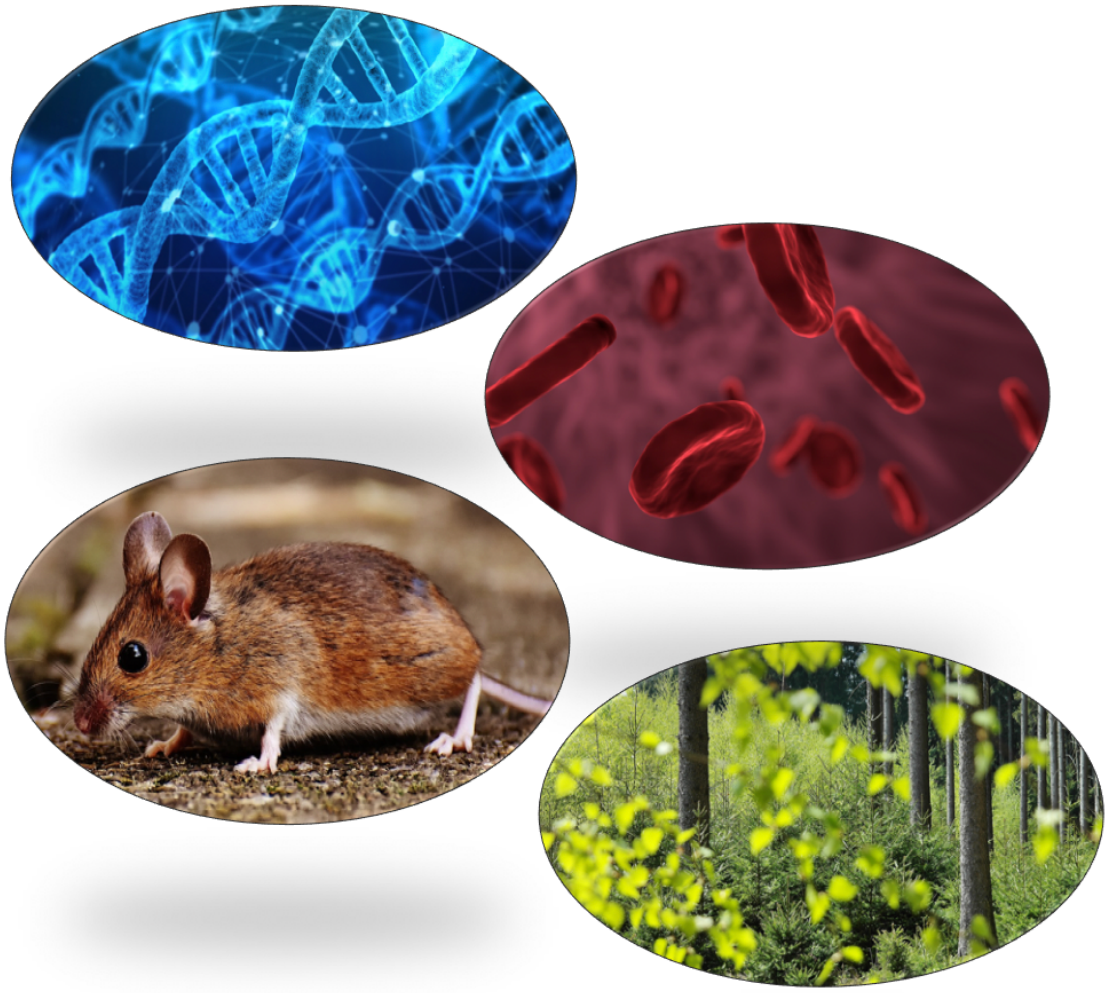
LABORATORY MANUAL
BIO 111
GENERAL BIOLOGY



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2023 – 2024

LABORATORY MANUAL TO ACCOMPANY BIO 111

CALIFORNIA POLYTECHNIC STATE UNIVERSITY
DEPARTMENT OF BIOLOGICAL SCIENCES
2023 – 2024

Welcome to BIO 111 Lab, General Biology. The lab section of this course will complement the lectures, providing you with hands-on experience with the incredible diversity of life on earth and the scientific process we use to gain knowledge of it. We hope that this insight will help spark your interest and appreciation for the organisms we share this planet with. In addition, you will acquire and hone important laboratory skills that will serve you well throughout your careers at Cal Poly and beyond. Over the next 10 weeks, you will learn the proper use of microscopes, the importance of neat and clear laboratory notebooks, the use of a variety of other important lab equipment and lab techniques, and the importance of hypotheses and predictions, among many other things.

And most importantly, we want you to appreciate and love biology as much as we do!

How to use this book

1. Read it! We have recently rewritten the entire lab manual. We believe that everything in it is important. Read it!
2. Pre-Lab Assignments: At the beginning of each lab, you will find a series of small Pre-Lab Assignments (indicated by a "⚠") that you will need to complete **BEFORE COMING TO LAB** each week.

Table of Contents

LABORATORY NOTEBOOK GUIDELINES

You will be required to purchase a notebook with blank lined pages to be used through the quarter as a Laboratory Notebook. Each lab exercise will have instructions for specific things to be recorded in your notebook. You will record observations and data obtained during experiments, you will create tables, and you will take notes on pertinent information found in displays and provided by your instructors. This notebook must be brought to lab and used every week. Your instructor may ask at any time to inspect your notebook, to ensure you are using it properly and recording necessary information. Your lab notebook will become a valuable tool for studying for quizzes and exams.

1. **Overall organization**
    a. Your lab notebook should be hard bound. A "Composition Book" available at the Cal Poly Bookstore is a good choice.
    b. You should create a table of contents on the first page.
    c. Write page numbers on each page.
    d. No loose paper. Record everything directly into the pages of your notebook.

2. **Lab exercise Organization & Structure**
    a. Begin each lab exercise on a new page (i.e., the end of Lab 2 should not be on the same page as the beginning of Lab 3).
    b. Each lab exercise must be dated.
    c. Each lab exercise must be titled.
    d. Objectives for each lab must be clearly stated at the beginning.
    e. Transitions between topics/activities must be clear and titled.

3. **Drawings & Tables**
    a. Must be titled.
    b. Important features must be labeled.
    c. Only use metric units on ALL scaled entries.
    d. Indicate total magnification you used in viewing a slide.
    e. Tables must be completely filled in.

4. **Discussion Questions & Observations**
    a. Makes clear what question/topic is being addressed.
    b. Answers/observations must be thoughtfully and completely stated.
    c. Use complete sentences

Lab Notebook Template

Lab 1: Introduction to the Process of Science and the Care and Use of Microscopes

26 September 2021

- Pre-lab terms/Questions
- Objectives (one for each activity or exercise)

Exercise 1: The Process of Science

A. Answers to discussion questions

Exercise 2: Observational Study of Plant Growth

A. Observations
   Week 1: Date
   Week 2: Date
   Week 3: Date
B. Discussion/Terms/Short Answer Questions

Exercise 3: The Care and Use of Microscopes

A. Definitions/functions of microscope parts
B. Drawings of images under microscopes (with titles, labels, and magnification)
C. Answers to discussion questions

Lab 1: Introduction to the Process of Science and the Care and Use of Microscopes

Pre-Lab Assignments

1. Read this week's laboratory exercises, including the instructions on the pages 4 – 5 about how to format your lab notebook.
2. On Canvas, watch the use and care of microscope video(s) and come to lab prepared to demonstrate proper microscope techniques and the major differences among microscope types.
3. In your lab notebook, define the following terms:
    - Independent Variable
    - Dependent Variable
    - Replication
    - Control
4. Check your lab Canvas to view this week's textbook reading assignment.

Learning Objectives

1. Understand the steps of the scientific method and how to use them to answer scientific questions.
2. Set up an observational study that we will revisit for several weeks.
3. Understand how to find an image under a microscope.

Exercise 1: The Process of Science

Introduction

In this exercise, you will gain knowledge and experience with the scientific method.

Typically, the scientific method consists of the following steps:

1. Ask a Question
2. Formulate a *hypothesis* (a general explanation of the observation)
3. Devise a *prediction* that is specific and testable based on the hypothesis
4. Conduct an *experiment* to provide evidence for or against your hypothesis
5. Analyze results and draw *conclusions – if hypothesis is not supported, think, reformulate question and/or hypothesis and try again.*
6. Report results

In groups of four, using the internet, whiteboards, and asking your instructor, discuss the following and write down your answers/notes in your lab notebook:

1. How do the steps in the scientific method relate to one another (e.g. how does one lead to the next)? How might the design and results of experiments change if the scientists conducting them desired a pre-determined outcome?
2. What is the difference between an independent and a dependent variable?
3. What is the role of a control in an experiment?
4. Why are replicates important in an experiment?
5. What is the difference between the classic definition of a theory and that of a scientific theory?

Exercise 2: Observational Study of Plant Growth

In this exercise, we will be conducting an experiment on the response of radish seedlings to light, which will tie into a later lab exercise on Photosynthesis.

The rapid germination of radish seeds (*Raphanus sativus*) allows us to investigate, in a period of just three weeks, how seedlings respond to different light conditions. Do seedlings need light to germinate? Under what conditions do seedlings "bend" toward a light source? You will observe seedlings set in different light conditions, and make observations on their response to the different conditions.

The light conditions we will observe in lab are as follows:

A. Seedlings are placed to the side of a light source and remain stationary.
B. Seedlings are placed to the side of a light source on rotating turntables.
C. Seedlings are placed directly under a light source.
D. Seedlings are placed in a dark cabinet, completely without light.
E. Seedlings are placed in a cabinet, behind a green filter
F. Seedlings are placed in a cabinet, behind a blue filter
G. Seedlings are placed in a cabinet, behind a red filter
H. Seedlings are placed in a cabinet, with no filter.

