers off, as the UV does not penetrate the plastic well. Expose **#1** to 15 seconds under UV.

4. Now remove the card from **#1** and **#2** and expose the plate for 15 seconds more. Remove the cards from #'s 1, 2, and 3, and irradiate it for 15 more seconds. Finally, remove all cards from the plate and add a final 15 seconds of UV to the plate.

Which quadrant will have had the most exposure? _____

What is the reason for using a red-pigmented bacterium? _____

Clean Thumb Test:

1. Divide a plate into thirds with a grease pencil.
2. Place your thumb lightly into wedge #1.
3. Wash your thumb with soap and place it on wedge #2.
4. Wipe your thumb with alcohol and place it on wedge #3. (Do not press too hard on the plate because you will break the agar.)

You will record all your results next week in your notebook.

You will have a total of 7 plates which you should wrap with tape and place in 37°C incubator. They will be removed in 2-3 days by your instructor and refrigerated until next week.

LAB 6

OSMOSIS & DIFFUSION

Goal: In this week's lab we will be performing four different experiments, looking at different aspects of diffusion and osmosis.

Pre-Lab Questions:

1. Define the following:

a. Brownian Motion

_____

_____

b. Diffusion

_____

_____

c. Facilitated Diffusion

_____

_____

d. Active Transport

_____

_____

e. Osmosis

_____

_____

f. Semi-Permeable

_____

_____

2. In today's lab we will be using dialysis tubing, which is made out of plastic with microscopic pores, as a semipermeable membrane. What property(s) would dialysis tubing potentially be selective for?

_____

_____

_____

3. An actual plasma membrane is composed of a phospholipid bilayer (polar heads, non-polar tails) with various channel proteins to act as pores. What are some properties that a plasma membrane could select for?_____

_____

_____

Experiment 1: Diffusion Rates and Temperature

In this experiment we will be comparing the rate of diffusion

Hypothesis: Based on your understanding of Brownian motion (from answering the pre-lab questions) what effect do you think temperature will have on diffusion rates and why?

_____

_____

_____

Procedure – Diffusion In a Liquid

1. Take 3 test tubes and label them 15℃, RT, and 37℃, These temperatures correspond to the temperatures of an ice water bath, room temperature and a water bath at 37℃,
2. Fill each tube ¾ full of tap water. Take a Pasteur pipet and add several drops of blue copper compound solution to the bottom of each tube (be careful not to shake the tube as you are adding the blue copper solution.)
3. Place each tube at the appropriate temperature and check them periodically. Record results (which diffuses fastest and slowest) in your notebook.

Analysis: Did the results match your hypothesis? _____

Experiment 2: Osmosis

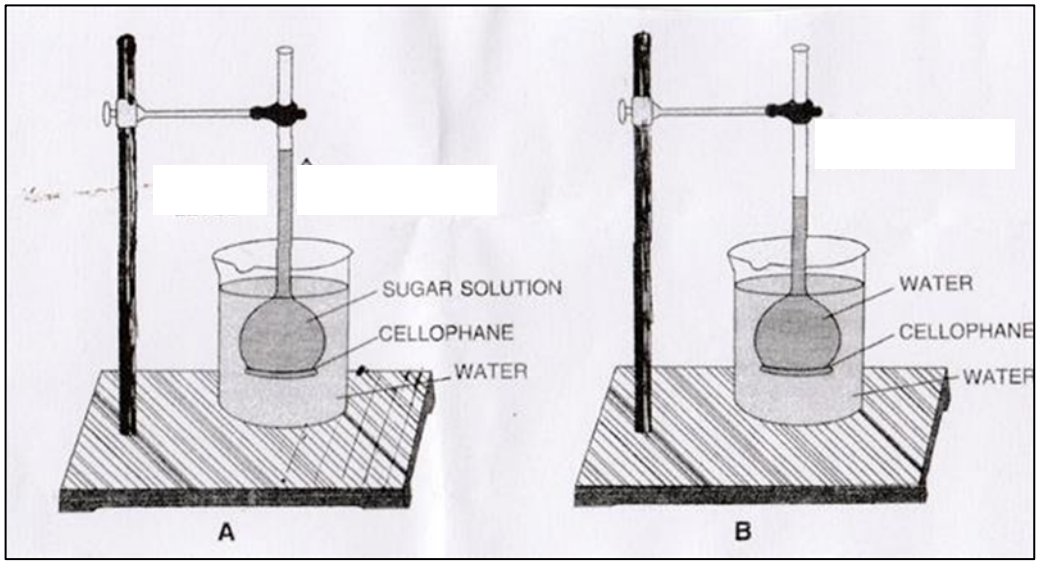
Hypothesis: If pure water and water with sugar dissolved in it were separated by semipermeable membrane that allowed water molecules through but not the sugar molecules, what would happen? Why?

_____

_____

_____

Experimental Setup



Procedure:

1. Place a pipet in the clamp
2. Take a 10-15cm length of dialysis tubing
3. Open the dialysis tubing and tie one end with a secure knot. Be sure to avoid leaks
4. Tie the other end to the pipet. You can use string
5. Pipet colored syrup in the top of the pipet until the dialysis bag is full, using a funnel.
6. Place your bag in the beaker of water.
7. Measure the distance of movement from the graduations on the pipet (Refer to the diagram for set up)

Analysis: Did your results demonstrate what you expected in your hypothesis? _____

Application: Would the syrup continue rising until all the pure water was used up? Why yes or why not? What if there was a super-long tube instead of a pipette in which the syrup can flow out?

_____

_____

_____

_____

Experiment 3: Selective Permeability, Diffusion, and Osmosis

We will be looking at the abilities of water, iodine molecules, glucose, and starch to diffuse across the dialysis tubing.

Research: *Look up online the diameters of the following molecules. Make sure to include the unit and to cite the URL of the source.*

a. **Water**:_ _____ **Source**:_____
b. **Iodine (I_2)**: _____ (*NOTE*: Look up the atomic radius of iodine and multiply by (4)
c. **Source**: _____
d. **Glucose ($C_6H_{12}O_6$)**: _____ **Source**: _____
e. **Starch:** _____ (*ask instructor what type of starch we are using*)
Source: _____

Hypothesis:

1. The pores in dialysis tubing are generally between 10-100 angstroms in diameter. Based on the data you collected in your research above, predict which molecules will be able to pass through the membrane and which ones will not:

_____
_____
_____

2. We will be starting with starch and glucose dissolved in water inside the dialysis tubing and iodine dissolved in water outside the tubing. Iodine reacts with starch to turn black. We also have glucose test strips and balances available. Describe how we will determine which molecule diffused where and where (inside or outside the dialysis tubing) we would expect each change to occur.

_____
_____
_____

Procedure:

1. Obtain a 20cm piece of tubing that has been soaking in water. Tie off one end of the tubing to form a bag. To open the other end of the bag, rub the end between your fingers until the edges separate.

2. Place 10-15ml of the 15% glucose/1% starch solution in the bag, leaving sufficient space for expansion of the contents in the bag. Record the color of solution in the table on the following page.

3. Test the glucose/starch solution for the presence of glucose using glucose test tape in a small test tube. Record the results in a table.

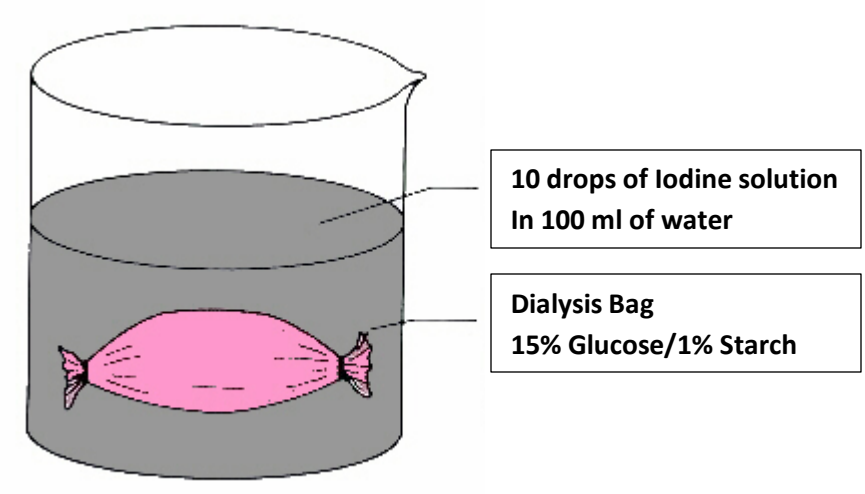
4. Fill a 250ml beaker with 100ml distilled water. Add approximately 20 drops of Iodine solution to the water (or enough to turn the water amber), and record the color in your notebook. Test this solution for the presence of glucose and record the results.

5. Rinse your dialysis bag, dry it with paper towels, and weigh. Record the weight in the table
6. Immerse the bag in the solution in the beaker leaving the ends sticking out

7. Allow your setup to stand for approximately 30 minutes. Record the final color of the solution in the bag, and of the solution in the beaker, in the table.

8. Remove your bag from the beaker, rinse, dry and weigh it. Record the weight.
9. Test the liquid in the beaker and in the bag for the presence of glucose. Record the results. To test the contents of the bag, carefully cut open your bag into a clean beaker.

	Initial contents	Solution Color Initial	Solution Color Final	Glucose Initial	Glucose Final	Weight Initial	Weight Final
Bag	15% glucose/ 1% starch						
Beaker	Iodine in water						



Setup of Dialysis

Analysis Of Results (*include these in your lab report*):

1. Which substance(s) are entering the bag, and which are leaving the bag? What experimental evidences support your answer?

_____

_____

_____

_____

2. Explain the results you obtained. Include the concentration differences in your discussion

_____

_____

_____

3. Did your results match your predictions in your hypothesis? What does it tell you about the properties that determines whether or not a particular particle pass through the membrane?

_____

_____

_____

Experiment 4: **Osmosis In Living Cells**

Plant cells are surrounded by rigid cell walls, and under normal conditions, the cytoplasm of the cell is closely pressed against these cell walls. Cells in this condition are said to be **turgid**.

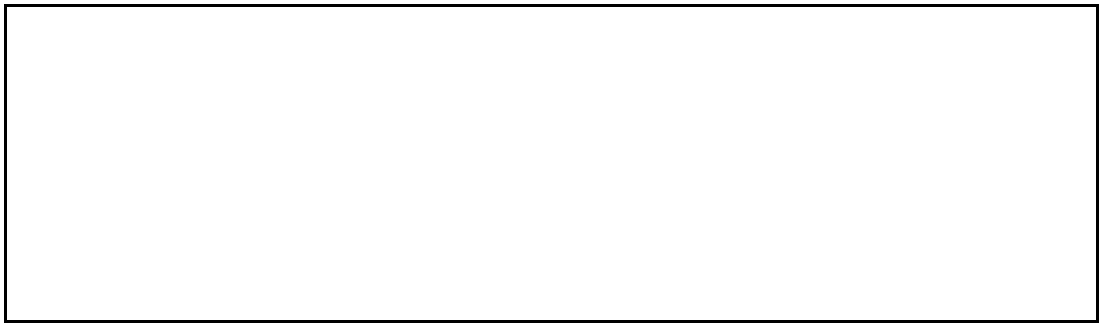
Hypothesis: The *Elodea* plant grows in "freshwater" ponds. The water has minerals and other solutes dissolved in it but at a much lower concentration than sea water. Assuming that glucose cannot pass directly through the cell wall and cell membrane of an *Elodea* cell, what will happen when the leaf is placed in:

Distilled (*purified*) water: _____

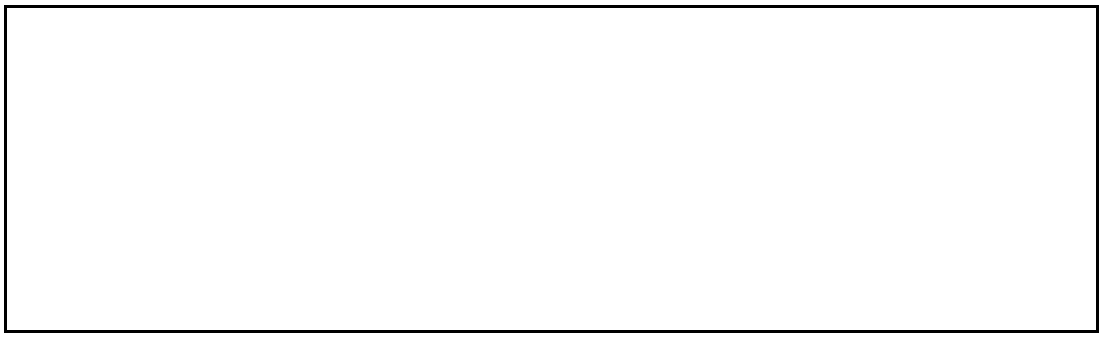
Concentrated glucose solution: _____

Procedure:

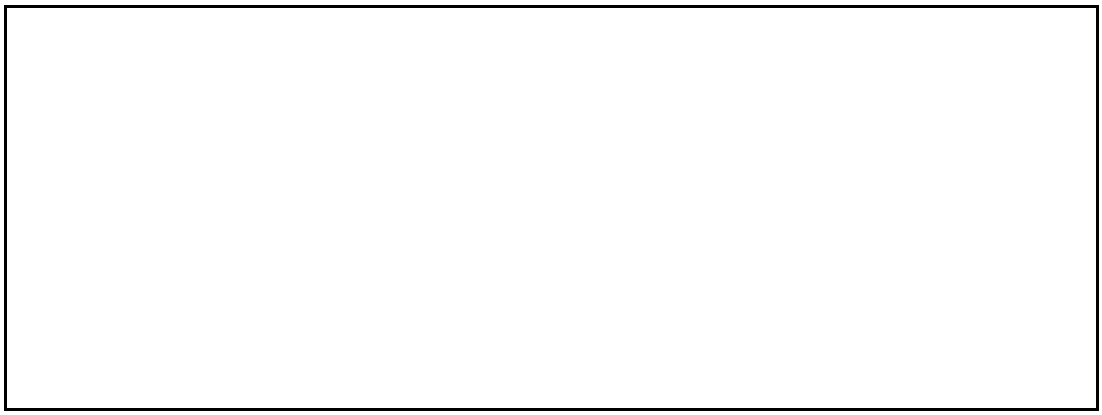
1. Mounting and sketching elodea tissue sample:
   Remove a leaf from an *Elodea* plant, peel off a piece of the epidermis or cut a very small piece from the tip, mount it in tap water on a microscopic slide then cover with coverslip. Using your compound microscope, identify the individual cells and note the position of the cytoplasm in relation to the cell walls. Sketch a few cells.