Note: Individual colors of light correspond with different wavelengths of light energy. Plants sense different wavelengths of light energy through a variety of light receptors in their cells. Not all wavelengths have the same meaning to plants. "White" light is a mixture of all the wavelengths of visible light. Note that a "green filter" only allows green light into the chamber. It is a common misconception, for example, that the "green filter" filters out green light. There is no substitute for actually looking at the light box itself to be clear on this!

Observations

Make the following observations for each light treatment and record them in your laboratory notebook during the first three weeks of lab.

1. Height of seedlings in centimeters. Measure height as the length of the stem from pot to the apical meristem.

2. Are the seedlings leaning away from the vertical? If so, does it appear that they are leaning toward a light source?

3. Color of leaves (yellow, pale green or green) and stem (white or red).

Exercise 3: The Care and Use of Microscopes

Introduction
Many biological observations require microscopes (think about cells!). There are several different types of microscopy commonly used in biology: light microscopy (uses visible light to form an image), electron microscopy (uses electrons to form an image), and confocal microscopy (uses lasers to form an image).

In Bio 111, we will only use light microscopy, using bright field light microscopes which shine the light directly into the objective lens. In this type of microscopy, the background appears bright and objects appear darker. Both of the microscopes you will be using this quarter have two lens systems: **the ocular lens** and the **objective lens**. The ocular lens is the lens closest to your eye that you directly look through, it magnifies at 10X while the objective lens is located in the nosepiece of the microscope. In **compound microscopes** there are generally four objective lenses that magnify at 4X (low power), 10X, 40X, and 100X (oil immersion, which we will not be using in this laboratory). Compound scopes invert the image you are viewing, which can take some getting used to. For instructions on use, care, storage, and tips for getting a good image on dissecting microscopes, go the appendix of this manual (page 47).

Using the Compound Microscope

Materials

Compound scope
Prepared slides
Sample from outside
Slides and coverslips
Immersion oil
Lens paper
Alcohol and lens paper
cone/Q-tips

Procedure
1. Carefully take a compound microscope from the cabinet. Carry it upright, using both hands: one under the base and the other holding the arm. Do not tilt the microscope backwards since the ocular lenses may fall out. You may need to clean the lenses and stage. ONLY use lens paper, because anything else will scratch the lenses. Wetting the lens paper with isopropyl alcohol will help.
2. Identify and familiarize yourself with the major parts of your microscope:

- Arm
- Base
- Coarse focus adjustment
- Condenser
- Condenser height adjustment
- Fine focus adjustment
- Light intensity control
- Light source
- Light switch
- Eyepiece/ nosepiece
- Objective lenses
- Ocular focus adjustment
- Iris diaphragm lever
- Slide adjustment knobs
- Slide holder
- Ocular lens
- Stage

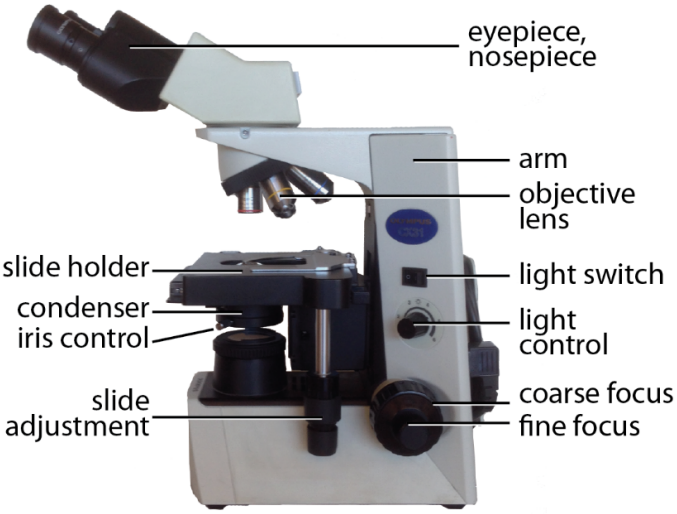


Fig. 1. The Compound Microscope

3. Obtain a prepared slide as directed by your instructor.
4. Place the slide in the slide holder. Position the slide on the stage in the center (over the hole where the light comes through) using the slide adjustment knobs.
5. Plug in and turn on the microscope; adjust the light to mid-range
6. See "Steps to Obtaining a Good Image on a Compound Microscope" (in appendix on page 47).
7. Observe and draw your slide using the 4X first, then the 10X, and 40X objectives.
8. Make a wet mount of an organism (your TA will show you how). Find this organism under the microscope and draw it in your lab notebook on the highest magnification at which you can get a clear image.

Lab Notebook Assignment

1 Title

2. Objectives

3. Exercise 1: The Process of Science
    a. Discussion Questions
        1. How do the steps in the scientific method relate to one another (e.g. how does one lead to the next)? How might the design and results of experiments change if the scientists conducting them desired a pre-determined outcome?
        2. What is the difference between an independent and a dependent variable?
        3. What is the role of a control in an experiment?
        4. Why are replicates important in an experiment?
        5. What is the difference between the classic definition of a theory and that of a scientific theory?

4. Exercise 2: Observational Study in Plant Growth
    a. Weekly Observations
    b. Discussion Question
        1. Using the scientific language you defined in your prelab and discussed in lab, what is the experimental purpose of each treatment (A – H)?

5. Exercise 3: The Care and Use of Microscopes
    a. Describe the function of the following parts of the compound scope: slide holder, condenser, iris control, eyepiece, objective lens, coarse focus, fine focus, slide adjustment
    b. Make a side-by-side comparison of the view through a microscope by drawing THE SAME slide under the 4X objective, the 10X objective, and the 40X objective.
    c. Draw the wet mount of an organism you made at the highest magnification at which you can get a clear image (make sure to indicate the magnification of your drawing).
    d. Discussion Questions
        1. How big is 1 µm relative to 1 mm? Relative to 1 cm?
        2. Calculate total magnification on a compound microscope when using 4x, 10x, 40x, and 100x objectives.

Lab 2: Unity and Diversity of Life

Pre-Lab Assignments

1. Check your lab Canvas to view this week's textbook reading assignment.
2. In your lab notebook, define and explain the cellular role the following terms:
   - Cell membranes
   - Enzymes
   - Nucleic acids
   - Ribosomes

Learning Objectives

1. Understand cells and cell components and be able to recognize or describe an organism based on its cells.
2. Remember taxonomic ranks and understand how and why they are used in organizing life in conjunction with phylogenies.
3. Survey all forms of life and recognize or describe the essential features of bacteria, plants, fungi, and animals.

Introduction:

Scientists sometimes discover previously unknown organisms when exploring nature. Once discovered, it is the duty of the scientists to identify the organism, and place that organism in the evolutionarily appropriate location within biological organizational frameworks). In this lab, we will examine the Unity of Life – the things that all life forms on earth have in common, and the Diversity of Life – the incredible diversity of organisms found on earth. You will learn about the phylogenetic tree of life, about taxonomy and the scientific classification system used to categorize organisms in the three domains of life. There will be six group activities and an extensive array of materials in both labs. Your instructor will lead you through the activities in the first portion of lab, and then you will be able to circulate through both lab rooms and study the materials at your own pace for the rest of the available time. Use your laboratory notebook to record notes and illustrations of the educational materials arrayed in both rooms. You will make observations about five new organisms, make hypotheses about what these organisms are, then collect data to support or reject your hypotheses. In order to have sufficient knowledge to make hypotheses, you need to collect some data about how different kinds of organisms are grouped together in the first place and the systems scientists use to organize life.

Exercise 1: Unity – Cell Theory & the Universal Cellular Components

In groups of three or four and using the whiteboards, discuss the following:

1. Cell theory states that all living organisms are composed of cells. What is a "cell"? What are the implications of cell theory?

2. What four cellular components are present in all living cells (hint: your prelab will help!)?

Exercise 2: Diversity – Prokaryotes vs. Eukaryotes

1. Look at prepared slides available to observe both Prokaryotic and Eukaryotic cells.
    a. Note any differences you observe between the two different cells.

2. Use a swab to sample your gum line and setup a wet mount slide to investigate animal cells (your cheek cells!) in your mouth.

Exercise 3: Diversity – Cellular Features

1. In groups of three or four, use the laminated cell parts provided by your instructor to create model cells for the following major lineages:

    a. Bacteria
    b. Plant
    c. Fungus
    d. Animal
    e. Virus

2. Draw these cells and viral structure in your lab notebook.

Exercise 4: Organizing Life

Evolutionary theory states that all life present on earth today descends from the same ancestor. Over evolutionary time, one form of life evolved into the millions of different species we see today, and millions more that have gone extinct. While many scientists have attempted to classify and organize all these different forms of life, modern scientists have accepted that life must be organized by how recently they split into different species in evolutionary time. We have two major ways to accomplish this: taxonomy and phylogenetics. Use the information present at the station to answer the questions below.

1. What are the three Domains of life? Rank the domains from most diverse (greatest number of species) to least diverse (smallest number of species), based on the Tree of Life poster.
2. What are the seven levels of taxonomic classification below Domain, starting with the broadest (most ancient evolutionary spit) and ending with the narrowest (most recent evolutionary splits)?
3. Which two taxonomic names are used for species identification?
4. If you had a group of organisms with known taxonomic rankings, how could you tell which of the organisms were more closely related relative to the others?

Exercise 5: Comparing and Contrasting Among Groups

All forms of life are grouped together based on key characteristics that they have in common. The three most important characteristics are:

1. Cellular Association
2. Mode of Nutrition
3. Specialized Cell Features or Cell Types

- **Cellular association can be:**
  - Unicellular: Free living individual cells
  - Colonial: Cells that live together in groups
  - Multicellular: Organism that has specialized groups of cells that depend on other groups of specialized cells and none would be able to function unless all specialized cells are present

- **Mode of nutrition can be:**
  - Autotrophic: Create food using energy from sunlight or non-organic reactions
  - Heterotrophic: Obtain food from organic compounds
    - Can be accomplished by:
      - Secreting enzymes into the environment and absorbing digested compounds
      - Engulfing food inside cells
      - Ingesting food particles into a multicellular structure or organ

- **Specialized cell features can include:**
  - Flagella, cilia, cell walls (or lack thereof), specialized organelles, and many more!

- **Specialized cell types can include:**
  - Guard cells, Nerve Cells, and many more!

Displayed in both lab rooms are examples of different forms of life. Go through these examples and identify what characters define different life forms (e.g., what characteristics must be present in an organism for it to be considered an “animal” or a “plant”?), then complete table 2 – 1.

Table 2-1. Major Lineage Comparisons

Organism	Cellular Association	Mode of Nutrition	Specialized Cell Features	Specialized Cell Types	Cool Fact
Bacteria				N/A	
Plant					
Microbial Eukaryotes					
Fungus					
Animal					

Exercise 6: Identifying Organisms

Now that you know more about that characteristics that define different group in the tree of life, observe the five mystery organisms in the classroom. Make a hypothesis based on your first impression of the organism, then collect data to support or reject that hypothesis. Your lab instructor will be able to answer some questions for you, but others you have to figure out on your own.

Mystery Organism #1

- Hypothesis: _____
- If your hypothesis is correct, what cellular features should be present in organism #1?

- Make observations of organism #1 and complete Table 2 – 2

Mystery Organism #2

- Hypothesis: _____
- If your hypothesis is correct, what cellular features should be present in organism #2?

- Make observations of organism #2 and complete Table 2 – 2

Mystery Organism #3

- Hypothesis: _____
- If your hypothesis is correct, what cellular features should be present in organism #3?

- Make observations of organism #3 and complete Table 2 – 2

Mystery Organism #4

- Hypothesis: _____
- If your hypothesis is correct, what cellular features should be present in organism #4?

- Make observations of organism #4 and complete Table 2 – 2

Mystery Organism #5

- Hypothesis: _____
- If your hypothesis is correct, what cellular features should be present in organism #1?

- Make observations of organism #5 and complete Table 2 – 2

Table 2-2. Mystery Organisms

Organism	Hypothesis	Cellular Association	Mode of Nutrition	Specialized Cell Features	Specialized Cell Types	Actual Lineage
#1						
#2						
#3						
#4						
#5						

Lab Notebook Assignment

1. Title

2. Objectives

3. Exercise 1: Define the following terms (if not present in the prelab):
    a. Cell
    b. Cell Theory (and its implications, as discussed in lab)
    c. Cell membranes
    d. Enzymes
    e. Nucleic Acids
    f. Ribosomes

4. Exercise 2:
    a. Labeled drawings of prepared slides of prokaryotes and eukaryotes.
    b. Labeled drawing of your wet mount investigation of animal and bacterial cells living in your mouth with an indication of the relative size.

5. Exercise 3: Drawings (with labels) of the bacteria, plant, fungus, animal cells and virus capsid.

6. Exercise 4: Answers to the lab manual questions.

7. Exercise 5:
    a. Drawings and notes from the stations about Bacteria & Archaea, Microbial Eukaryotes, Fungi, Plants, and Animals.
    b. Table 2 – 1 completely filled in

8. Exercise 6: Table 2-2 completely filled in with correct organism information and identities.

9. Discussion Questions
    a. Which domain is the most diverse? Why do you think that might be?
    b. Which domain is the most morphologically diverse? Why do you think that might be?
    c. What data was the most crucial to you when identifying the mystery organisms?
    d. If you an unknown organism can photosynthesize, can you automatically categorize it as a plant? Why or why not?

Lab 3: Cellular Respiration

Pre-Lab Assignments

1. Check your lab Canvas to view this week's textbook reading assignment.
2. In your Lab Notebook, please give the zoological definition of the following terms: endothermic, ectothermic, homoeothermic, heterothermic, body temperature, respiration rate, and metabolic rate.

Learning Objectives

1. Distinguish and describe homeotherms, heterotherms, ectotherms, and endotherms.
2. Understand and compare how thermoregulation strategy influences metabolic rate and different temperatures.

Exercise 1: Endothermic Homeotherms

In this activity, you will investigate how endothermic homeotherms physiologically respond to changes in environmental temperatures. First, we will measure the respiration rate (a proxy for metabolic rate) of a mouse at room temperature and then at a colder temperature.

1. Based on what you learned from your pre-lab assignments, you should construct a hypothesis about how you think the mouse will respond physiologically to the colder environment.

   HINT: Your hypothesis should include how the metabolic rate of the mouse (measured as the respiration rate) and the mouse's body temperature will or will not change as the mouse is moved from room temperature to the colder environment. Make a hypothetical graph of the results.

2. Obtain the bent glass tubing-rubber stopper-syringe-jar assembly that will serve as a respirometer.

3. Obtain a tea ball with soda lime.

4. Insert the stopper into the jar. Then use a syringe with the thin metal tip to add dye to the top of the S-Shaped glass tube (manometer), until the dye level in the S-tube is about half way up the "S". Place a rubber band around the S-tube to mark the level of the dye.

5. Place some paper towels in the jar, then place a mouse in the jar.

   NOTE: These mice are not pets and may bite if handled improperly. Follow the directions of your instructor when handling live specimens in lab.

6. Make sure the stopper makes a tight seal. **Do not hold the stopper by the glass tubing manometer, it is fragile and will break. Handle the assembly only by the stopper!**

7. Fill the syringe with air from the room to about 2 ml past the 10 mL line. Your instructor will show you how. Attach the syringe snugly into the fitting on top of the jar.

8. Slowly push the syringe plunger to the 10 mL line. This causes the pressure inside the jar to be increased above the pressure outside the jar. Thus, the liquid dye in the S-tube will be displaced such that the level of the dye closest to the stopper will be below the rubber band and the other side will be above the rubber band.

9. As the mouse respires, it will consume O_2 and give off CO_2. Since the CO_2 is absorbed by the soda lime, each unit of O_2 consumed will decrease the pressure in the jar. Thus, as the mouse continues to respire (consumes O_2) liquid dye in the U-tube will be drawn inward.

10. At the moment the levels of dye are in the S-tube are at an equal level on both sides, record the time. **This will be your start time!!**

11. Continue to introduce more air into the respirometer by depressing the syringe plunger until all of the air has been used.

12. When the liquid dye in the S-tube is at an equal level on both sides, record the time. **This is your end time, that is, the time it took the mouse to use the O_2 in 10 ml of air.**

13. Record the difference between the start and stop time in table 3-1.

14. Next, carefully remove the syringe from the stopper. Also loosen the stopper between runs to give the mouse some fresh air.

15. Next, record the time it takes a mouse to consume the O_2 in 10 ml of air when the mouse is subject to cold. Place the jar **without the mouse**, in a bucket of ice. Pack ice all the way around the jar. Set the stopper loosely on top of the jar. Allow at least five minutes for the temperature inside the jar to equilibrate.

16. Place the mouse in the jar, insert the syringe and repeat steps 4-13.

Table 3-1. Seconds to Consume 10 ml of Air

Trial	Room Temp.	Cold Temp.
1		
2		
3		
Average		

Exercise 2: Ectothermic Heterotherms

For this activity, you will investigate how ectothermic heterotherms physiologically respond to changes in environmental temperatures. For this activity, we will be using goldfish and measuring their respiration rate (a proxy of metabolic rate) in room temperature water and water that has been chilled by ice. Our experimental apparatus is not accurate enough to detect differences in oxygen use by aquatic species, thus we must infer these differences based on the rate at which the fish "gulps" water to move across its gills. To conduct this experiment, follow the following steps:

1. Based on what you learned in your pre-lab assignments, you should construct a hypothesis about what differences in respiration rate you think the goldfish will display in warm vs. cold environments.

2. Place a goldfish in a beaker with 600 mL of water from the "water source" tank and move them to your lab table.

3. Allow the goldfish to acclimate to its new environment for 2 minutes by not disturbing or bumping the table or beaker.

4. After the acclimation period, begin your observations by counting the number of "gulps" for 60 seconds. Wait one minute.

5. Repeat step four two more times and record all these data in table 3-2.

6. Obtain a second beaker of 600 mL of water and add ice to the water until a thermometer reads 10 ˚C/50 ˚F.

7. Move your fish to the cold beaker of water and allow the fish to acclimate for two minutes.

8. Repeat step four above three times using the fish in the cold water and record all these data in Table 3-2.

9. Once your data is collected, move the fish as quickly as possible back to the room temperature water.

10. Put the fish and the room temperature water back into the source tanks. Dump the cold water down the sink.

Table 3-2. Number of "gulps" over 60 seconds.

Trial	Room Temp.	Cold Temp.
1		
2		
3		
Average		

Lab Notebook Assignment

1. Title

2. Objectives

3. Tables 3-1 and 3-2 completely filled in.

4. Graph of the results of exercise 1 and exercise 2.

5. Discussion Questions:
    a. Infer, based on your graphs, what this data shows about the metabolic rate of mice (endotherms) and goldfish (ectotherms) in different temperatures. How do the variables of body temperature, respiration, and environmental temperature relate to metabolic rate for each?
    b. Place each of the following organisms where they belong within the matrix of endothermy, homeothermy, ectothermy, and heterothermy.
        - Frog
        - Mouse
        - Human
        - Snake
        - Dog
        - Camel
        - Arctic Fish
        - Tropical Fish
        - Salmon

Lab 4: DNA and Biotechnology I

 **Pre-Lab Assignments**

1. Check your lab Canvas to view this week's textbook reading assignment.
2. In your lab notebook, please define the following terms:
    - Transposon
    - Exons
    - Introns
    - Regulatory sequences
    - DNA isolation
    - Polymerase chain reaction
    - Gel electrophoresis.
3. Do a Google search for the Alu family of transposons and find out how many base pairs long they are.
4. Learn about the Alu-PV92 transposon by visiting the website: http://geneticorigins.org/
5. Answer the following questions:
    1. On which chromosome is the Alu-PV92 transposon located (if it is present)
    2. How many base pairs is the Alu-PV92 transposon specifically?
    3. How can Alu insertions be used to track human history and evolution?