2. Now treat the cells with a concentrated solution of 35% sucrose by placing a drop or two of the solution next to the coverslip and wicking it under the coverslip. Observe the cells and record any changes in the position of the cytoplasm relative to the cell wall.



3. What would happen if you now placed these cells in distilled water? This process is called deplasmolysis. Do this by wicking distilled water under the coverslip. Sketch your observations and record your results:



Analysis:

1. Did your observations match your hypothesis?

_____

_____

2. What would have occurred if glucose was able to diffuse easily through the cell membrane of elodea?_____

_____

_____

3. What would have occurred differently had we used an animal cell instead of a plant cell?_____

_____

_____

LAB 7

MITOSIS AND MEIOSIS

Goal: Learn to identify mitotic and meiotic stages and understand the pathways. Learn what is occurring at the level of DNA nucleotide sequence from the cell exterior for each stage.

Pre-Lab Questions:

1. How does mitosis relate to meiosis?

_____

_____

2. At what stage does genetic recombination occur?

_____

3. Identify two cell types that undergo meiotic division.

_____

_____

4. What is the advantage to having meiosis in nature? What would life on earth lose if we had only mitosis?

_____

_____

_____

Background

An important characteristic of living cells is their ability to divide and produce two genetically identical daughter cells. Prior to division, cells undergo a growth process in which molecules (*ex*: fats, nucleic acids) are synthesized from food molecules using energy derived from respiration. Organelles (cell organs) must also allocate to daughter cells. This is a step beyond simple molecular synthesis.

The eukaryotic cellular organelles to be aware of today are plasma membranes, ribosomes, mitochondria and chloroplasts, cell membrane and cell wall, vacuoles, nucleus and nucleolus. Other features to track are chromosomes, nucleotides, centromere and the spindle. Before dividing, a cell contains hundreds of mitochondria. Cells in balanced, continuous growth double their components and then divide these components in half, producing two equally-full daughter cells. This also applies to vacuoles and lysosomes.

Most cells have only one nucleus and its division involves a special mechanism. The important contents of the nucleus are the chromosomes, the carriers of hereditary information. Different organisms have different numbers of chromosomes in their nuclei: for example, the donkey has 66; humans have 46, and fruit flies, 8.

Mononucleated cells have one center for its nuclear genomic material. This is where the DNA that codes for the organism is housed condensed into chromosomes. During the growth period, these chromosomes make copies of themselves. Each duplicated chromosome consists of a single long DNA molecule that is copied, folded, and wrapped around structures called nucleosomes. These are composed of proteins called histones. Each microscopic chromosome in an onion cell contains a DNA molecule that is about a meter long! In addition to having a nuclear genome a somatic cell has a much smaller, separate genome

in each mitochondria or chloroplast. Mitochondrial genes are relatively easy to sequence because the genome is small. Mitochondrial genes tend to evolve more quickly than nuclear genes.

Mitosis

Mitosis is the process by which the nucleus equally divides its contents to form two daughter nuclei. As a cell enters mitosis, its nuclear envelope breaks down. A spindle forms. The chromosomes then line up at the center of the cell. Spindle fibers radiating from opposite poles of the cell attach to each of the two chromatids. The fibers attach to each chromatid at the centromere, a locally constricted region of a chromosome where chromatids are held together and a kinetochore plate is found. As a cell progresses through mitosis, the centromere splits, and the spindle fibers pull the separated sister chromatids to opposite poles of the cell. The cell then divides along the centerline, producing two daughter cells that each have a copy of all the chromosomes that were in the mother cell prior to growth and division.

Chromosomal Anatomy

Following mitotic nuclear division (*karyokinesis*), the cytoplasm pinches in two (*cytokinesis*). Fundamentally different cytokinesis mechanisms are found plants and animals. In animal, the cytoplasm divides by constricting inward (furrows). A contractile ring of actin squeezes the cell in half. In plants, there is no constriction process. Instead, endoplasmic reticulum and Golgi apparatus-derived membrane vesicles migrate to the cell center and form a plate (*phragmoplast*) across the center of the mother cell. These vesicles fuse together and with the plasma membrane, forming the ends of two new daughter cells.

Question:

How is division completed in plants vs animals?

_____

_____

_____

Experiment

In this exercise you will observe mitosis in animal and plant cells and identify different mitotic stages.

Fish Blastula Procedure:

1. Obtain a prepared slide of a whitefish blastula and the darkly stained chromosomes in several of the cells.

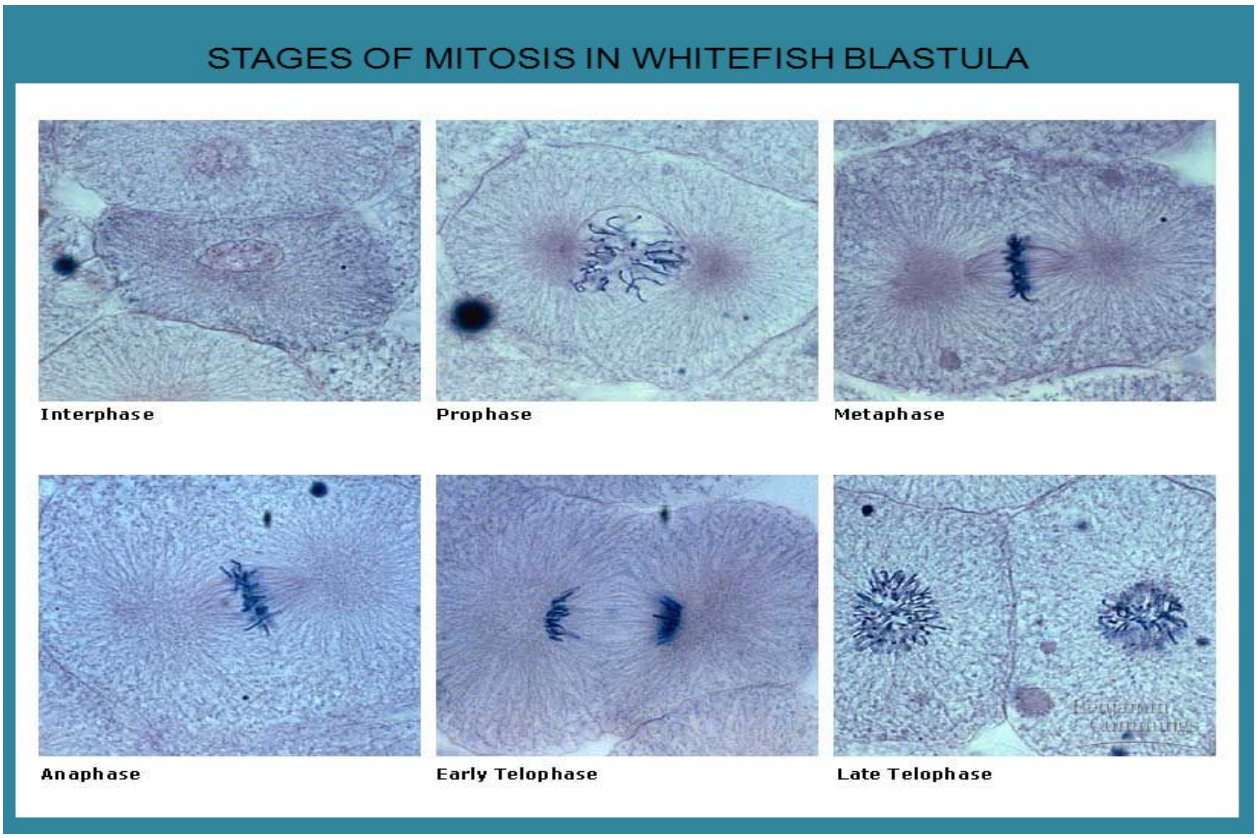
2. Center a cell containing chromosomes in the field of view and observe it first under medium power (40x) and then with the high-power objective (100x).

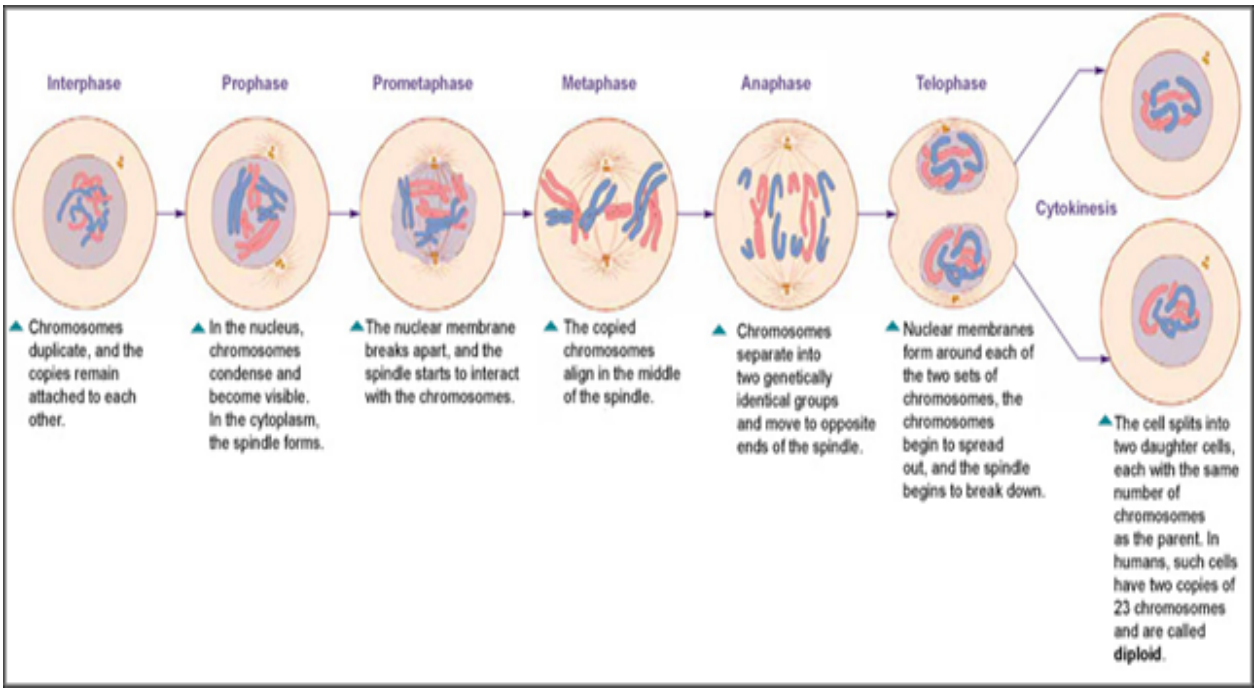
Sketch the cell, indicating such features as the spindle, chromosomes, centromere, and asters.

3. Locate another cell in which the chromosomes are visible. Chances are that the chromosomes are not aligned in the same patterns as in the previous cell. During mitosis, the chromosomes undergo stereotyped movement in the cell, ensuring that each daughter cell will obtain a full chromosome complement. In a blastula, the cell divisions are not synchronized, so different cells may be in different stages of mitosis or may not even be dividing at the time tissue was fixed and prepared.

4. Over the next 20 minutes, look at various cells on the blastula slide and identify the mitotic stages. You should find examples of interphase, prophase, metaphase, anaphase, and telophase. Use the diagram.

Sketch each stage and label the structures:





Onion Root Tip Procedure:

1. Obtain a slide of a longitudinal section of an onion root tip. Cells just behind the tip divide by mitosis as the root elongates. Study this area on your slide and identify cells in interphase, prophase, metaphase, anaphase, and telophase. Sketch cells in these stages.