Learning Objectives

1. Remember and understand the steps in determining genotypes.
2. Remember the steps in Polymerase Chain Reaction and understand what is happening in each step and why it is necessary.
3. Learn to follow a clear cellular biology protocol.

Introduction:

In the next two labs you will take on the duties of a real-life lab technician by following and recording a very specific protocol to determine whether or not you have the Alu-PV92 transposon present in your chromosomes! In order to determine your genotype for the Alu-PV92 transposon you must complete three major steps:

1. DNA Isolation: Obtain cheek cells and isolate the DNA from the other cellular material.
2. Polymerase Chain Reaction (PCR): Amplify the specific region of your DNA that may or may not have PV92.
3. Gel Electrophoresis: Visualize your amplified DNA sample to determine your genotype.

Working in groups of two and using the step-by-step directions posted around the room, you will start by isolating the DNA found in **YOUR** cheek cells and will end by placing your DNA sample in the PCR machine.

Note: Although you will be working in groups of two, each student will be using their own sample, derived from their own cheek cells.

Exercise 1: DNA Isolation & Polymerase Chain Reaction (PCR)

In this activity you will be following and recording a very specific protocol. To obtain usable results **it is critical** that you perform each step correctly and accurately. Be very careful to follow the instructions. Try your best to understand and perform the steps correctly by yourself. However, if you are uncertain, seek advice from your instructor. Working in groups of two and using the step-by-step directions posted around the room, you will start by isolating the DNA found in **YOUR** cheek cells and will end by placing your DNA sample in the PCR machine.

Lab Notebook Assignment

1. Title

2. Objectives

3. Carefully written stepwise description of the procedures in activity 1.
    a. *For each step, be sure to note what the overall purpose of that step is in terms of the overall goal of DNA isolation.

    Example procedure:

        1. Add 400µl of ____
            a. This buffer serves to ……..

4. Write out the three steps of Polymerase Chain Reaction (PCR), in order, and what is occurring in the DNA sample during each of these steps.

Lab 5: DNA and Biotechnology II

 **Pre-Lab Assignments (Same as last week; you <u>do not</u> need to do this prelab twice)**

1. Check your lab Canvas to view this week's textbook reading assignment.
2. In your lab notebook, please define the following terms:
    - Transposon
    - Exons
    - Introns
    - Regulatory sequences
    - DNA isolation
    - Polymerase chain reaction
    - Gel electrophoresis
3. Do a google search for the Alu family of transposons and find out how many base pairs long they are.
4. Learn about the Alu-PV92 transposon by visiting the website: <u>http://geneticorigins.org/</u>
5. Answer the following questions:
    1. On which chromosome is the Alu-PV92 transposon located (if it is present)
    2. How many base pairs is the Alu-PV92 transposon specifically?
    3. How can Alu insertions be used to track human history and evolution?

Learning Objectives

1. Analyze DNA gels for genotype, fragment length, and infer phenotype from these data.

Introduction:

In this lab you will complete the third major step of the DNA and Biotechnology labs, gel electrophoresis. We know that the size of the Alu-PV92 transposon is 300bp long, and the size of the amplified intron alone is 550bp. Thus, an intron that contains the 300bp transposon would be a total of 850bp long. How, then, does one determine the actual size of their own amplified DNA product? Gel electrophoresis takes advantage of the *negatively charged* phosphate backbone of DNA to separate the amplified DNA products you created last week. First, the DNA samples are loaded onto a gel, and an electric current is applied. Because DNA is uniformly negatively charged, the DNA samples will migrate through the agarose gel matrix toward the positive electrode at varying rates, depending on the overall length of the DNA segment. What size of segments do you think will migrate through a matrix faster, and which will take longer? Usually, a sample of DNA of known sizes, called a **ladder**, is run in the same gel as a size reference for the unknown samples.

Exercise 1: Gel Electrophoresis

1. Prepare a two percent gel as follows:
    - Weigh out 0.40 grams of agarose. Be careful to note the decimal point here!
    - Add agarose to a 50 ml flask containing 20 mL of TAE buffer.
    - Microwave on high for 30 seconds.
    - Use tongs to remove the flask (it will be very hot!) and swirl the contents to ensure all of the agarose is dissolved.
        - Your instructor will add 2µl of 1 mg/ml ethidium bromide directly to your agarose before it has cooled, swirl to mix. When cool, pour solution into gel casting tray.

2. When the gel is solidified (~10 minutes), remove the dams and comb and pour TAE buffer into the reservoirs until there is about one millimeter of buffer above the gel.

3. Your lab instructor will add 5 µl of a 100 bp ladder to one well to demonstrate pipette technique.

4. Each student in the group is to add 10 µl of their own PCR product into a well. Make sure you write down which well you placed your DNA into!

5. Secure the gel tank cover in place, attach electrical cables, set the power supply to 85 volts, and press the start button.

6. When the loading buffer dye is between half and three quarters across the gel, turn off the power supply.

7. Still wearing gloves and eye protection, carry the try containing the gel over to the Gel Doc 2000. Use a spatula to transfer the gel on to the transilluminator of the Gel Doc 2000.

8. Use the 100 bp ladder to determine the size of each DNA band. From the results determine your genotype with respect to the Alu-PV92 insert.

9. Obtain a print of the get containing your results from your TA

Lab Notebook Assignment

1. Title

2. Objectives

3. Tape your gel picture into your notebook and label it as follows:

    Write:

    - +/+ under each lane that shows DNA from an individual who has the ALU-PV92transposon on both copies of chromosome 16.

    - +/- under each lane from someone who has the transposon on only one copy of the chromosome.

    - -/- under each lane from someone who does not have this particular transposon at this site.

4. Put a * above your DNA. What is your genotype for Alu-PV92?

Lab 6: Genetics

Pre-Lab Assignments

1. Check your lab Canvas to view this week's textbook reading assignment.
2. Do a Google search for ABO blood typing and familiarize yourself with the concept of the ABO blood group system.
3. Please define the following terms:
    - Antibody
    - Antigen
4. In your Lab Notebook, please define the following terms:
    - Heterozygous
    - Homozygous
    - Recessive
    - Homozygous dominant

Learning Objectives

1. Remember the Hardy-Weinberg equations, understand what they mean, and be able to use them in novel circumstances
2. Distinguish between alleles that are or are not in Hardy-Weinberg equilibrium and what that means for evolution of that allele
3. Understand red blood cell antigens and how they determine blood type
4. Understand which blood types can give or receive transfusions
5. Read or create blood type tests

Introduction:

For introductory and background information on genetics please see the assigned reading above. The Hardy-Weinberg law of genetic equilibrium provides a mathematical model for studying evolutionary changes in allelic frequency within a population. In this lab exercise, you will apply this model by using your class as a sample population. In 1908 G. Hardy and W. Weinberg independently proposed that the frequency of alleles and genotypes in a population will remain constant from generation to generation if the population is stable and in genetic equilibrium. Five conditions are required in order for a population to remain at Hardy-Weinberg equilibrium:

1. A large breeding population
2. Random mating
3. No change in allelic frequency due to mutation
4. No immigration or emigration
5. No natural selection

Exercise 1: Hardy-Weinberg Simulation

During this activity, you will calculate the frequency of genes in your lab section that control a person's ability to taste phenylthiocarbamide, or PTC. The ability to taste PTC or not are phenotypes, determined by underlying genotypes. Since humans are diploid, the genotypes for each gene consists of two alleles, or alternate versions of that gene. A person who has at least one allele (T) is able to taste PTC, whereas a person with only the alternate allele (t) is not able to taste PTC. Therefore, we know that T is dominant to t.

Looking at individuals, a taster could be either homozygous dominant (TT) or heterozygous (Tt). A non-taster, however, can only be homozygous recessive (tt). To assess gene frequency, it is necessary to look at a population (group of individuals). For the PTC tasting trait at the population level, there are only two different alleles in the population:

T = PTC taster OR t = PTC non-taster

Since there are only two alleles in this case, the total number of T alleles plus the total number of t alleles must add up to 100% of the PTC alleles in the population. The frequency (expressed as a decimal, e.g., 10% = 0.10) of T alleles in the population plus the frequency (expressed as a decimal, e.g., 90% = 0.90) of t alleles in the population add up to one.

In Hardy-Weinberg calculations, the frequency of a dominant allele is designated p, and the frequency of a recessive allele is designated q. If the population as a whole contains 100% of the alleles, or 1, this can be represented by the following equation:

$$\boldsymbol{p + q = 1}$$

Where:

- **p** = the frequency of the dominant allele
- **q** = the frequency of the recessive allele

If you square both sides if this equation, you obtain the equation below, which relates the frequency of alleles (p and q) in a population to the frequency of genotypes in the population.

$$(p + q)(p + q) = (p + q)^2 = \boldsymbol{p^2 + 2pq + q^2 = 1}$$

Where:

- $\mathbf{q^2}$ = The frequency of the population that has the homozygous recessive genotype (tt)
- $\mathbf{p^2}$ = The frequency of the population that has the homozygous dominant genotype (TT)
- **2pq** = The frequency of the population that has the heterozygous genotype (Tt)

If you know the number of students in the class that are homozygous recessive (q^2), and the total number of students in class, you can calculate p^2 and 2pq.

Part I: Calculating Initial Gene and Allele Frequencies

1. Obtain a piece of PTC test paper and determine if you are a taster or a non-taster.

Number of Tasters: _____

Number of Non-Tasters: _____

Total Number of Students in Class (Population): _____

2. Calculate q^2:

$$q^2 = \frac{\textbf{Number of Non-Tasters}}{\textbf{Total Number of Students in Class}} = \_\_\_\_\_ =$$

3. Calculate q:

$$q = \sqrt{q^2} =$$

4. Once you have q, you can calculate p:

$$p + q = 1 \; rearranges \; to \; p = 1 - q = 1 - \_\_\_\_\_ =$$

5. Now that you have p and q, you can use these numbers to calculate genotype frequencies. Enter values for p^2 and 2pq in Table 4-1, "First Generation."

6. Fill in the number of students column of Table 4-1 using the following equation:

Number of Students with a Given Genotype = ***Frequency of the Genotype*** x ***Total Number of Students in Class***

Table 6-1: First Generation

Genotype	Genotype Frequency	Number of Students
Homozygous recessive (q^2)		
Homozygous dominant (p^2)		
Heterozygous (2pq)		

Part II: Simulating the Creation of New Generations

1. Once Table 4-1 is filled in, your lab instructor will arbitrarily design genotypes to the tasters. Why must these genotypes be assigned and cannot be definitively known?

2. Record your genotype here: _____

3. To create a new generation, you will randomly pick, without regard to the sex of any classmate or any other bias, another person to mate with anywhere in the room. *Your instructor will help with the mixing and random mating*.

4. Each partner is to provide a gamete. If you are homozygous dominant, then you can only contribute a T. If you are homozygous recessive, then you can only contribute a t. If you are heterozygous, then you can contribute a T or a t. In this case, you should randomly choose the allele you contribute (T or t). Your instructor will give you paper alleles to use while "mating." To have two offspring, you must mate twice with your partner. Make sure the second mating event is also random with respect to the game you contribute!

Table 6-2: The Second Generation

	Your Contribution	**Your Partner's Contribution**	**Final Genotype**
Offspring #1			
Offspring #2			

5. One partner will now adopt the genotype of the first offspring, and the other will adopt the genotype of the second offspring. Now each set of partners are siblings.

6. For the third generation, pick another partner at random and repeat the mating procedure described above.

7. Repeat the random mating technique for six generations and record the genotype for each generation in Table 4-3 below.

Table 6-3. Third Through Sixth Generations

	Third Generation	**Fourth Generation**	**Fifth Generation**	**Sixth Generation**
Your Genotype				

Part III: Calculating Hardy-Weinberg Equilibrium

1. Your instructor will survey the class to find each student's final genotype.

2. After all of your classmates' new genotypes are reported, complete Table 4-4, using the following equation to calculate genotype frequencies:

$$\textbf{Genotype Frequency} = \frac{\textbf{Number of Students with Genotype}}{\textbf{Total Number of Students in the Class}}$$

Table 6-4. Sixth Generation

Genotype	Genotype Frequency	Number of Students
Homozygous recessive (q2)		
Homozygous dominant (p2)		
Heterozygous (2pq)		

3. Compare the genotype frequencies of the first and sixth generations. Where there any changes? _____
4. If you saw changes, what does this mean with regard to this allele being in equilibrium?

5. Which of the assumptions of Hardy Weinberg equilibrium do you think your class is most likely to have violated?

Exercise 2: Blood Types

Part I: What is a blood type?

There is one gene that governs the ABO blood type, with three alleles (A, B, and O) relatively common in human populations. People with an A allele produce an enzyme that adds a specific sugar (N-acetylgalactosamine) to the surface of a red blood cell (RBC), or erythrocyte. People with the B allele produce a slightly different version of the enzyme that recognizes a different substrate; the enzyme therefore adds a different sugar (galactose) to the surface of RBCs. The O allele encodes a nonfunctional enzyme that cannot add either sugar to the RBCs. The different blood types in the ABO system therefore are based on differences in sugars (carbohydrates) found on RBCs. The + and – associated with blood types is in reference to another sugar called the Rh factor. For the Rh sugar, you either have it or you don't, so if you are blood type A with the Rh factor, your blood type is A+. However, if you have type A sugars (antigens) on your RBCs but do not have the Rh factor, you would have blood type A-.

Procedure:

1. Working in groups of three or four and using the whiteboards, draw out RBCs of all the possible blood types: A +/-, B+/-, AB+/-, O+/-.
2. Draw out all the possible RBCs in your lab notebook.

Part II: Donating and Receiving Blood Transfusions

An important component of your immune system is the presence of antibodies in your blood that bind to foreign objects, such as bacteria, viruses, eukaryotic pathogens, or any other substance that does not belong. When these antibodies bind to foreign objects, it stimulates an immune response. The sugars that are present on your RBCs allow your immune system to differentiate between your blood and someone else's blood and your immune system produces antibodies that bind to RBCs that display incorrect sugars. The type of antibodies your immune system uses to bind to RBCs causes agglutination, or clotting. Because we have antibodies in our blood that will bind to antigens, we cannot donate RBCs to anyone who does not have the sugars we do. If we did, the antibodies of the recipient of the blood transfusion would bind to the new sugar type on RBCs and agglutinate in the blood stream. In this activity, you will gain experience with who can donate and receive blood from each other. Fill in table 4-5 below.

Procedure:

1. In your groups of three to four and using the whiteboards, discuss what antibodies a person of each ABO blood type would *produce*.
2. Discuss what antibodies a person of each ABO blood type would be sensitive to (i.e., agglutinate in the presence of that antibody).
3. Discuss what might happen to person who receives a blood transfusion of the wrong blood type.
4. Based on your discussion, decide which blood types can donate and receive transfusions from which blood types.
5. Complete Table 4-5

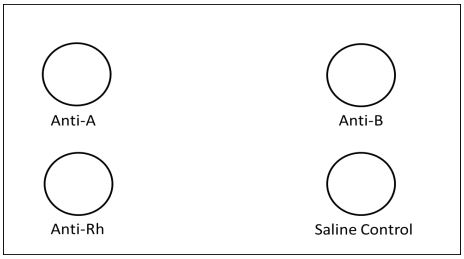
Table 6-5. Transfusion Compatibility Summary Table

Blood Type	Genotype		Can Donate Blood To	Can Receive Blood From	Antibodies Produced	Antibodies That Cause Agglutination
	ABO	Rh				
A+						
A-						
B+						
B-						
AB+						
AB-						
O+						
O-						

Part III: Reading a Blood Type Test

The test to determine your blood group is called ABO typing. Your blood sample is mixed with antibodies against type A and B blood. The sample is checked to see whether or not the blood cells stick together (agglutinate) in the presence of these antibodies. If blood cells stick together, it means the blood reacted with one of the antibodies, which means the blood sample had antigens in it (sugar on the red blood cells) that the antibodies recognized, bound to, and caused agglutination. This is a type of present / absent test, meaning that if the antibody used to mix with the blood sample causes agglutination, then the specific antigen for that antibody was present in that sample. On the other hand, if the antibody used to mix with the blood sample does not cause agglutination, then the specific antigen for that antibody was absent in that sample.

A blood test plate is arranged on a panel similar to the picture below. Draw out this image on your white boards and draw out what you expect would result if the person being tested was A+, A-, AB+, and O+.



Part IV: Blood Type Test

This activity is **optional**; however, you are responsible for all of the preceding information and will be expected to answer questions about this material on quizzes and exams regardless if you do the actual test in lab today or not.

1. Obtain the following from the side counter: two alcohol swabs, lancet, placemat, two toothpicks and a plastic test card.

2. Wash your hands with soap and water. Then scrub your chosen finger with the alcohol swab.

3. Prick your figure with the lancet to get a blood sample.

4. Place a few drops of blood into each of the four circles on the plastic test card.

5. Clean your sampled finger with the second alcohol swab.

6. Place a drop of anti-A antibody onto the blood sample in the circle labeled Anti-A

7. Place a drop of anti-B antibody onto the blood sample in the circle labeled Anti-B

8. Place a drop of anti-Rh antibody onto the blood sample in the circle labeled Anti-Rh

9. Place a drop of saline solution onto the blood sample in the circle labeled Control.

10. Use the toothpicks to mix the blood with the antibody in each circle. Use a fresh toothpick tip to mix each sample.

11. It typically takes a few minutes for agglutination to occur. It sometimes helps to gently agitate the solution with the toothpick again.

12. With help from your classmates and instructor, interpret your results.

13. When finished, place the lancet and toothpicks in a sharps container. Role the cotton swabs and plastic test card up in the placemat and discard into the bio hazard bag.

Lab Notebook Assignment

1. Title

2. Objectives

3. Exercise 1: Hardy-Weinberg Simulation
    a. Tables 6-1, 6-2, 4-3, and 6-4 completely filled in.
    b. Hardy-Weinberg Discussion Questions
        i. How did genotype frequencies change in a small breeding population? Why?
        ii. How would non-random mating events change genotype frequencies? What type of selection would this be?
        iii. How would mutation events change allelic frequencies?
        iv. How would immigration and/or emigration change genotype frequencies?
        v. Would genotype frequencies change if some of the individuals in the population (class) did not survive to mate in all six generations? What type of selection would this be?

4. Exercise 2: Blood Types
    a. Draw out RBCs of all the possible blood types: A +/-, B+/-, AB+/-, O+/-.
    b. Drawing of the results of a blood test from a person with blood type A+, A-, AB+, and O+.
    c. Table 6-5 completely filled in.
    d. Bloody Typing Discussion Questions
        i. What blood type is the universal recipient? Why?
        ii. What blood type is the universal donor? Why?