2. Look at a cell in late telophase. Can you see a cell plate forming? _____

The line of vesicles forming across the center of the long axis of the spindle is called the *phragmoplast*.

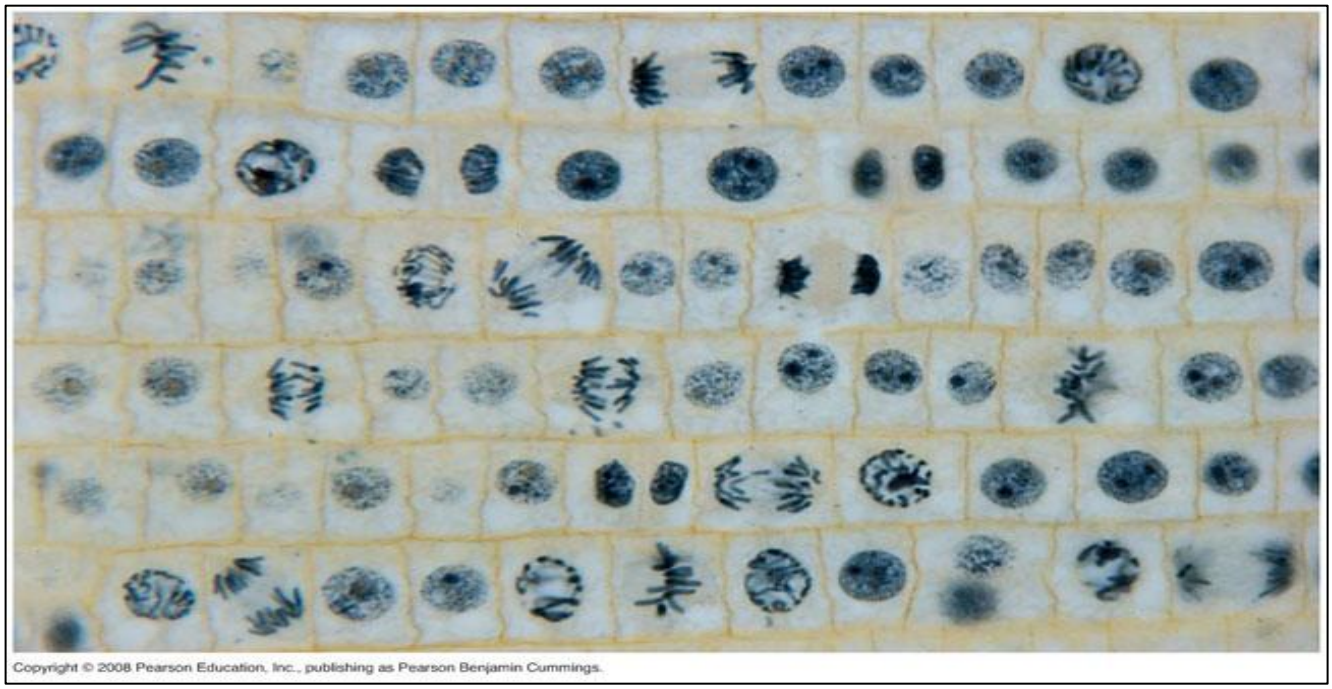
3. Mitosis lasts for about 90 minutes in onion root tip cells. Each of the four phases takes a different amount of time. The phase lasting the longest will be the most commonly observed.

Which phase did you observe most often?

_____

_____

Your slide should look similar to the image below:



Meiosis

Meiosis is a form of nuclear division, which produces daughter cells having half the number of chromosomes found in the parent cell. In sexually reproducing species, meiosis must occur or the number of chromosomes would double with each fusion of egg and sperm. If meiosis did not occur in humans, a sperm and an egg would each contribute 46 chromosomes to a fertilized egg, so that it would have 92. Since mitosis always produces daughter cells with the same number of chromosomes as the parent cells, all cells in the new individual would also have 92 chromosomes. If this individual mated with another of the same generation, the third generation of fertilized eggs would have 184 chromosomes. Obviously, if this exponential progression continues, there soon would not be a cell large enough to contain the increasing number of chromosomes in each generation.

Note that there are two parts to meiosis. In meiosis I, the homologous chromosomes line up side by side during metaphase, whereas in meiosis II, there is half the number of chromosomes and they line up singly at metaphase. Understanding these differences in alignment is the key to understanding the differences between meiosis and mitosis and the differences between meiosis I and II.

A cell that begins meiotic cell division has previously undergone a period of growth chromosome replication. Each chromosome consists of two chromatids joined at the centromere. This configuration is called a dyad. As this cell enters meiotic prophase I, the homologous chromosomes are paired and lie side by side as a tetrad (also called a bivalent) of four chromatids, in much the same way that one might hold four skeins of yarn clenched in one hand.

This pairing process, called synapsis, involves forming a connection between adjacent chromatids in tetrads. During this pairing process, the homologous chromosomes reciprocally exchange parts in a process called crossing over. This means that parts of the maternally derived chromosome actually pass and bind to the paternally derived chromosome and vice versa. The net result is that each member of the homologous pair becomes a mosaic of parts derived from both the maternal and paternal chromosomes. In effect, new gene combinations, or chromosomes, are created. Crossing over has profound implications as an additional source of evolutionary variation in sexually reproducing species.

As prophase I ends, the recombined chromosomes unravel except at points of attachment called the chiasmata. Eventually the chromosomes separate, lining up side by side at the center of the cell in metaphase I. while these events are occurring, the nuclear membrane breaks down and the spindle forms.

At the end of metaphase I, the homologous chromosomes in each pair separate from each other and move to the opposite poles of the cell during anaphase I. Cytokinesis, the division of the cytoplasm marks the end of meiosis I. Each daughter cell produced has half as many chromosomes as the starting cell, but each of these chromosomes is a combination of maternally and paternally derived genes because of crossing-over and each chromosome consists of two chromatids.

In the second part of meiosis, these chromosomes again line up at the center of the cell in metaphase II, and then proceed through anaphase II and telophase II. The result is the separation of the sister chromatids and the production of a total of four cells, each with the haploid number of chromosomes. Each chromosome at this point consists of one chromatid, sometimes called a monad, in which the genes have been recombined as a result of crossing over. (See diagrams at the back of this manual and below).

Meiosis in *Ascaris* and In A Flower Experiment

In *Ascaris*, a genus of roundworm, meiosis occurs in in the gonads. This is true for most mammals. Diploid cells in these organisms undergo meiosis, producing haploid gametes, or egg and sperm. Because the chromosomes in *Ascaris* are small, crossing over is not easily seen and will be studied separately. In *Ascaris*, the production of oocytes, through a process called gametogenesis, or oogenesis, starts in a tubular ovary.

As the developing oocytes descend from the ovary to the oviduct, meiosis occurs; different stages can be observed by looking at different regions of the reproductive tract or uterus, meiosis is completed and a sperm nucleus, which had penetrated during fertilization, fuses with the egg nucleus. A tough shell forms around the fertilized egg, or zygote, as these events occur. Inside the shell, the zygote divides by mitosis producing the embryo.

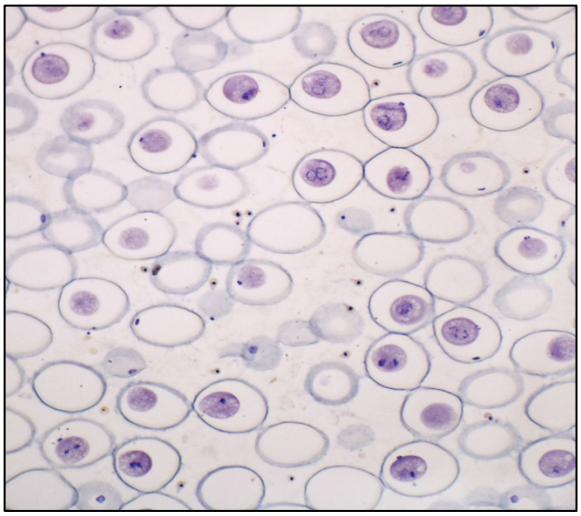
Ascaris is found in the intestines of pigs. These worms reproduce sexually and have separate sexes and internal fertilization. *Ascaris* is used to demonstrate meiosis because the diploid number of chromosomes is only four. This means that the chromosomes can be easily counted during the normal meiotic stages, an ideal characteristic for learning the stages of meiosis. The chromosomes are small, however, and careful observation is required.

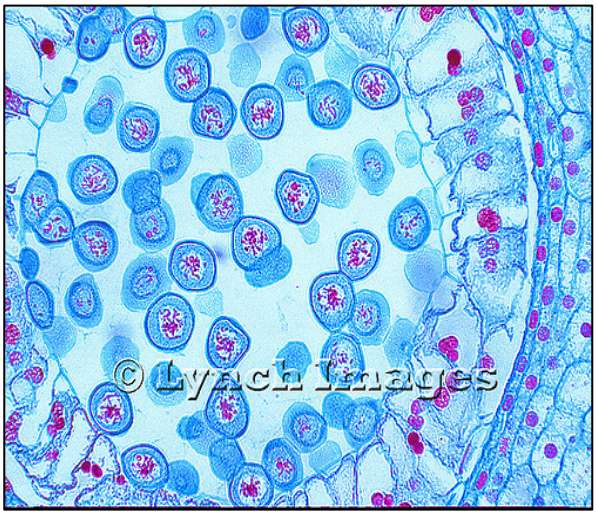
Procedure:

1. Obtain slides from your slide box labelled *Ascaris* bivalent and univalent. The bivalent corresponds to meiosis I and univalent to meiosis II.

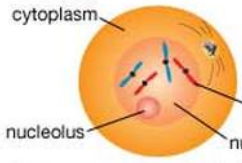
Draw pictures of the slide, showing in detail, the distribution of chromatic material.

Stages should be identifying based on your illustration and notations. When finished with the roundworm, do the same exercise for a *lily anther* (reproductive organ in some flowering plants). Examples of what the slides will look like are included below, as well as a diagram of the meiotic process in detail.

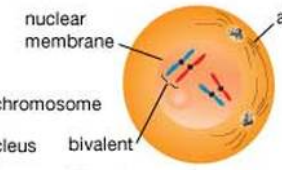




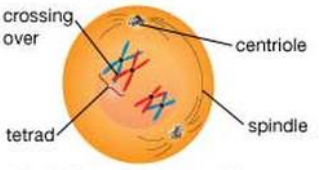
Meiosis, or sex cell division



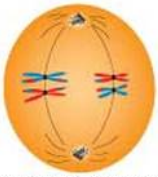
At the onset of meiosis, DNA strands thicken into chromosomes. Homologous, or like, chromosomes begin to approach each other.



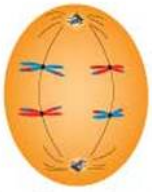
Homologous chromosomes pair to form bivalents. The centrioles divide and move to opposite poles of the cell.



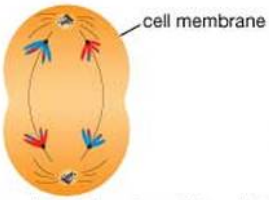
The bivalents duplicate to form tetrads, or four-chromatid groups. The nuclear membrane disintegrates. Crossing over (recombination) occurs.



In metaphase I, the tetrads, attached to spindle fibers at their centromeres, line up at mid-cell.

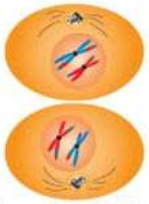


In early anaphase I, the tetrads separate, and the paired chromatids move along the spindle to their respective centrioles.

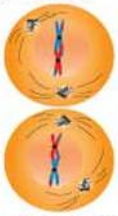


In late anaphase I, the chromatids have almost reached the spindle poles. The cell membrane begins to constrict.

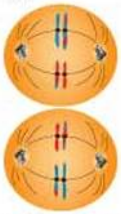
In telophase I, nuclear membranes enclose the separated chromatids. The cell membrane completes its constriction.



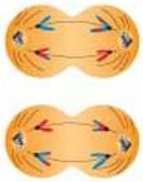
The first meiotic division ends. There are now two cells, each with the same number of chromatids as the parent cell.



Prophase II begins. In the second meiotic division, homologous chromatids do not duplicate but merely separate.



In metaphase II, the chromatids line up at mid-cell. The centrioles and asters are at the poles. A spindle has formed.



In anaphase II, the now-separated chromatids approach their respective poles. The cell membrane begins to constrict.

Telophase II has been completed. There are now four cells, each with half the number of chromosomes of the parent cell.

LAB 8
MENDELIAN GENETICS

Goals: To observe the laws of Mendelian genetics, introduce statistical analysis, and the laws of heredity.

Pre-Lab Questions:

1. Define the following:

a. Gene:_____

_____

b. Allele_____

_____

c. Law of Segregation _____

_____

d. Law of Independent Assortment _____

_____

e. Monohybrid Cross_____

_____

f. Dihybrid Cross_____

_____

g. Crossing Over_____

_____

h. Dominant and Recessive_____

_____

i. Codominant_____

_____

j. Incomplete Dominance_____

_____

2. If two plants heterozygous for a particular trait that has dominant/recessive alleles breed, out of 100 offspring, how many are likely to express:

a. The dominant phenotype? _____

b. The recessive phenotype? _____

3. Two plants heterozygous for green peas (as opposed to the recessive yellow) and heterozygous for smooth peas (as opposed to recessive wrinkled) breed and produce 100 offspring. How many are likely to produce the following phenotypes:

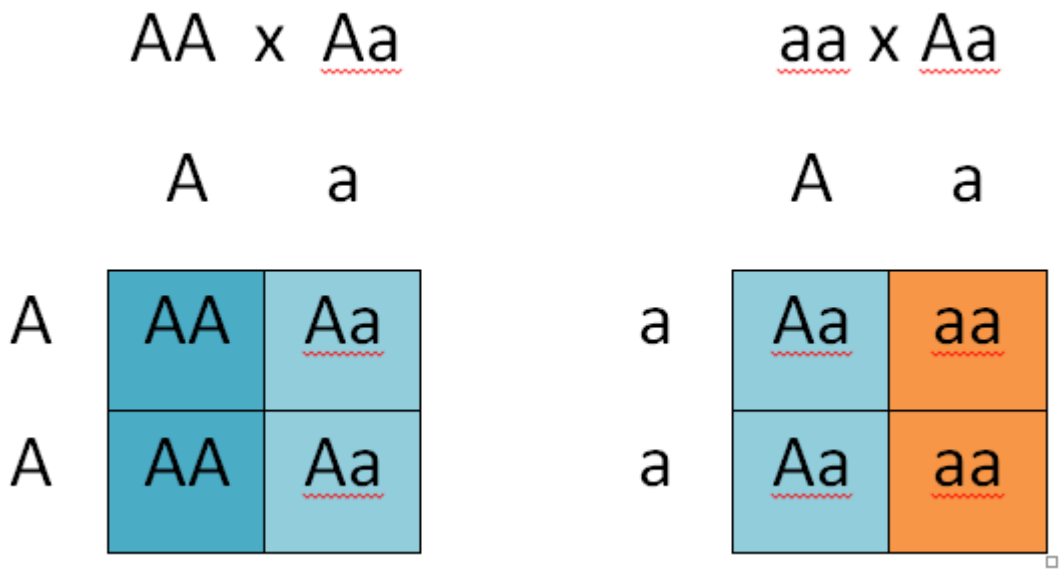
a. Green and Smooth _____
b. Green and wrinkled _____
c. Yellow and Smooth _____
d. Yellow and wrinkled _____

Experiment #1: Corn Exercise

a. Monohybrid Cross

In the first exercise we are testing the hypothesis that the parents that produced this Indian corn (remember that the kernels are seeds and thus offspring) are heterozygotes. This is a monohybrid cross because the only trait we are examining is color (mono), and both parents are assumed to have a dominant and a recessive gene for this trait (hybrid). We will not be looking at other phenotypic traits at this time.

Punnett squares: An easy tool for figuring out the likelihood that offspring will have a particular genotype or phenotype. Reflecting the segregation of genes in gamete development and their recombination in fertilization, the alleles are separated and allowed to recombine in any possible combination.



Procedure:

1. Count the number of purple kernels and the number of yellow kernels in ten rows on the corn cob. Mark the kernel in the row that you start with, with it pin to help you keep track of the number of rows. **DO NOT** remove the kernels from the ear of corn.
2. Write down the total number of purple kernels, the total number of yellow kernels. Also write the total class counts for both colors, in your notebook and on the blackboard. Copy the class data in your notebook.

Group #	purple	yellow	Total (P+Y)
1			
2			
3			
4			
5			
6			
7			
8			
9			
Total Class			

Statistical Analysis of the Collected Data

The Chi-Square is a statistical tool used to test how reasonable your hypothesis is. For instance, perhaps the genes do not sort independently (are on the same chromosome), or one or both parents are homozygous for the trait, or there was a rare mutation in the 'parent or offspring, or you only tested a very small sample. If your differences are significant, you could reject your hypothesis, or begin again and try to explain why the statistics in your first experiment were not reliable.

Degrees of Freedom:

The number of pieces of information needed to know the outcome. For example, in a coin toss, to know the result you only need to know one piece of information: which side it wasn't. If you were told it wasn't tails, you could deduce that it was tails. This would be 1 degree of freedom. If someone were at

a traffic light, you would need to know that it was not red or yellow to deduce that it was green. This is 2 degrees of freedom.

Application:

1. Based on this, what would be a general mathematical principle for calculating the degrees of freedom based on the possible outcomes of a situation?

_____

2. In the monohybrid cross that we looked at for corn, how many degrees of freedom are there?

_____

Procedure:

Do a Chi-square (x^2) analysis of the class data from the class total.

$$x^2 = \Sigma(\frac{(O-E)^2}{E})$$

0= Observed number of kernels

E = Expected number of kernels.

1. Which color, purple or yellow is dominant? How do you know?

_____

_____

2. To get the number of **expected kernels**, multiply the expected ratios by the total number of kernels you observed.
3. Fill in Punnett Square for yellow and purple corn, assuming both parents are heterozygous.

4. Based on your Punnett Square, what is the ratio of dominant to recessive?

_____

5. Using the dominant/recessive ratio and your answer to which color is dominant, determine, from the total class values:

a. Expected number of purple_____

b. Expected number of yellow_____

6. The actual counts you got are the observed values. Plug your numbers into the chi-square formula and figure chi-square.

7. Use the Chi-square table and look under the number of Degrees of Freedom that you determined earlier to figure out if your data varies significantly from the expected values. If your data fall below the 5 % probability value, your data would be said to differ significantly from the expected values given the hypothesis that the parents were heterozygotes. Your data would then be called significant, and you would reject the hypothesis that the parents were heterozygous for the chosen alleles. If the Chi-square value falls above the 5% probability values, then it is very likely that any deviations you had from a perfect ratio were due to chance alone. Do the Chi-square analysis with your data and the class data.

a. What is your p value? Is your hypothesis supported?

_____

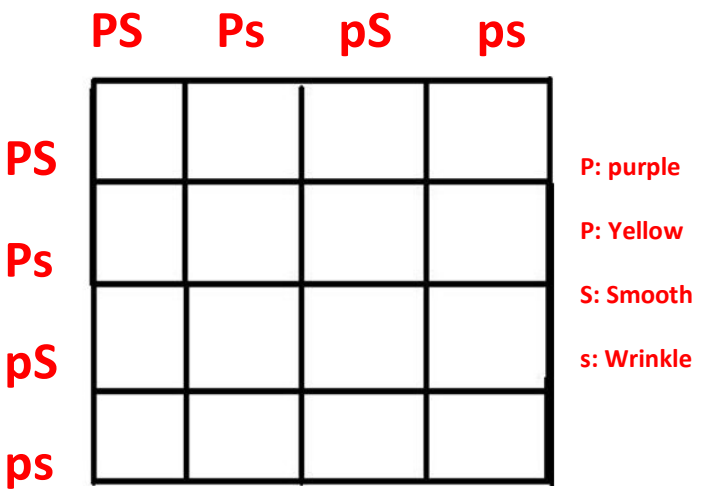
_____

Table of Values of Chi-Square

		Hypothesis is supported							" Not Supported		
		Differences are insignificant							" Are Significant		
p =		0.99	0.95	0.80	0.50	0.30	0.20	0.10	0.05	0.02	0.01
Degrees of freedom	1	0.0002	0.0039	0.064	0.455	1.074	1.642	2.706	3.841	5.412	6.635
	2	0.0201	0.103	0.446	1.386	2.408	3.219	4.605	5.991	7.824	9.210
	3	0.115	0.352	1.005	2.366	3.665	4.642	6.251	7.815	9.837	11.341
	4	0.297	0.711	1.649	3.357	4.878	5.989	7.779	9.488	11.668	13.277
	5	0.554	1.145	2.343	4.351	6.064	7.289	9.236	11.070	13.388	15.086

B. Dihybrid Cross

In this exercise you will examine a dihybrid cross in corn plants. Di = 2 traits, dominant and recessive genes for each in both parents (hybrid). The two traits are color and texture. Make a Punnett Square in your notebook based on the following example, and fill it in with expected genotypes and phenotypes.



Procedure:

1. Count 10 rows of kernels as before but note there are four classes. DO NOT remove any of the kernels from the corn cobs.
2. Put your data on the board and copy the class data into your notebook.

Group #	Purple smooth	Purple wrinkled	Yellow smooth	Yellow wrinkled	Total kernels
1					
2					
3					
4					
5					
6					
7					
8					
9					
Total Class					

3. Which trait, smooth or wrinkled is dominant? How do you know?

_____

_____

4. Fill in a Punnett Square to determine the number of kernels you would expect of each phenotype combination

5. Using the determined ratio from the Punnett Square and your answer to which traits are dominant, determine, from the total class values:

a. Expected number of purple smooth_____
b. Expected number of purple wrinkled_____
c. Expected number of yellow smooth_____
d. Expected number of yellow wrinkled_____

6. Do a Chi-square analysis of your data and the class data.

a. With 2 different alleles, how many degrees of freedom are there? Think of the number of phenotype outcomes that are possible. _____
b. What is your p value? Is your hypothesis supported? _____

Experiment #2 Human Genetics (*not included in lab report*)

Determining Human Phenotypes and Genotypes

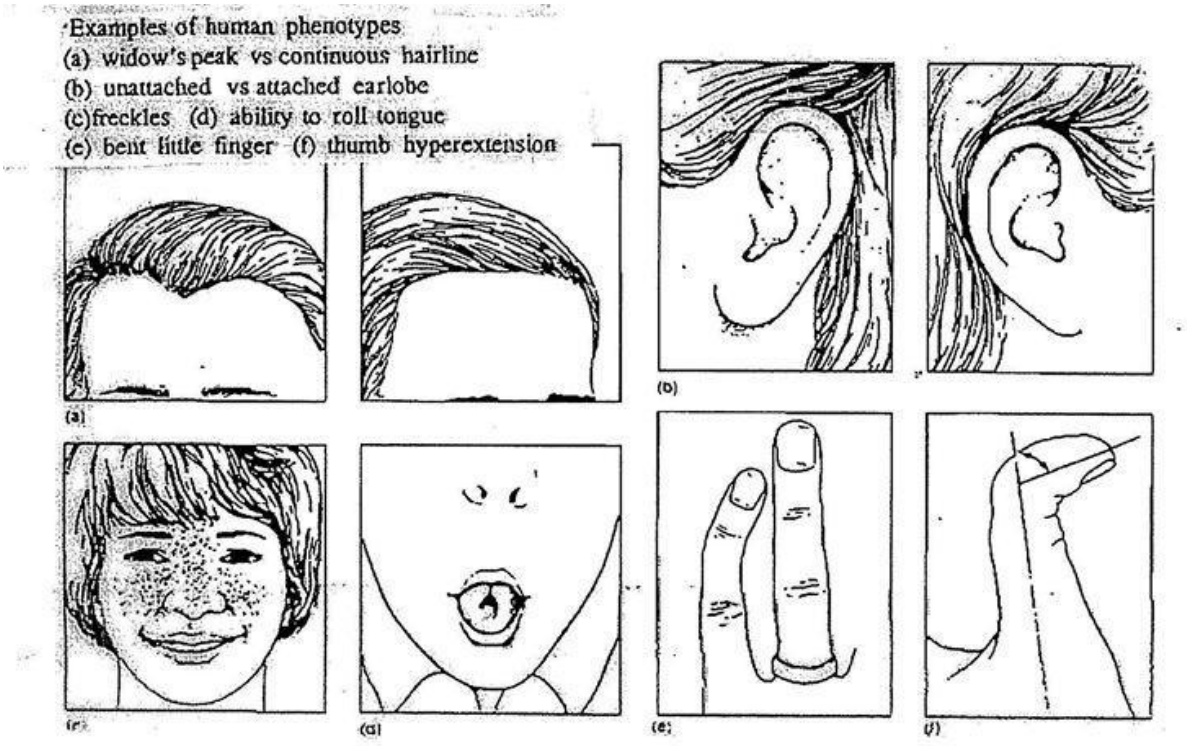
Work with your partner and determine each other's phenotypes for the inherited characteristics given in the diagrams. Record your phenotypes in a table. Directions for blood-typing are given later.

*** Tally the class's phenotypes for these traits in a table on the whiteboard. Then record the tallies and calculate the relative frequency of these traits in your class. The table shows some of the phenotypes that are included in your sample data.**

Look over the frequencies of the dominant and recessive phenotypes in the class data.

Is it true that dominant phenotypes are always the most common in a population?

1. Widows peak (d)
2. Unattached lobes (d)
3. Freckles (d)
4. Eye color brown (d)
5. Tongue rolling (d)
6. PTC tasting (d)
7. Interlacing left over right (d)
8. Little finger bent (d)
9. Thumb can be bent backward (d)
10. Cleft in chin (d)



Your instructor has added several characteristics, i.e. PTC tasting, eye color, chin cleft, interlacing fingers, beyond those pictured above.