Lab 7: Evolution

 **Pre-Lab Assignments**

1. Check your lab Canvas to view this week's textbook reading assignment.
2. In your lab notebook, please describe the difference between convergent and divergent evolution.
3. Watch the video titled "Evolution Prelab" on your lab Canvas.

Learning Objectives

1. Understand what evolution is and be able to recognize or describe the five mechanisms of evolution (natural selection, sexual selection, mutation, migration, genetic drift) and how population size influences each.
2. Differentiate between divergent and convergent evolution and understand how to recognize and/or describe each.
3. Understand the general process of human evolution and be able to recognize close human relatives.
4. Explain the example of evolution in action from class and what major conclusions about evolution can be drawn from these examples.

Introduction:

Evolution can be defined in many ways, depending on the level of organization at which one is working. For example, evolution is any change in the frequency of a single genetic allele in a population over only a few generations. Contrastingly, the development of an entirely new species, which involves many genes and occurs over the course of hundreds to thousands of years is also evolution. In any case, **evolution** is defined as genetic change over time.

Evolution is one of the best-supported theories in all of biology, providing an explanation for life's diversity as well as its unity. It provides insight concerning the relationships of various organisms to each other and to their environment, and makes testable predictions. At the same time, accepting the scientific evidence for evolution does not imply the rejection of religious ideas. Religious ideas about life's origins often depend on unique, supernatural factors, which are outside the realm of science. In this laboratory, we will simply simulate different mechanisms of evolution and examine the evidence for evolution.

Exercise 1: Mechanisms of Evolution

Introduction

In this exercise, we will illustrate the process of evolution by simulating the different mechanisms by which evolution occurs. **Evolution** is defined as a change in genetic frequencies in a population over time. Genetic frequencies are influenced by multiple factors and can be altered by several mechanisms: natural selection, sexual selection, mutation, migration, and genetic drift. In the activities below, you will illustrate how each process leads to evolution.

Activity 1: Natural Selection

Natural selection is the most widely cited mechanism of evolution. **Natural selection** is the process in which certain individuals are better able to survive in an environment due to some advantage. For example, maybe they are better at escaping predators or finding food. As a result of this advantage, these individuals produce a higher number of offspring than other individuals. Because different individuals are making unequal contributions to the next generation, genetic frequencies slowly change over time (e.g. evolution occurs). You can see how this works by performing a simple experiment.

Evolution by Natural Selection in a Small, Stable Population:

1. From your TA, obtain an "environment" and a "population" of beads.
2. One student will take the role of "Mother Nature," and will be in charge of distributing beads into the environment. The other students in the group will be "predators," selecting beads to "eat" during each round.
3. While all "predators" close their eyes, "Mother Nature" should spread beads of each color randomly through the environment. The relative frequencies of each color should be equal, and the total number of beads in the environment should be twice the number of "predators" in the group. For example, if your group has five "predators", you should place ten beads in the environment, two of each color.
4. With the lights off, predators should open their eyes and take one bead from the environment as quickly as possible.
5. "Mother Nature" then allows the beads to reproduce: for each bead remaining in the environment, place one more bead of the same color in the environment.
6. Record the number of each bead color still present in the environment in Table 7-1.
7. Repeat steps 3 through 6 four more times and record your final bead counts in Table 7-1.
8. Record the final bead counts for each environment in table 7-2.

Table 7-1. Bead color frequencies after "predation events" in a small population.

Environment Color:						
Predation Event	**Orange Beads**	**Blue Beads**	**Green Beads**	**Pink Beads**	**Tan Beads**	**Total Beads**
Start	2	2	2	2	2	10
1						10
2						10
3						10
4						10
5/End						10

Table 7-2. Bead color frequencies after final "predation event" by environment color

Environment Color	Orange Beads	Blue Beads	Green Beads	Pink Beads	Tan Beads
Halloween					
Pirate					
Tiger					
Safari					
Owl					

Evolution by Natural Selection in a Large/Growing Population:

1. From your TA, obtain an "environment" and a "population" of beads.
2. One student will take the role of "Mother Nature," and will be in charge of distributing beads into the environment. The other students in the group will be "predators," selecting beads to "eat" during each round.
3. While all "predators" close their eyes, "Mother Nature" should spread beads of each color randomly through the environment. The relative frequencies of each color should be equal, and the total number of beads in the environment should be more than twice the number of "predators" in the group. For example, if your group has five "predators", you should place 15 beads in the environment, three of each color.
4. With the lights off, predators should open their eyes and take one bead from the environment as quickly as possible.
5. "Mother Nature" then allows the beads to reproduce: for each bead remaining in the environment, place one more bead of the same color in the environment.
6. Repeat steps 3 through 6 four more times and record your final bead counts in Table 7-3. Do not put away any beads at this time (we need them for the next activity)!
7. Record the final bead counts for each environment in table 7-4.

Table 7-3. Bead color frequencies after "predation events" in a Growing Population

Environment Color:						
Predation Event	**Orange Beads**	**Blue Beads**	**Green Beads**	**Pink Beads**	**Tan Beads**	**Total Beads**
Start	3	3	3	3	3	15
5/End						170

Table 7-4. Bead color frequencies after final "predation event" by environment color

Environment Color	Orange Beads	Blue Beads	Green Beads	Pink Beads	Tan Beads
Halloween					
Pirate					
Tiger					
Safari					
Owl					

Genetic drift is the random change of alleles in a population. This can be due to the death or migration of all individuals that possessed those alleles or simple chance. Smaller populations experience genetic drift at higher rates than larger populations because they have fewer redundant individuals (that is, fewer individuals that have any particular allele, which leads to reduced variation). Compare the final relative bead frequencies in each environment when the population was small and stable vs. when the population was large and growing. Under which circumstances were more alleles lost (or more variation maintained)?

Activity 2: Migration

Migration occurs when populations of individuals travel from one location to another. This can occur seasonally, like when many North American birds travel south for the winter, or it can occur more randomly, like when seeds carried by wind colonize a new island. To illustrate how migration can cause evolution, perform the following activity.

Evolution via Migration

1. Before you begin, take note of the current bead frequencies of your environment.
2. Your TA will simulate a migration event. Are the relative bead frequencies the same in the source population after the migration event? Are the relative bead frequencies the same in the destination population after the migration event?

Activity 3: Mutation

Mutations are errors that occur during DNA replication. For a mutation to lead to evolution, this DNA replication error must occur in the cell giving rise to sperm or eggs (it is not possible to pass on to offspring a mutation in a skin cell, for example). Mutations in sperm and eggs can be negative or positive, and whether and how they alter genetic frequencies can depend on many factors. To illustrate this concept, perform the activity below.

Evolution via Natural Selection on a New Mutation

1. For this activity, you will be doing another round of "predation" events. Just like before, one team member will be "Mother Nature" and the rest will be "predators."
2. "Mother Nature" will set up the environment in exactly the same way as Activity 1 (Natural Selection in a Small, Stable Population), but this time one of the tan beads will undergo "mutation" and change its color.
3. Perform five rounds of predation events and reproduction (see steps 3-7 from activity 1) and record your findings in Tables 7-5 and 7-6.

Table 7-5. Bead color frequencies after "predation events"

Environment Color:							
Predation Event	**Orange Beads**	**Blue Beads**	**Green Beads**	**Pink Beads**	**Tan Beads**	**"Mutated" Beads**	**Total Beads**
Start	2	2	2	2	1	1	10
5/End							10

Table 7-6. Bead color frequencies after final "predation event" by environment color

Environment Color	**Orange Beads**	**Blue Beads**	**Green Beads**	**Pink Beads**	**Tan Beads**	**"Mutated" Beads**
Halloween						
Pirate						
Tiger						
Safari						
Owl						

Activity 4: Sexual Selection

Sexual selection occurs when one sex (the "choosey sex") only chooses to mate with individuals of the opposite sex (the "chosen sex") that possess some preferred trait. Mates that possess this preferred trait will leave many offspring; mates without this trait will leave few or zero offspring. Just like in natural selection, this leads to unequal contribution to the next generation and a change in gene frequencies over time. Your TA will show you some videos of the kinds of traits animals use when choosing mates. Based on these videos, discuss the following questions with your group and write down your answers in your lab notebook:

1. Are the traits (coloration, singing, dancing, etc.) beneficial for the animal's survival? If the answer is no, why would they evolve to have them?
2. How could preferences for these traits evolve in the choosy sex?
3. What kind of information do you think individuals of the chosen sex may communicate with these traits?

Exercise 2: Types of Evolution

For this exercise, you will visit several stations (in both lab rooms) that will highlight various concepts associated with evolution. You should spend a minimum of 20 minutes at each station. Pay close attention to the differences among the types of evolution.

While at each station, take careful notes in your laboratory notebook about the examples given. You should note:

- Similarities and differences among the types of evolution.
- How you will tell these apart from one another on a quiz or exam.

Any other information provided at the stations.

Evolution Stations

1. Convergent Evolution
2. Divergent Evolution
3. Human Evolution
4. Evolution in Action

Lab Notebook Assignment

1. Title

2. Objectives

3. Table 7-1, 7-2, 7-3, 7-4, 7-5, and 7-6 completely filled in and copied into your lab notebook

4. Answers to Discussion Questions
    a. Compare the final bead color frequencies between the different environment colors for exercise 1, activity 1 (Natural Selection in a Small, Stable Population). Did each environment lead to the same relative color frequencies? How did environment color influence the final frequencies?
    b. In activity 1, did you observe higher occurrences of genetic drift in small populations or growing populations? Why?
    c. Compare the final frequency of "mutation" beads in each environment. Was there a difference? Why or why not?
    d. In the migration activity, were the relative bead frequencies the same in the source population after the migration event? Were the relative bead frequencies the same in the destination population after the migration event?
    e. Sexual Selection Discussion Questions:
        1. Are the traits (coloration, singing, dancing, etc.) beneficial for the animal's survival? If the answer is no, why would they evolve to have them?
        2. How could preferences for these traits evolve in the choosy sex?
        3. What kind of information do you think individuals of the chosen sex may communicate with these traits?

5. Convergent Evolution:
    a. Definition
    b. Sketches and descriptions of at least three examples of convergent evolution at the station. For each example, what was the selection pressure that caused unrelated organisms to evolve similar structures?
    c. Answers to the two discussion questions at the station.

6. Divergent Evolution:
    a. Definition
    b. Sketches of the bone structures in at least three of the example organisms.
    c. Answers to the four discussion questions at the station

7. Human Evolution
    a. What are the major genera in the human evolutionary tree?
    b. Answers to five discussion questions at the station (on poster).

8. Evolution in Action
    a. Answers to discussion questions at the station about antibiotic resistance (four, on poster, two on card), artificial selection (two, on poster), and the Galápagos finches (three, on poster).

Lab 8: Photosynthesis

Pre-Lab Assignments

1. Check your lab Canvas to view this week's textbook reading assignment.
2. In your Lab Notebook, state the function of the following terms: epidermis, mesophyll, palisade cells, spongy cells, stomata, guard cells, and spectrophotometer.
3. Review your observations and findings from the activity **"Plant Seedlings and their Response to Light"** that was initiated during the first week in lab.

Learning Objectives

1. Recognize and describe the different parts/cells in a leaf and their role in photosynthesis.
2. Learn and remember which wavelengths/colors or light are used by plants in photosynthesis, and how that differs in other photosynthesizing organisms.
3. Analyze or create absorption spectra.

Introduction

In this lab, we will learn about the process of photosynthesis. The pre-lab assignments provide background and introduction material.

Exercise 1: Leaf Structure

Working in groups of two or three, using your pre-lab assignment, provided microscope slides, the models available in the room, and the internet, draw and label each of the following with structure and function (if not present in prelab):

1. Epidermis
2. Mesophyll (Palisade Mesophyll and Spongy Mesophyll)
3. Stomata
4. Guard Cells

Exercise 2: Different Colors of Light and Photosynthetic Efficiency

In this activity, you will investigate how differences in light color (wavelength) relate to photosynthesis. Specifically, you will test what colors (wavelengths) works best for photosynthesis and which don't. **Chlorophylls** are pigments that absorb light, and so we must isolate these pigments so we can see what colors of light (wavelengths) are used for photosynthesis and which are not.

Part I: Create a Hypothesis

1. Working in groups of 3 to 4, create a hypothesis about which colors of light (red, blue, or green) you think are most and least used in photosynthesis.

Part II: Isolating Chlorophylls

1. Obtain a four-inch strip of chromatography paper.

2. Following your lab instructor's demonstration, obtain a spinach leaf and fold it twice so you have four layers of leaf. Place the folded leaf over the chromatography strip about 3 cm from one end. Roll the edge of the glass vial over the leaf with just enough pressure to crush the cells. Place "juice" from the crushed cells should form a green band on the paper strip.

3. For each group there is a ring stand holding four test tubes. Each tube contains 1.5 mL of solvent (nine parts petroleum ether to 1 part acetone). Using forceps, insert your strip into the solvent, with the crushed cells placed at the bottom of the tube.

4. Firmly place a rubber stopper on the top of the tube and twist while inserting the stopper for a snug fit. Now wait and observe while the solvent carrying pigments, travels up the chromatography paper.

5. When the solvent has reached the top of the paper (about ten minutes), use forceps to remove the strip and immediately replace the stopper in to the test tube (the solvent is highly volatile and evaporate quickly without the stopper in place!).

    i. **Note: There are two different chlorophylls: a blue-green one called Chlorophyll A (Chl_a) and a yellow-green one called Chlorophyll B (Chl_b). The yellow pigments running to the top are carotenoids and can be ignored for this experiment.**

6. Use scissors to cut out the Chl_a and the Chl_b from the strip. Your instructor will explain how this is done.

7. Place your groups Chl_a and Chl_b bands into two separate test tubes filled with 3 mL of 85% acetone. The acetone will extract the chlorophylls from the chromatography paper into solution.

8. After the pigments are extracted into solution, pour each extraction into its own cuvette.

Part III: Measuring Absorbance of Chl_a and Chl_b

Now that we have isolated the pigments, we can use a machine called a spectrometer to measure the degree to which each pigment absorbs different wavelengths (colors) of light.

1. Turn on your spectrometer.

2. Put the blank cuvette of acetone into the reader.

3. Use the stylus to press the red bar on the screen. Hit "calibrate." Allow the spectrometer to complete its warm up cycle.

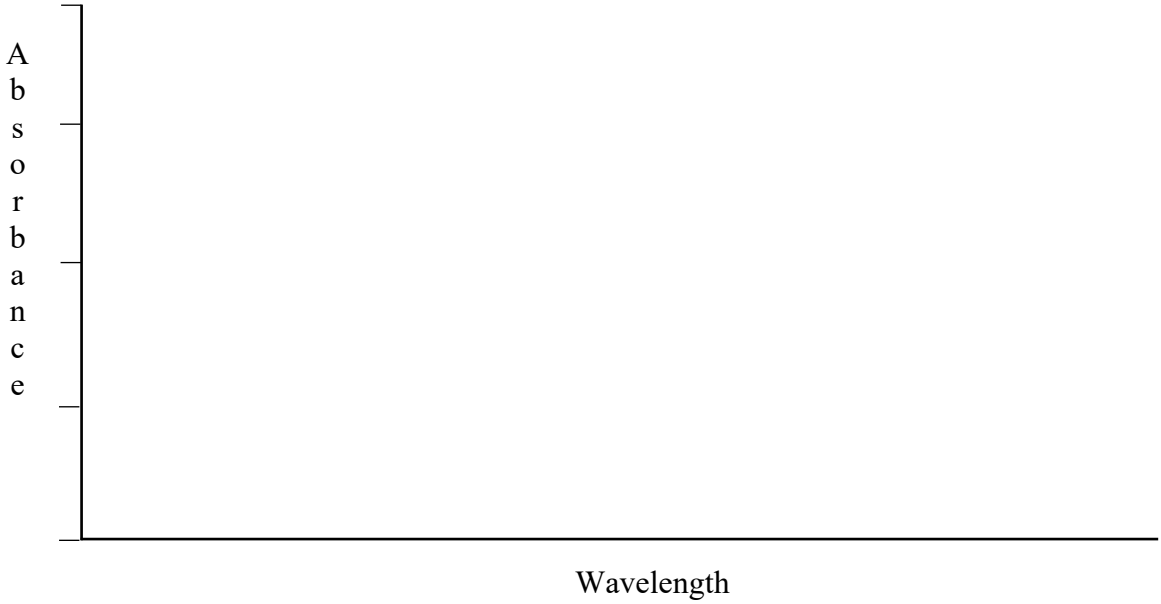
4. Use the stylus to press "finish calibration."

5. Put the Chl_a sample in the reader. Press the green arrow on the screen with the stylus.

6. Draw the graph that appears on the screen on Graph 3-1 below. Make sure to label the axes with light wavelengths and colors and to label the line you draw as Chl_a.

7. Remove the Chl_a cuvette and replace it with the Chl_b cuvette. Repeat step 6 with Chl_b.

8. Turn off your spectrometer and clean all your cuvettes.



Graph 8-1. Wavelength vs. Absorbance of Chl_a and Chl_b from spinach leaves.

Exercise 3: Photosynthesizing with Other Pigments

For this activity, we are going to look at photosynthetic organisms that primarily use pigments other than chlorophylls a and b.

1. Make a wet mount of the sea water sample. This water has been especially sampled to increase the population of the plankton (microscopic organisms that live in sea water). Draw what you see under the microscope.

2. Observe and draw the red and brown algae samples out on the lab benches. Pay close attention to the colors of the algae. Based on your observations and the results of activity 2, what wavelengths of light do you think red algae and brown algae absorb for photosynthesis respectively?

Exercise 4: Plant Modifications

For this activity, you will through the different rooms of the plant conservatory and make observations about the types of plants you see and how they have been modified over evolutionary time to maximize their photosynthetic efficiency. Below is a table with three types of plants and three photosynthetic resources. Fill in this table indicating whether that type of plant lives in an environment where that resource is abundant or limited. Copy this table into your lab notebook.

Table 8-1. Limiting and Abundant Resources in Different Environments

Resource	Arid Room	Tropical Forest	Cloud Forest
Water			
Sunlight			
Gases			

As you go through the rooms in the greenhouse, think about the limiting and abundant factors associated with a plant's ability to photosynthesize. For each environment, write down at least two adaptations that you observe in plants that may be related to maximizing photosynthetic efficiency.

Lab Notebook Assignment

1. Title

2. Objectives

3. Exercise 1
    a. Drawing of a leaf cross section with the following structures labeled with their name and function (if not present in prelab):
        - Epidermis
        - Mesophyll
            - Palisade Mesophyll
            - Spongy Mesophyll
        - Stomata
        - Guard cells

4. Exercise 2
    a. Hypothesis
    b. Graph 8-1 completely filled in
    c. Conclusion based on the results of the experiment. Did the results support the hypothesis?

5. Exercise 3
    a. Drawings of plankton, red algae, and brown algae
    b. Hypotheses and explanations for which wavelengths the algae primarily use for photosynthesis.

6. Exercise 4
    a. Table 8-1 completely filled in.
    b. Notes about plant photosynthetic adaptations – taking photos and taping them into your notebook would be a great way to remember your plants.

7. Discussion Questions About Plant Observation Experiment (Started in Lab 1):
    a. Define the following terms:
        - Phototropism
        - Germination
        - Chlorophyll
    a. Based on your observations, describe the photosynthetic responses of the radish seedlings to each wavelength (color) of light.
    b. In phototropism, what part of a young radish seedling appears to bend? Why would this part of the plant bend, rather than other tissues?
    c. For the seedling that was grown in the dark (Treatment D), did you observe vertical growth? If so, why do you think a seedling plant would grow in the dark?