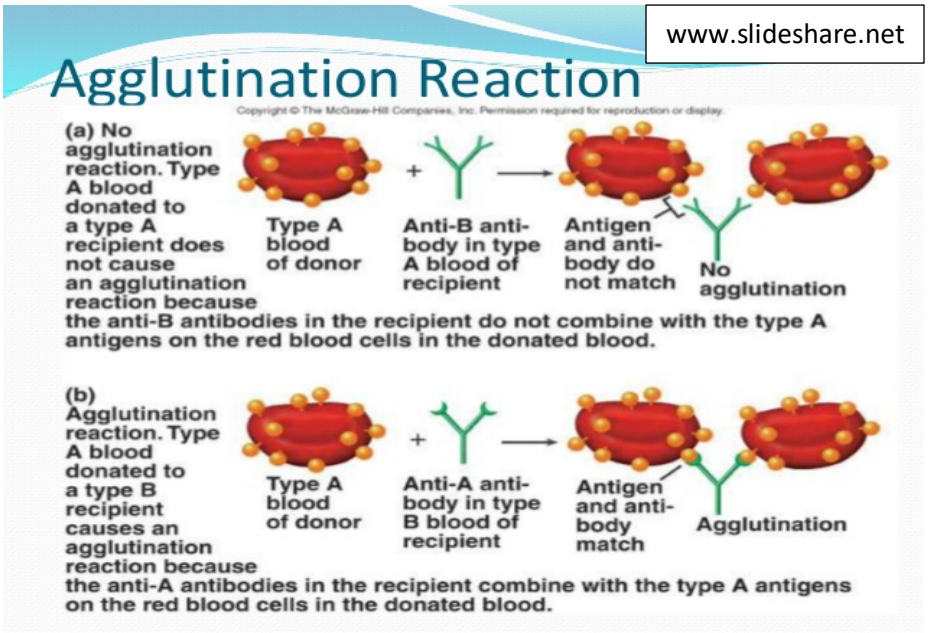
Experiment 3: Blood Type

Human cells have a number of different membrane-bound molecules on different cells. One type is carbohydrate molecule on red blood cells. This can be the "A" molecule or the "B" molecule. The alleles for each are codominant, meaning someone heterozygous for the A and B alleles will express both. One can also have a null, or "O" allele.

Genotype	**Phenotype**
AA or AO	A
BB or BO	B
OO	O

A separate membrane-bound protein is the rhesus factor, or Rf. This is a single allele gene and one is either positive or negative for it.

The immune system is adapted to recognize antigens, or membrane bound molecules, that are from the individual's cells and attack cells with foreign antigens. This is how the immune system fights pathogens while not attacking the body's own cells. The B cells of the human body will produce antibodies against the foreign antigen, leading to their destruction.



Hypothesis: Predict what blood types would be accepted in a transfusion by people with the following blood types and who they can donate to:

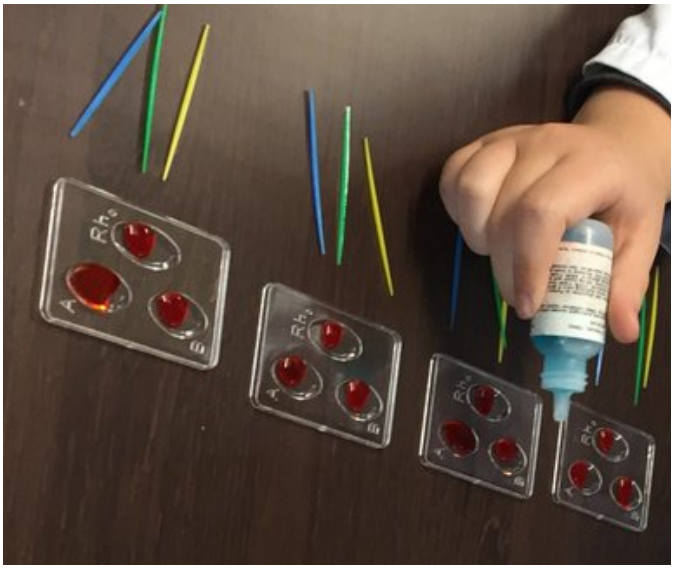
Host blood type	Accepted	Can donate to...
A+		
A-		
B+		
B-		
O+		
O-		
AB+		
AB-		

Blood Type Procedure:

Since we are unable to type our own blood, hemagglutination will be demonstrated to you with pre-screened, typed, synthetic blood.

Diagram of Antigen-Antibody Reaction. The complimentary shapes of each set of antigens and antibodies cause agglutination by linking blood cells together.

Add a drop of anti-serum to the appropriate circle on the typing cards.



Analysis:

Observe the mixtures against a light background with bright light, watching for the appearance of red granules which indicates that the blood cells are agglutinating or clumping.

It helps to gently rock the card back and forth. A model of what happens during agglutination is shown. What is the difference between agglutination and clotting? Do the same for Rh which is another factor that must be considered when blood is typed. You are either Rh" or Rh'.

Questions for Lab Report:

1. Explain why dominant traits are not always the most common traits in a population.

2. Explain why people with type O blood are called universal donors, and those with type AB are called universal recipients.

Lab 9

Gene Expression of Beta Galactosidase

Goal: To see the function of the Lac operon and to determine which gene of the Lac operon, if any, is mutated in the bacterial sample that you are using.

Pre-lab questions:

1. Bacteria are classified as (circle one) Prokaryotes/Eukaryotes
2. Fill in the blanks: DNA is _____ (process) into mRNA.
3. The mRNA is then _____(process)into polypeptides.
4. Define the following terms:

a. Lactose_____

b. Beta galactosidase_____

c. Gene_____

d. Polycistronic_____

e. Polymerase_____

f. Promoter_____

g. Operon_____

h. Operator_____

i. Repressor_____

j. CAP_____

k. IPTG_____

5. Look up and write the function of the following genes:

a. Lac I_____

b. Lac Z_____

c. Lac Y_____

d. Lac A_____

6. Define the following types of operons and determine which category the Lac operon falls into:

a. negative inducible operon_____

_____

_____

b. negative repressible operon _____

_____

c. positive inducible operons_____

_____

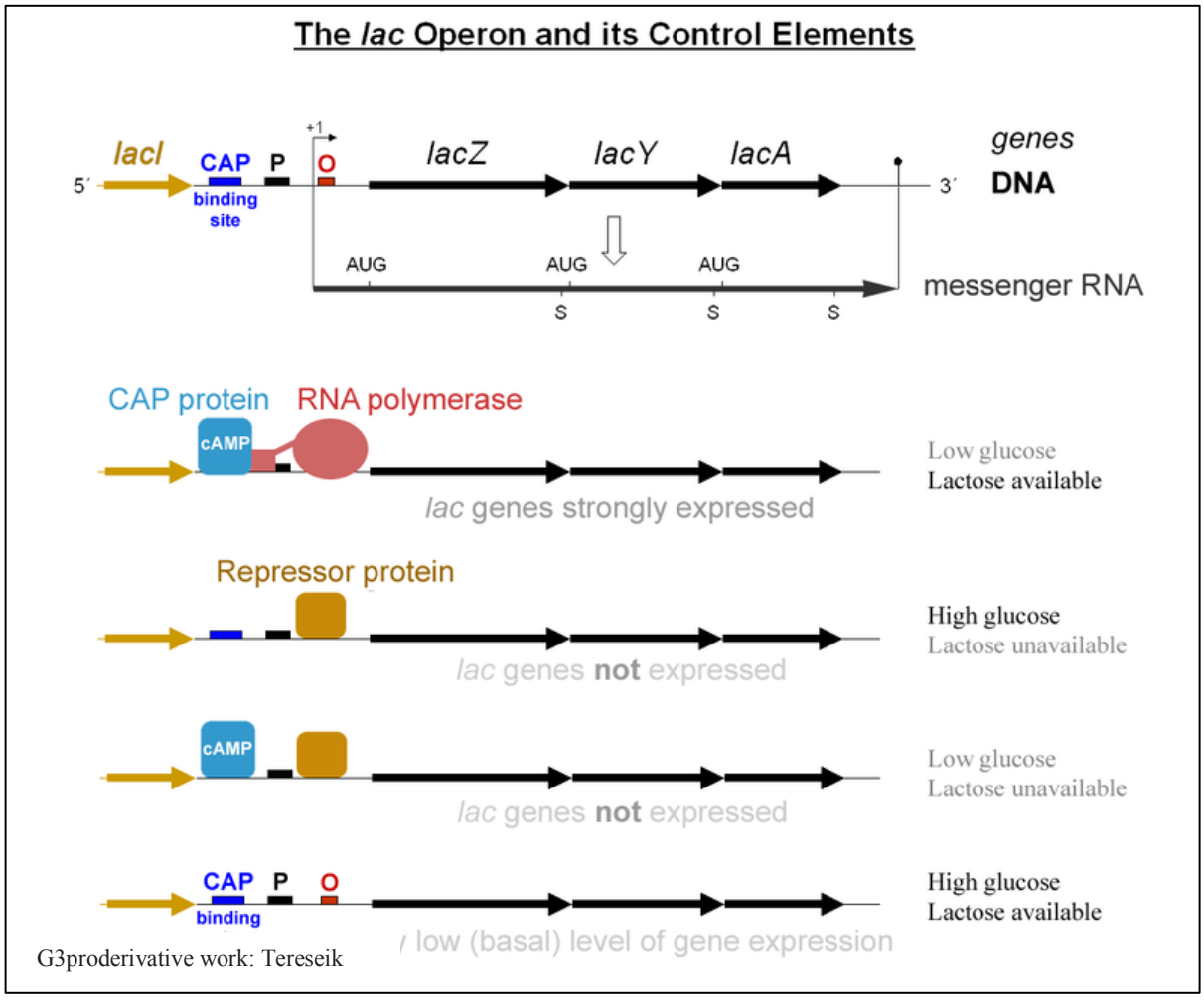
d. positive repressible operons_____

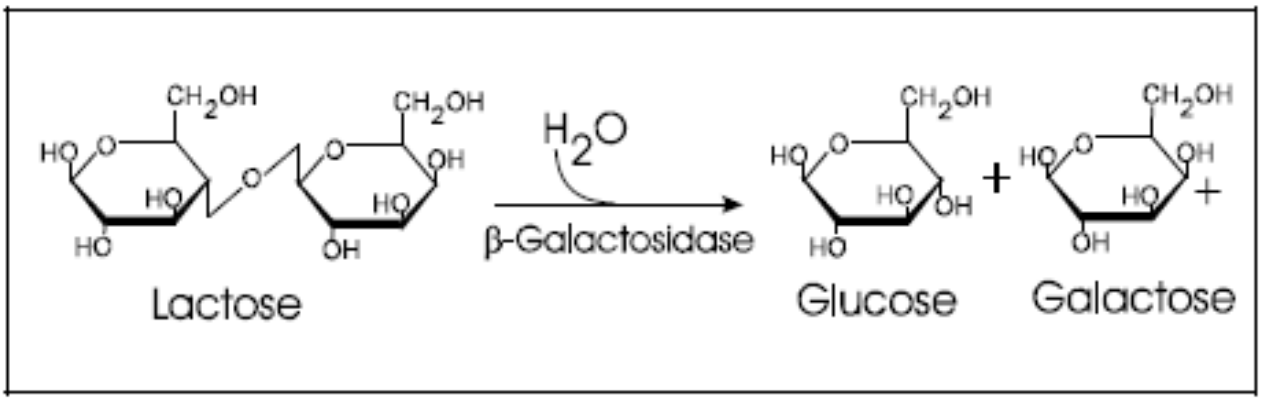
_____

e. What type(s) of operon, from the above options, is the Lac operon? _____

_____

Lactose is a disaccharide, made of glucose and galactose monomers linked by a covalent bond. It is not directly usable in cellular respiration but can be broken into galactose and glucose by hydrolysis, catalyzed by enzymes like beta galactosidase. It takes energy to express beta galactosidase, so bacteria have operons that allow for expression when there is lactose present and no glucose present.





Control systems: Notice that the lac operon has two control systems, one for determining the presence of lactose and the other the presence of glucose.

1. What does the lack of glucose lead to? _____

   _____

2. What action does the presence of lactose lead to? _____

   _____

In today's lab we will be focusing on the lactose present/absent control system. We will determine possible mutations in bacteria that lead to expression or lack thereof of beta galactosidase. We will be using a chemical called ONPG to indicate the expression of beta galactosidase. It will turn yellow when exposed to beta galactosidase.

Materials:

You will begin with **three unknown strains** of *E. coli* bacteria:

1. Normal (wild-type). *E coli* (lac+),
2. Lacking the repressor protein (*lac1*-), and
3. Lacking the galactosidase enzyme (*lacZ*-).

We will expose each strain of bacteria to three environmental signals (lactose, glucose and a chemical inducer (IPTG)). Then we will determine the identity of each unknown based on how much beta-galactosidase it makes in response to the environmental signal.