Lab 9: Ecology Fieldtrip to Poly Canyon

Pre-Lab Assignments

1. Check your lab Canvas to view this week's textbook reading assignment.
2. Bring laptop to class.
3. Wear sturdy walking shoes and bring necessary equipment for hiking (water, sunblock, hats, sunglasses, layers, etc.)

Learning Objectives

1. Recognize the identity of local plant life and how water shapes their appearance.
2. Understand and differentiate between the different types of communities in Poly Canyon.

Introduction:

Ecology is the part of biology that is concerned with the interactions of organisms with each other, and with the physical and chemical features of the environment. In this lab, we will learn about the primary factors that contribute to and affect plant growth, thus laying the foundation for each community we will visit. In particular, temperature, available moisture, and soil composition can have great effects on the composition of a community. Geographically, the amount of water available to plants varies with features such as slope of the land, exposure to the sun and wind, the depth and kind of soil, and drainage patterns. Since most of the precipitation in San Luis Obispo comes in the relatively mild winter months (December - March), think about what this could mean for plant communities throughout the seasons.

A **plant community**, or a group of plant species growing together in a certain kind of physical habitat, typically has one or more **dominant species**. These dominant species will generally exhibit a greater effect on the types of plants that grow in a plant community, and the animals that interact with that community. This dominance may be due to shading of other plants, breaking down and recycling of nutrients, and various other factors.

We will look at the leaf structure of various plants and learn how variations in leaf, root, and stem structure help plants adapt to and thrive in a variety of environments. Pay careful attention to plant/animal/microorganism interactions that can occur within each community. In addition, pay attention to the invasive species your instructor points out, and how they affect the plant and animal composition in each community.

Exercise 1: Researching for Presentations

Before going outside, your lab instructor will assign pairs of students a plant or community to research. Each pair of students will be responsible for researching important information about their assigned plant or community and presenting this information to the class when outside in Poly Canyon. Using the internet, look up the pertinent information about your assignment. On the next page are a few important questions to ask.

Plant Questions:

1. How do you recognize individuals of this plant species?
2. What is the plant's native range? Is it native to California or is it from somewhere else? If it's not native, how did it get here?
3. How much water does this plant need to survive (High/intermediate/low)? How big/thick are the leaves?
4. What adaptations does this plant have to survive in its environment? For example, if it lives in a very dry environment, how does it conserve water? How does it use water if it lives in a very wet environment?
5. Include at least one cool fact or any interesting information you find out while doing research.

The possible presentation assignments are listed below with more ideas for information to include in your presentation that is specific to each plant. In your presentation outside, you can include this information as well as answers to questions 1-5 above.

Plant Species

1. Poison Oak
    a. How do you recognize poison oak?
    b. What should someone who has contacted poison oak do?
2. California Bay-laurel
    a. What common cooking ingredient is a close relative of this tree?
    b. During your presentation, you should grind up a leaf in your hands and have your classmates smell it. Be careful, though, as it may cause eyes to water!
3. Fennel
    a. During your presentation, you should grind up some leaves in your hands and have your classmates smell it.
4. Eucalyptus
    a. Why were eucalyptus planted in California?
    b. Why does eucalyptus shed bark?
5. California Sycamore
    a. What happens to California Sycamore when it is attacked by anthracnose fungus?
6. Yucca
    a. How many times in its lifetime does yucca reproduce?
    b. What is the relationship between yucca and the yucca moth?
7. Coast Live Oak
    a. What kind of root systems do coast live oaks create with mycorrhizal fungi?
8. California Coastal Sage & Black Sage
    a. During your presentation, you should grind up some leaves in your hands and have your classmates smell it.
    b. How do the seeds germinate?

Exercise 2: Field Trip to Poly Canyon

In this activity, we will walk up Poly Canyon road to view live examples of the plants and communities that you have just researched. You should try to spot your assigned plant or community, but your lab instructor will point them out for you as well. Take careful notes and ask questions as you will be responsible for any of the information given on the field trip and found in this manual on the final exam.

During your field trip in Poly Canyon, your TA will discuss different plant communities that exist in Poly Canyon. Fill in the table below with information about each type of community.

Table 9-1. Poly Canyon Plant Communities

Community Name	Description	Plants	Water Availability	Unique Features
Riparian				
Rocky Outcrop				
Coastal Scrub/Chaparral				
Grassland				

Lab Notebook Assignment

1. Title
2. Objectives
3. Exercise 1: All notes taken while researching your assigned presentation.
4. Exercise 2: All notes recorded during the field trip to Poly Canyon.
5. Exercise 2: Table 9-1 completely filled in.
6. Discussion Question: Among different plant communities, discuss the relationship between leaf size relative to:
    a. water availability
    b. soil type
    c. slope exposure/light exposure

Appendix: Microscope User Guide

IMPORTANT TIPS FOR USING COMPOUND MICROSCOPES

- You should ALWAYS use the lowest magnification objective lens to bring the object into focus. The lower magnification lenses are furthest from the stage and therefore less likely to hit the slide on the stage and damage the slide or the lens.
- Use both eyes. You won't be able to see in 3D like on a dissecting scope, but you will be able see better.
- Set the eyepieces apart to match your eyes as with the dissecting scope.
- Focus on the object using the scopes focus knob, while looking through the eyepiece that does NOT have its own focus control on it. Then use the focus control on the other eyepiece so both eyes are focused (diopter adjustment). Once you do this, changes in focus using the scopes focusing knob will correctly affect both eyepieces.

- Make sure the light is on, the slide is set using the slide holder (i.e. correctly placed on the stage), and the iris is closed.
- Once you have achieved focus using the shortest objective, ONLY use the FINE focus with the 40X and 100X objectives (if you use the coarse focus you are much more likely to hit the slide with the objective, potentially damaging both.
- The lenses of the compound microscope are **parfocal**; that is, the specimen will be in general focus when switching from objective to objective.
- Immersion oil must be used for the 100x objective, but **ONLY** the 100x objective. DO NOT put the 40x objective in oil!
- After using immersion oil, you must clean the 100x objective lens with lens paper and isopropyl alcohol ***immediately***.

STEPS TO OBTAINING A GOOD IMAGE ON A COMPOUND MICROSCOPE

1. ALWAYS start with the lowest power 4X objective.
2. Raise the condenser to its highest position.
3. Raise the stage to its highest point using the coarse focus knobs. By doing this, you simplify the process. You will only need to turn the coarse focus in one direction to find correct focus of the specimen.
4. Confirm that the slide specimen is in the middle of the scope's light beam. You may need to remove the slide and confirm where the specimen is located by holding it up to a light or by using a dissecting scope. It's also a good time to assess whether the slide needs to be cleaned!
5. Obtain a clear focused view of your subject using the coarse focus knob.
   Tip: If you have trouble finding the specimen, move the mechanical stage knob back and forth while lowering the stage simultaneously. Focus on something that moves as a result of moving the stage knob.

   Tip: Still having trouble? Focus on the edge of the cover slip first, then shift over to the specimen. Focusing on the edge of the cover slip will get you into the correct plane for the specimen.

6. Make sure that the two oculars are focused properly to provide a clear stereo view:
    a. Focus on the specimen through the right ocular using the fine adjustment.
    b. Focus on the specimen through the left ocular using the ocular diopter focus adjustment.
    c. Now use both eyes to look through the oculars. If the diopter is properly adjusted, you should have a clear stereo view when looking through both oculars?
    d. If not, try this: Adjust the distance between the oculars. The distance between people's eyes varies!
    e. Experiment with the absolute distance between your eyes and the ocular lenses.
7. Observe the specimen at increasing magnification up to the 40X objective by changing to more powerful objectives one at a time.
8. If you can't get the 40X image clear, the lens may be dirty and may need cleaning.
9. At any magnification, experiment with the iris diaphragm to improve contrast. It will almost always be best as closed as possible.
10. Never use the 100X objective as this requires adding a special oil to the slide.

CLEANING SLIDES AND MICROSCOPES

1. Remove the slide from the stage carefully. You may need to rotate the objective lens out of the way.
2. Clean all objectives and eyepieces using lens paper with a small amount of isopropyl alcohol. ONLY use lens paper on objective and eyepiece lenses.
3. If you have used the oil immersion lens, you will probably need a little more alcohol to remove the oil from both the oil immersion objective AND the slide you were examining with oil.
4. Store prepared slides in the container where you obtained them. Clean and dry off blank slides and coverslips. Store blank slides and coverslips in the containers provided. **Be careful not to leave cover slips on counter tops or sinks!** They are hard to see and are a serious hazard that can easily cause nasty cuts to you or someone else.

STORING MICROSCOPES

1. Make sure the light is turned off before unplugging the microscope.
2. Clean the stage with a paper towel if needed.
3. Wrap the power cord around the base of the scope.
4. **ALWAYS** place the scope back in the correct cubby. Numbers on the bottom of the cubby and on the scope must correspond. The arm/neck/body tube of the scope must be facing outward (see figure 1b-4).

HOW TO PUT THE COMPOUND MICROSCOPE AWAY

1. Rotate the objective lens in use away from the specimen on the stage.
2. Remove the slide from the stage and store or dispose of it properly.
3. Rotate the 10X objective into place, lower the condenser, center the stage, turn the light intensity to low or mid-range, turn off and unplug the microscope.
4. Clean all objectives, in particular the 100X oil lens using lens paper.
5. Clean the stage with a paper towel.
6. Wrap the power cord around the base of the microscope.
7. Place the microscope in its corresponding numbered storage cubby hole.

Fig. 1b-4. Microscope storage