IPTG is a chemical that, like lactose, can bind to the repressor protein and cause it to release from the operator (an inducer). IPTG, unlike lactose, is not hydrolysable by β-galactosidase. Therefore, its concentration remains constant during an experiment. (Wikipedia)

Hypothesis: Predict the relative beta galactosidase expression (low, medium, high) you would expect to see for each strain under each condition:

	Lactose	Glucose	IPTG
Wild-type			
LacI-			
LacZ-			

Procedure:

1. You will need to label 12 small tubes: For each of the three unknown strains (A, B, C) you will need four tubes:

• Control tube

• Lactose tube

• Glucose tube

• Chemical inducer tube

Tips:

- Be sure to label each tube with unknown strain (either A, B or C), the condition (control, lactose, glucose and chemical), and something that identifies your group (e.g. A+L, A+ G, A+C and A control)
- You don't have to worry about using sterile techniques, because we won't be growing the bacteria for very long.
- Be very careful to use a different pipette for each solution and for each strain of bacteria to avoid cross contaminating reagents.

2. Add 1.5ml of growth media to each tube

3. Add 0.6ml of the appropriate bacterial strain (A, B or C) to the tubes.

Tips:

- Be sure to swirl the culture before taking your 0.6ml of bacteria
- Measure carefully
- After everything is set up, all the tubes should have the same amount of liquid in them. If they do not, you've made a mistake. Redo the tube that has a different volume of liquid.

4. Add 0.15ml of the appropriate sugar or chemical inducer (IPTG) to the appropriate tubes.

Add 0.15ml of distilled water to the control tubes. Mix each tube by carefully flicking the bottom of it with your finger.

5. Incubate all your tubes in an incubator at 37°C for 85 minutes.

6. After incubation add one drop of detergent (Sarkosyl) from the dropper bottles to each tube. To mix, flick the bottom of the tube carefully. The detergent will lyse the bacteria (cause them to burst) and allow the beta-galactosidase enzyme that the bacteria have produced to be free in the solution.

7. Add 0.8ml of 1%ONPG (a substrate for beta-galactosidase) to each tube and mix again by flicking carefully. ONPG will be on ice because it must stay cold.

8. Incubate the tubes at 37°C for 15 minutes. Some tubes will begin to tum various shades of yellow. Add 1ml of Stop (1M Na_2C0_3) to each tube to stop the reaction.

9. Make notes on the shade of yellow in each tube. You may want to place the tubes in front of a piece of white paper to help distinguish the color.

Results: Fill in the shades of yellow in the table below:

	Lactose	Glucose	IPTG
A			
B			
C			

Analysis: Which strain was which? Fill in the appropriate letter and **explain** how you can tell:

Wild-type_____

Lac I-_____

Lac Z-_____

In your lab report also include any inconsistent results and explain why you might have expected something different. Don't forget the CAP/cAMP glucose control system is also in effect.

Fruit Flies

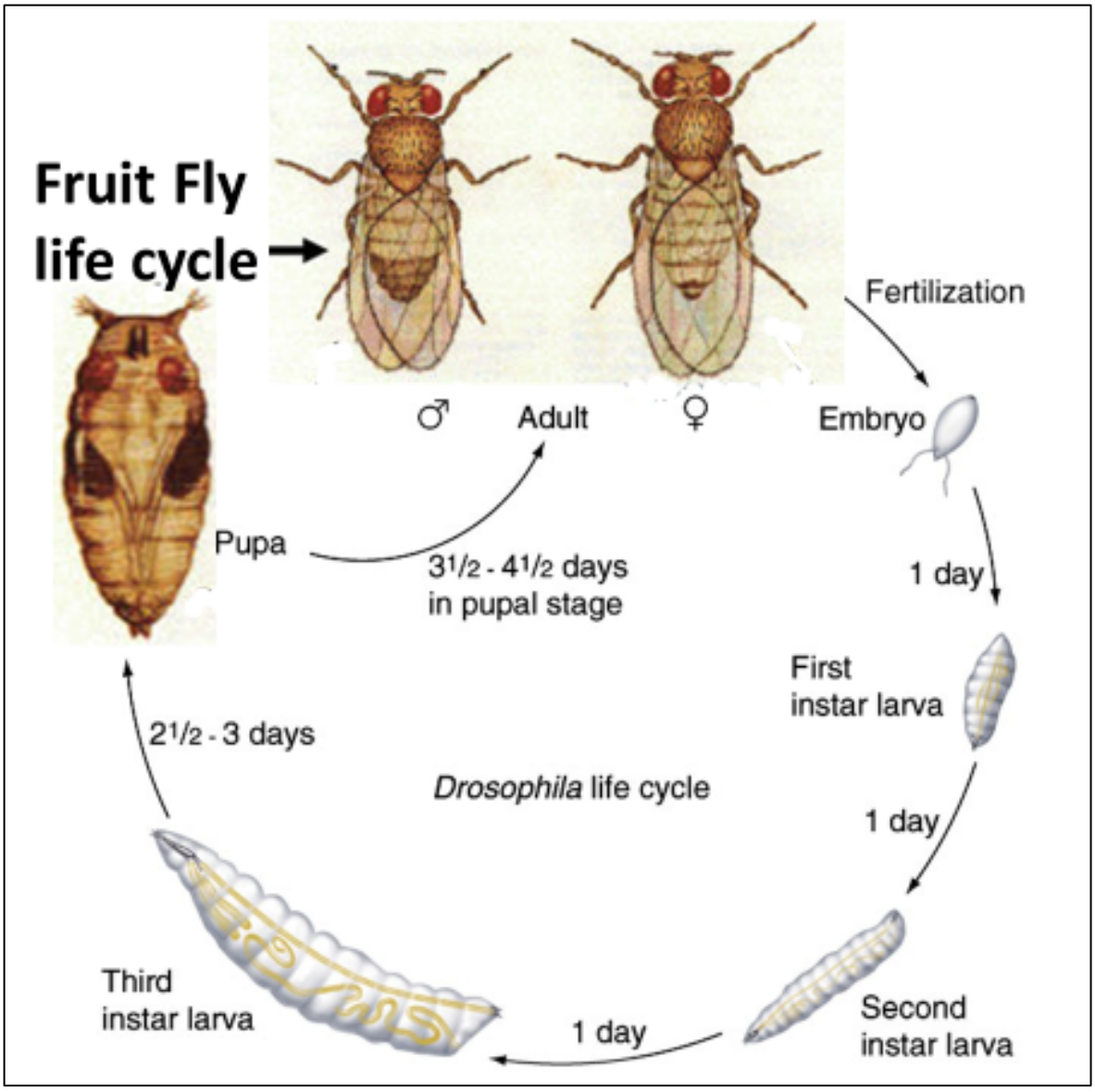
While we are incubating the bacteria, we will take a look at some fruit flies with different phenotypes.

Drosophila melanogaster

One of the classic tools of genetic research is the fruit fly, *Drosophila melanogaster.* This organism has been used in genetic studies for nearly 70 years and has played an important role in the development of our knowledge of heredity. *Drosophila melanogaster* has been a particularly important eukaryotic genetic system for such studies because it has a very low chromosome number. The haploid (N) number of chromosomes is 4 and the chromosomes are designated X (I), 2, 3, and 4. The 2, 3, and 4 chromosomes are the same in both sexes and are referred to as autosomes to distinguish them from the X and Y sex chromosomes. *Drosophila* females are characterized by having two X chromosomes while *Drosophila* males have an X and a Y chromosome.

The Y chromosome does not contain alleles of genes present on the X and so male *Drosophila* have one dose of each sex-linked gene while females have two. The 4^{th} chromosome is so small, constituting only about 5% of the total genome, that, for all practical purposes, it can be ignored. Thus, almost the entire genetic content of the *Drosophila* genome resides on only three chromosomes.

Another characteristic of *Drosophila* that makes it an excellent genetic research tool is its short generation time. At 25° C, *Drosophila* culture will produce a new generation in 10 days: one day in the egg (embryo) stage, five days in-the larval stage, and four days in the pupal stage.



To begin:

I. Anesthetize the flies as demonstrated by your instructor by using your fly pad.

2. When the flies have become immobilized on the fly pad you can examine them and move them with a paintbrush under the stereoscopic microscope.

3. From the vial of wild type flies, identify 3 females and 2 males and return the rest to their appropriate vial. The following criteria may be used to distinguish adult males and females.

a. Size- the female is generally larger than the male.

b. Shape of abdomen- the female abdomen is larger and more pointed than the male abdomen.

c. Abdominal pigmentation- alternating dark and light dorsal bands can be seen on the entire rear portion of the female abdomen; the last few segments of the male are uniformly pigmented.

d. External genitalia- on the ventral portion of the abdomen, the female has anal plates and lightly pigmented ovipositor plates. The male has anal plates and a darkly pigmented genital arch and penis.

e. Sex comb- on males, there is a tiny brush -like tuft of hairs on the basal tarsal segment of each foreleg. This is the most accurate method for sexing males.

4. Next you will examine flies with mutations; each mutation is on a different chromosome.

a. White eyed flies- chromosome # 1 (sex-linked)

b. Apterous- chromosome # 2

c. Sepia flies - chromosome # 3

Finally:

d. Double mutant - chromosomes 2 and 3

Also, be sure to look.at the bar eyed flies to see a noticeable change in eye shapes.

Analysis

Draw Punnett squares for *a*, *b*, and *c*. Draw as if *each of these mutant flies were mated with a wild type male and female*. Then, do a Punnett square for a cross, which would involve two F1 flies. Figure out the phenotypic ratios for each cross. Also, cross your double mutant with wild type male and female and do an Fl cross for that as well. Put all the results in your notebook.

Lab 10

Molecular Genetics

Goal: In this lab activity we will be performing experiments with DNA, looking at it on a macroscopic scale and inserting foreign DNA into bacteria and observing the results. These techniques are commonly used in modern molecular biology and are the basis for the field of genetic engineering.

Pre-Lab questions:

1. Define the following terms:

a. Bacterial transformation_____

b. Plasmid_____

c. Selecting agent_____

d. Antibiotic_____

e. Competent bacteria_____

f. Cloning_____

g. Bacterial colony_____

2. Look up and list some common lab methods for bacterial transformation:_____

_____

3. Look up and define the following terms for inserting foreign genes into *eukaryotic* cells:

a. Transfection_____

b. Viral Transduction_____

Background: Approximately 1 in every 10,000 cells successfully incorporates plasmid DNA in preparations having average competency. However, there are a large number of cells in a sample (typically $1x10^9$) that only a small fraction needs to be transformed to obtain colonies on a plate. The same volume of recovered cells plated on selective (contains antibiotic) and nonselective agar medium will yield vastly different numbers of cells. The nonselective medium will have many more growing cells that form a bacterial lawn.

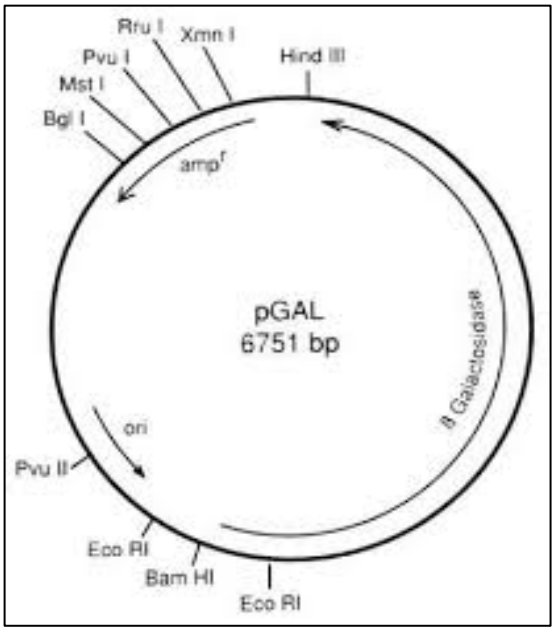
Many different plasmids serve as useful tools in molecular biology. One example is the pGAL plasmid present in multiple copies in specified host *E. coli* host cells. It contains 6,751 base pairs and has been

genetically modified. In the cell, the plasmid does not integrate into the bacterial chromosome but replicates autonomously. The pGAL plasmid contains the *E. coli* gene which codes for p-galactosidase in the presence of artificial galactosides such as 5-Bromo-4-Chloro-3-indolyl-I3-D-galactosidase (X-Gal). pGAL colonies appear blue when X-Gal is cleaved by β-galactosidase and forms a colored product.

The proprietary plasmid, pGAL (Blue Colony) was engineered by EDVOTEK. Plasmid pGAL carries the complete gene for β -galactosidase. Since the host *E. coli* does not contain a β -galactosidase gene, only cells transformed by the pGAL plasmid will produce the functional β-galactosidase enzyme. Cells that express β-galactosidase will cleave X-Gal and the pGAL transformed colonies will be blue.

In addition to the expression and cleavage of X-Gal by β-galactosidase, transformation by pGAL is also demonstrated by resistance to ampicillin. *E. coli* host cells used in this experiment are not naturally resistant to ampicillin. The plasmid pGAL contains the gene which encodes for 13-lactamase that inactivates ampicillin. *E. coli* cells transformed by pGAL will express the resistance gene product p-lactamase as an extracellular enzyme excreted from *E. coli* cells. Once outside the cell, the enzyme diffuses into the surrounding medium and inactivates ampicillin.

With time, small "satellite" colonies may appear around a large blue colony. Cells in the small "satellite" or "feeder" colonies are not resistant to ampicillin and have not been transformed with the pGAL plasmid. They are simply growing in a region of agar where β -lactamase has diffused and inactivated the antibiotic ampicillin. The number of satellite colonies increases if the concentration of ampicillin is low, or the plates have incubated for longer.



Lab Instructions *One partner will carry out this experiment while the other while the other is preparing the bacteria in Experiment #2

Experiment #1 DNA Extraction from Strawberries

(credit to Mr. Chanokh Berenson (YC 2013) for writing this lab procedure)

Background: DNA is normally too small to see without the help of a microscope.

Strawberries, however, have so much DNA that it is possible to see their DNA with the unaided eye. A chromosome is an organized package of DNA found in the nucleus of the cell. Strawberries have **8 (!)** copies of each chromosome. For comparison, humans only have two copies of each chromosome. In addition, ripe strawberries produce enzymes (pectinases and cellulases) which help break down cell walls and expose their DNA

Purpose: The purpose of today's lab is to extract and isolate DNA from Strawberries.

Materials:

• 1-2 strawberries (about the volume of a golf ball)

• 2 Graduated Cylinders

• 10mL DNA Extraction Buffer (soapy salty water)

• About 20 mL ice cold 91% isopropyl alcohol

• 1 Ziploc TM bag

• 1 Clear Test Tube

• 1 Filter Funnel with Filter Paper

• 1 Beaker

• 2 Pipettes

DNA Extraction Buffer

• 50 ml dishwasher detergent

• 15 grams sodium chloride (2 teaspoons)

• Water to 1 liter

Procedure:

1. Prepare a filter funnel with moist filter paper and carefully place it onto a Beaker.

2. Fill Graduated Cylinder #1 with 10mL of the DNA Extraction Buffer (soapy salty water).

3. Remove the green leaves from the strawberries and dispose of the green leaves in the

waste bin.

4. Place strawberries into a Ziploc TM bag and seal the bag shut.

5. With your hands, squish the strawberries for a few minutes to make a strawberry pulp.

Be careful not to squish so hard that you open or damage the Ziploc TM bag.

6. Once the strawberries are squished, add 10 mL of the DNA Extraction Buffer (soapy salty water) from your graduated cylinder and squish the Strawberry + Buffer mixture for a few more minutes. Try not to make a lot of soap bubbles. The soap breaks up the

phospholipid bilayers of the Strawberry cells.

7. Pour the soapy salty Strawberry Pulp into the Filter Funnel over the Beaker. Do not force any strawberry pulp through the filter. Collect at least 3 mL of liquid.

8. Remove the Filter Funnel from the top of the Beaker and set the Filter Funnel down on the workstation very carefully.

9. Transfer the Strawberry Extract Solution from the Beaker into a clear test tube with a

pipette. Be very careful not to drop the Beaker.

10. Very carefully and with a pipette, add 20mL ice-cold isopropyl alcohol to the top

Strawberry Extract Solution in the tube. Drip the isopropyl alcohol carefully down the

side of the tube so that it forms a separate layer on top of the strawberry liquid.

Do not bump the test tube.

11. Watch for about a minute. What do you see? Record your observation:

_____

_____

12. After the DNA appears, spin and stir the transfer pipette in the tangle of DNA to wrap the DNA around the pipette.

13. Pull out the pipette and look at the DNA on the pipette. The fibers are millions of DNA strands. Describe what you see:

_____

_____

_____

14. Remove the filter paper from the filter Funnel and dispose of the filter paper,

strawberry pulp, Ziploc TM bag, and the pipette with the DNA in the blue waste bin.

15. Rinse out the Funnel, Beaker, and Test Tube and return them back to your station.

Analysis/Conclusion:

1. What is the function of DNA?

_____

_____

_____

2. Was the DNA Double Helix observable in this experiment? Why or why not?

_____

_____

_____

3. What is the purpose of the Soap in this experiment?

_____

_____

_____

4. Do you think this experiment would work to extract and isolate DNA from Human cheek cells? Why or why not?

_____

_____

Experiment #2: Bacterial Transformation

Hypothesis:

1 Bacteria transformed by the p-Gal plasmid_____

2 On a plate that has X-gal and ampicillin on it, what would you expect to see if the following bacteria are plated?_____

a. Bacteria that are naturally resistant to ampicillin without being transformed by p-Gal_____

b. Bacteria that have not been transformed and are sensitive to ampicillin_____

2. What would you expect to see if you plated a bacterial sample that had been transformed with p-Gal on a plate that had X- gal but no ampicillin? Remember that most bacteria in the sample don't actually get transformed. _____

_____

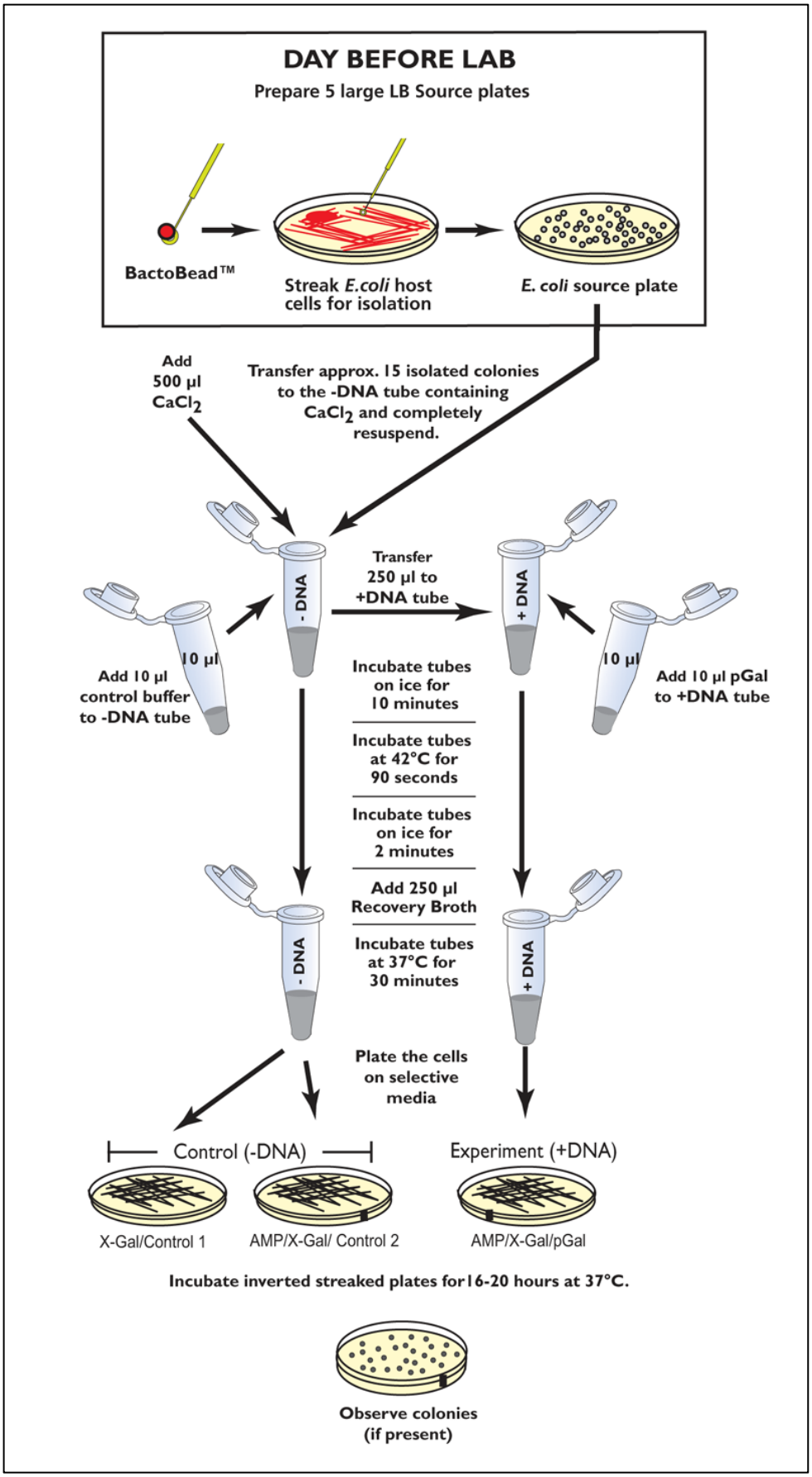
EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of the biologic process of bacterial transformation by plasmid DNA.

This experiment demonstrates the acquired Lac' phenotypic trait of the' transformed bacterial cells as shown by the presence of blue bacterial colonies.

BRIEF DESCRIPTION OF EXPERIMENT:

In this experiment, students will transform host bacterial cells with a plasmid DNA. The transformants acquire antibiotic resistance and exhibit a blue color due to the incorporation and expression of β-galactosidase and ampicillin resistance genes. IPTG is not required since pGAL™ contains the intact β-galactosidase gene. The number of transformants will be counted and the transformation efficiency will be determined.


DAY BEFORE LAB
Prepare 5 large LB Source plates
BactoBead™
Streak E.coli host cells for isolation
E. coli source plate
Add 500 µl $CaCl_2$
Transfer approx. 15 isolated colonies to the -DNA tube containing $CaCl_2$ and completely resuspend.
- DNA
Transfer 250 µl to +DNA tube
+ DNA
10 µl
Add 10 µl control buffer to -DNA tube
10 µl
Add 10 µl pGal to +DNA tube
Incubate tubes on ice for 10 minutes
Incubate tubes at 42°C for 90 seconds
Incubate tubes on ice for 2 minutes
Add 250 µl Recovery Broth
Incubate tubes at 37°C for 30 minutes
- DNA
+ DNA
Plate the cells on selective media
Control (-DNA)
Experiment (+DNA)
X-Gal/Control 1
AMP/X-Gal/ Control 2
AMP/X-Gal/pGal
Incubate inverted streaked plates for16-20 hours at 37°C.
Observe colonies (if present)

SETTING UP THE TRANSFORMATION AND CONTROL EXPERIMENT

1. Put your initials or group number on the tubes labelled “pGAL DNA” (contains 25ui of plasmid DNA) and “Control Buffer” (contains 25ul of buffer). Place them back on ice.
2. Set up the Control:

- Using a sterile transfer, pipet, transfer 0.25 ml (250ui) of cell suspension from tube “Cells” to the tube “Control Buffer”
- Carefully place the pipet back into the wrapper.
- Cap the tube: mix by tapping. Put the tube back on ice.

3. Set up the transformation:

- Using the same pipet from Step 2, transfer 0.25ml (250ui) of cell suspension from the tube “Cells” to the tube “pGAL DNA”
- Cap the tube: mix by tapping. Put the tube back on ice.

4. Incubate the cells prepared in steps 1 – 3 on ice for 10 minutes.
5. Place both transformation tubes at 42^{o}C for 90 seconds. This heat shock step facilitates the entry of DNA in bacterial cells.
6. Return both tubes to the ice bucket and incubate for 1 minute.
7. Add 0.75 ml of the recovery broth to the tube “Control Buffer”

   Add the recovery broth with a sterile 1 ml pipet. Avoid touching the cells with the pipet.

8. Add 0.75 ml of the recovery broth to the tube “pGAL DNA”

   Quick Reference:

   DNA and component cells are combined in a 0.25 ml suspension. After the cells have incubated with the DNA, growth medium (recovery broth) is added. Bacterial cells continue to grow through the recovery process, during which time the cell wall is repaired. Cells recover and begin to express the antibiotic resistance gene.

9. Incubate the closed tubes in a 37^{o}C water bath for 30 minutes for a recovery period.

10. While the tubes are incubating, label "3"

    Agar plates as indicated below. Write on the bottom or side of the petri plate.

- Label one plate: X-GAL/Control 1
- Label one plate: AMP/X-GAL/Control 2
- Label one plate: AMP/X-GAL/pGAL
- Put your initials or groups initials on all plates.

11. After the recovery period, remove the tubes from the water bath and place them on the lab bench. Proceed to plating the cells for

    PLATING THE CELLS

    Plating cells from the tube labelled "Control":

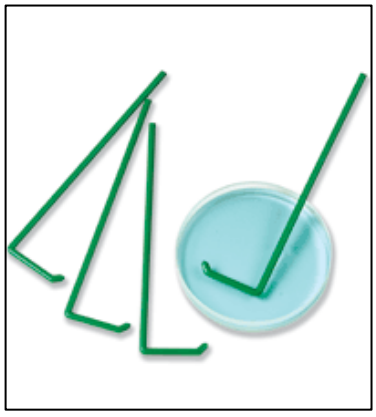
12. Use a fresh, sterile 1 ml pipet to transfer recovered cells from the tube "Control Buffer" to the middle of the following plates:

- 0.25 ml to the plate labelled X-GAL/Control 1
- 0.25 ml to the plate labelled AMP/XGAL/Control 2

13. Spread the cells over the entire plate with a sterile spreader.

14. Cover both control plates and allow the liquid to be absorbed.

To avoid contamination when plating, do not set the lid down on the lab bench. Lift the lid of the plate only enough to allow spreading. Be careful to avoid gouging the spreader into the agar.



Plating cells from the tube labelled "pGAL DNA"

15. Use a fresh, sterile 1ml pipet to transfer recovered cells from the tube "pGAL DNA" to the middle of the following plate.

16. 0.25 ml to the plate labelled AMPX/X-GAL/pGAL

17. Spread the cells with a sterile inoculation loop.

18. Cover the plate and allow the liquid to be absorbed (approximately 15 – 20 minutes).

19. Stack your group's set of plates on top of one another and tape them together. Put your initials or group number on the taped set of plates.

20. Place the set of plates in a safe place designated by your instructor.

21. After the cell suspension is absorbed by the agar (approximately 15-20 minutes), you or your instructor will place the plates in the **inverted position** (agar side on top) in a 37^{o}C bacterial incubation oven for overnight incubation (15-20 hours)

If the cells have not been absorbed into the medium, it is best to incubate the plates upright. The plates are inverted to prevent condensation on the lid, which could drip onto the culture and may interfere with experimental results.

The following week you will view the incubated plates. You will then record and analyze your results. Draw and/or photograph observations. Explain what they mean in terms of the learning objectives of this experiment. When finished, dispose of plates in biohazard bins.

ANSWER THESE QUESTIONS BEFORE ANALYZING RESULTS

1. On which plate(s) would you find (only) genetically transformed cells? Explain.
2. What is the purpose of the control plates?
3. Why would one compare plates AMP/X-GAL and AMP/X-GAL/pGAL?

Data Collection

Consider the results you obtained on your transformation and control plates.

- Transformation Plate: +DNA
- AMP/X-GAL/pGAL
- Control Plates: -DNA
- X-GAL/Control 1
- AMP/X-GAL/Control 2

4. Draw and describe. Record the following for each plate:

- How much bacterial growth do you observe? _____
- What color are the bacteria? _____
- Why do different members of your class have different transformation efficiency values? _____

  _____
- If you did not get any results, what factors could be attributed to this fact? _____

  _____

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Did you observe any satellite colonies? Why are the satellite, and feeder colonies white?
2. Why did the competent cells which did not receive DNA (control) fall to grow on the plates containing ampicillin?
3. Why are there so many cells growing on the X-GAL plate? What color are they?
4. What evidence do you have that transformation was successful?
5. What are some reasons why transformation may be unsuccessful?

LAB 11

ANIMAL DEVELOPMENT & PHYSIOLOGY

Goal: See living chicken embryos and use comparative anatomical approach to learn the developmental pathway of a bird. Witness blood flow at the cellular level. Produce detailed accounts of your observations that could be used as a study tool and to impart your findings effectively to somebody who wasn't in lab.

Pre-Lab Questions:

1. What is the purpose of a yolk?

_____

_____

2. Which of the five major embryonic processes forms the brain?

_____

3. List three organs that arise from each; ectoderm, mesoderm, endoderm.

_____

_____

_____

4. List five animals that reproduce extra embryonically.

_____

_____

Background

An animal's embryonic development can be divided into five major processes:

(1) **Gametogenesis:** The process in which cells undergo meiosis to form gametes.

(2) **Fertilization:** The action or process of fertilizing an egg, female animal, or plant, involving the fusion of male and female gametes to form a zygote.

(3) **Cleavage**: Is a number of mitotic divisions that partition the large cytoplasm of the fertilized egg into smaller cells

(4) **Gastrulation**: A morphogenic cellular movement that produces an embryo with three layers of cells

(5) **Organogenesis:** The process whereby specific organs develop from the primary germ layers. The germ layers are ectoderm, mesoderm and endoderm in animals that are triploblastic. That includes amphibians, reptiles, birds, mammals and fish. Learn examples of organs that differentiate from each layer.

The physical characteristics of eggs are related to the environment in which an animal lives, the place where the embryo develops, and the stages in the life cycle of the species. Eggs of animals that live in

aquatic environments generally have gelatinous coats which protect the egg from physical trauma and infection. Yolk supplies the embryo with sufficient energy to achieve the development stage of self-feeding. After a chicken hatches, it has about 24 hours before it needs to eat. Some other reptiles have an external yolk plug upon hatching and can go even longer without food.

See the turtle hatchling in the figure below:

Animals that release their eggs for development outside of the female have eggs covered by hard shells that provide protection against physical injury and desiccation. However, such a protective device is not without its problems. The embryo inside must exchange O_2 and CO_2 with the environment, must have sufficient energy and raw materials to develop to an advanced stage, and must have means of disposing of nitrogenous wastes. Inside such eggs, four extraembryonic membranes grow out from the embryo, surround it, and function in gas exchange waste storage, and nutrient procurement. Before class, look up same animals that reproduce this way.

In animals, the way in which an egg undergoes development is strongly influenced by the volume and distribution of yolk. Four types of eggs are found in the animal kingdom. Isolecithal eggs have yolk uniformly distributed throughout the egg and are characteristic of starfish and mammals. Mesolecithal eggs have a moderate amount of yolk localized in one hemisphere of the egg and are found within the clade Amphibia. Telolecithal eggs are characteristic of birds, reptiles, and fish. Telolecithal eggs have large yolks. The dividing portion of a telolecithal egg is restricted to a small disc area on the surface of the yolk. Finally, in centrolecithal eggs, found among insects, the dividing portion of the egg surrounds a large central yolk mass.

Chick Development

You will study eggs that are at five different stages of development. They will range from 3-21 days of age.

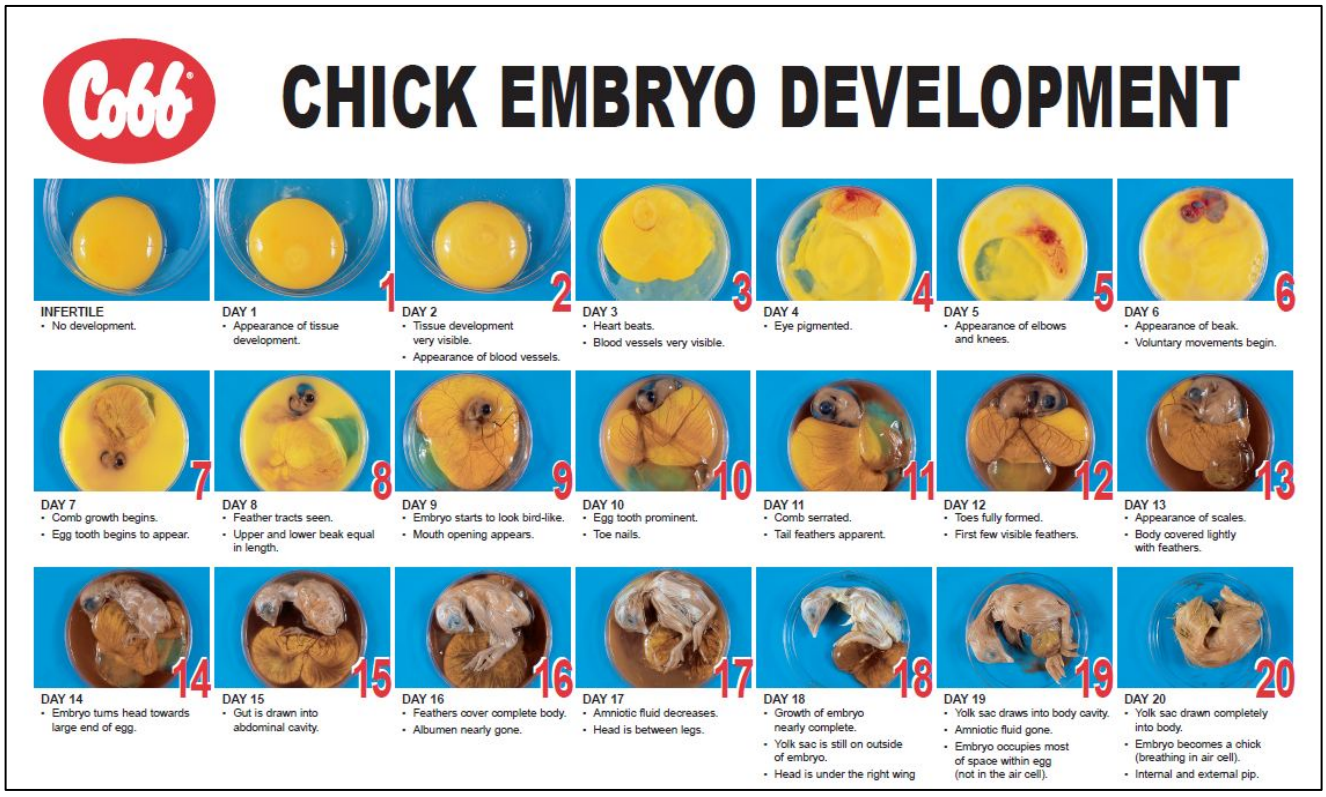
The very early stages; 18 – 72 hrs cannot be observed by opening a hard shelled egg because the embryo is still in the chicken during this time. Using prepared microscope slides to see what happens during the early hours of development. There are three slides to study.

Since the eggs of birds (also fish and reptiles) are telolecithal and contain large amounts of yolk, the nucleus and cytoplasm that will divide are confined to a small disc shaped area called the blastodisc that sits on top of the yolk. Fertilization occurs at the blastodisc where cell cleavage is confined. The large yolk does not divide. The developing chick embryo floats atop the yolk during development.

As the cleavage divisions occur in the blastodisc, a flat layer of cells called the blastoderm is produced. As the cleavage continues, the blastoderm becomes several cell layers thick with a fluid-filled space, the subgerminal space, developing between it and the yolk. The blastula is formed when the cell layers of the blastoderm-split into an upper epiblast layer and a lower hypoblast layer. The cells of the epiblast layer reorganize themselves and move to positions that commit them to different developmental fates, which are best represented in a fate map.

As development continues cells in the center of the long axis of the pear-shaped blastoderm migrate down from the surface and away from the center area in a process that is equivalent to gastrulation. These movements result in recognizable stages called the primitive streak and primitive groove. Examine the large models of chick development to visualize these stages.

<u>You may refer to the guides below when examining the embryos</u>:



Chicken Embryonic Development Procedure:

Take your egg of 72 or 96 hours and follow the procedures outlined below:

1. Pick up an egg from the group, maintaining its horizontal position as you carry it back to your bench. Place the egg, in a horizontal position, in the glass dish that is lined with cotton.

2. Place Scotch tape along the long axis of the egg, so that it covers most of the "top" of the egg.

3. Cover the rounded end of the egg with a small piece of scotch tape.

4. Puncture the rounded end of the egg that is covered by the tape with a 200 gauge needle or similar. Insert the needle into the egg so that it is pointing down. Be careful of your fingers!

5. Withdraw 0.5ml of albumen. This allows the embryo (if there is one) to move away from the upper surface of the egg, where you will be cutting out the window. Save the syringe and the albumen.

6. **CAREFULLY** puncture the tape-covered top of the egg with the tip of your scissors. The location of the puncture should be about a half an inch off-center.

7. Proceed to cut out an oval opening. Pull up with your scissors so that you are keeping them as far away from the embryo and vitelline envelope as possible.

8. The size of the opening depends on the size of the egg; it should be about the size of a quartet. With forceps, remove the shell cap, exposing the window.

9. If you are going to observe the embryo for more than a couple of minutes while it is still in the egg, you will need to prevent dehydration; add several drops of the chick saline to the surface of the embryo. If you are going to immediately explant the embryo, do not add any saline. It will prevent the filter paper doughnut from adhering to the vitelline envelope.

10. With the forceps, gently pick off any lumps of albumen on the egg's surface until it appears almost dry.

11. Place a filter paper doughnut on the blastoderm so it frames the embryo. The filter paper *will* stick to the vitelline envelope, holding the embryo in the center. You will then cut the filter paper off the surface of the egg, lifting the embryo with it. When you finally free the filter paper, the embryo should be framed and held within the filter paper. It is then transferred to the dish of warm saline.

- With forceps, gently position a filter paper doughnut on top of the vitelline envelope, framing the embryo. Gently pat the doughnut in place with the tips of the forceps. Wait a minute to allow the vitelline envelope to adhere to the filter paper.
- While you wait, fill one of the small dishes with about a quarter of an inch of warm saline and place the dish on the stage of your dissecting microscope.
- Hold one edge of the doughnut with the tips of the forceps and cut the vitelline envelope along the edge of the doughnut with a pair of scissors. Slowly work your way around the rim of the doughnut, carefully checking to see that the vitelline envelope adheres to it.
- Gradually lift the doughnut-the embryo should remain within the center of the doughnut.

Quickly transfer the explant to the small dish with the warmed chick saline. You can keep the embryo "upright" so the ventral (right side) is facing up. Alternatively, you can flip the explant over so the surface that was facing the yolk (the dorsal or the left side) is now facing upwards.

4. Place the dish on the stage of the dissecting microscope.

5. Sketch the embryo. Label the largest features: brain, lens, optic cup, heart, anterior (wing) limb bud, and posterior (leg) bud. Be precise in your labelling.

6. In your lab notebook, describe the flow of blood through the heart.

Question: What is the direction of blood flow? Be precise.

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