A Laboratory Manual

of

Small-Scale Experiments

for the

Independent Study of

Introductory Biology

Accompanies HOL LabPaq: BK-105

A Laboratory Manual of Small-Scale Experiments for the Independent Study of Introductory Biology 090308

Published by: **Hands-On Labs, Inc.**
3203 W. Hampden Ave.
Sheridan, CO 80110
Phone: Denver Area: 303-679-6252
Toll-free, Long-distance: 866-206-0773

www.LabPaq.com

E-mail: info@LabPaq.com

Printed in the United States of America.

ISBN: 978-1-866151-22-2

The experiments in this manual have been and may be conducted in a regular formal laboratory or classroom setting with the users providing their own equipment and supplies. The manual was especially written, however, for the benefit of independent study students who do not have convenient access to such facilities. It allows them to perform college and advanced high school level biology experiments at home or elsewhere by using the LabPaq, a collection of experimental equipment and supplies specifically packaged to accompany this manual.

Use of this manual and authorization to perform any of its experiments is expressly conditioned upon the user reading, understanding and agreeing to fully abide by all the safety precautions contained herein.

Although the author and publisher have exhaustively researched many sources to ensure the accuracy and completeness of the information contained in this manual, we assume no responsibility for errors, inaccuracies, omissions or any other inconsistency herein. Any slight of people, organizations, materials, or products is unintentional.

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PREFACE

Micro-scale and small-scale laboratory experiments are simply those that use small quantities of chemicals and specially scaled down equipment. Micro- and small-scale experiments have become very popular because they reduce the safety risks inherent in traditional laboratory experimentation. They also reduce the cost of chemicals and waste disposal problems. These are the reasons increasing numbers of high schools, colleges, and universities are switching to micro and small-scale laboratories. Prof. Hubert Alyea of Princeton University and Prof. Stephen Thompson of Colorado State University have successfully used small-scale experiments in their lab classes for many years.

Web courses, telecourses, and similar independent study and distance learning methods are growing in popularity in higher education. However, sciences must include an experimental lab component if students are to experience hands-on learning and inquiry, an essential part of studying the sciences. Micro- and small-scale techniques allow independent study students to perform real science experiments in a non-laboratory setting. This enables independent study students working from their homes to complete fully accredited laboratory experiences in a variety of sciences including biology, microbiology, anatomy & physiology, chemistry, physics or geology.

This laboratory manual consists of micro- and small-scale experiments designed to augment any advanced high school or college level biology course. While the manual's experiments can be and are being successfully performed in regular biology laboratories, these experiments were specifically designed to be performed in non-laboratory settings in conjunction with a formal biology course. This has been made possible by the development of the LabPaq, a collection of experimental equipment and supplies specifically designed to accompany this manual.

Before beginning these experiments, independent study students are encouraged to visit a formal laboratory facility at their nearest community education center for a general tour and safety orientation. Exposure to full-scale facilities and equipment will help students to better understand the micro- and small-scale experiments they will be expected to perform.

ACKNOWLEDGEMENTS

Hands-On Labs wishes to gratefully acknowledge the kind, thoughtful, and diligent support and work of the following individuals who generously contributed their professional time, expertise, and energies toward the development of this lab manual.

Cynthia Alonzo, Biology Professor, Colorado Consortium of Colleges Online

Ann Anderson, Hands-On Labs, Inc., Sheridan, Colorado

Linda Jeschofnig, Adjunct Professor, Colorado Mountain College and

President, Hands-On Labs, Inc., Sheridan, Colorado

Dr. Peter Jeschofnig, Science Chair and Professor, Colorado Mountain College

Robert Kelly, Biology Professor, Colorado Mountain College

Laura Riege, Science Department Chair, Laurel Springs School, Ojai, California

Richard, Stevenson, Hands-On Labs, Inc., Sheridan, Colorado

Graphic Illustrations are by JoNet Designs, Evergreen, CO.

www.jonetdesigns.com

INTRODUCTION

Important Information to Help Students with the Study of Biology

Welcome to the study of Biology: Don't be afraid of taking this course. By the end of the semester you will be really proud of yourself and wonder why you were ever afraid of the B-word, Biology! After taking their first biology class, most students say they thoroughly enjoyed it, learned a lot of useful information that will benefit their future, and only regret not having studied biology sooner.

Biology is not some mystery science comprehendible only to eggheads. Biology is simply the study of living organisms. You will find it easier to understand the world we live in and to make the multitude of personal and global decisions that affect our everyday lives after you have learned about the world surrounding you. Things like how organisms interact with each other and the environment, their varying characteristics, and how all these things affect each other and you, will become clear as you work through the experiments contained in this manual. Plus, having biology credits on your transcript will certainly be impressive, and your knowledge of biology may create some unique job opportunities for you.

This lab manual was designed to accompany any entry level college or advanced high school level biology course. It can be used by all students, regardless of the laboratory facilities available to them. These experiments have been and continue to be successfully performed in regular biology laboratories, but with the BK-105 LabPaq, independent study students may perform experiments at home or at small learning centers that do not have formal laboratories. Throughout the manual there are references about campus-based and independent study, but all of the information in this manual is equally relevant to both types of students.

Micro- and Small-scale Experiments

You may be among the growing number of students who are taking a full-credit biology course through independent study. If so, you can thank the development and perfection of micro and small-scale experimental techniques for this breakthrough. Experimentation is essential and fundamental to fully understanding the concepts of biology. In the past, biology courses required all classes to be conducted on a campus because experiments had to be performed in the campus laboratory. This was due, in part to the potential hazards inherent in some traditional experimentation.

These elements of danger, plus increasing chemical and material costs and environmental concerns about chemical and biological material disposal, have made high schools, colleges and universities reexamine the traditional laboratory methods

used to teach subjects such as chemistry and biology. Scientists began to scale down the quantities of chemicals used in their experiments and found that reaction results remained the same, even when very tiny amounts of chemicals were used. Institutions also discovered that student learning was not impaired by observing small-sized reactions.

Over time, more and more traditional chemistry and biology experiments were redesigned for micro- and small-scale techniques. Dr. Hubert Alyea of Princeton University is one of the primary pioneers and most prominent contributors to the development of micro- and small-scale experimentation. He not only reformatted numerous experiments, he also designed many of the techniques and much of the equipment used in micro- and small-scale chemistry and biology today.

With decreased hazards, costs and disposal problems, micro- and small-scale experimentation techniques have been quickly adapted for use in scholastic laboratories. As these techniques continue to be further refined it, it has become possible to perform basic experiments in the classroom and eventually outside the classroom. This slow but steady progression of micro- and small-scale techniques now makes it possible for independent study students to take full-credit biology courses since they can fully perform experiments at home.

How to Study Biology

Biology is not the easiest subject to learn, but neither is it the hardest. As in any other class, if you apply yourself responsibly, read your text conscientiously, and complete your assignments thoughtfully, you will learn the material. Here are some basic hints for effectively studying biology - or any other subject - either on or off campus.

Plan to Study:

- Schedule a specific time and establish a specific place in which to study without interruptions or distractions.
- Think of studying as your job. You have a specific time and place to work. Studying should be no different.
- Distractions such as television and friends are not allowed at work, nor should they be allowed until after you've finished your studies. Complete your assignments; then reward yourself with some time to relax and enjoy yourself.

Get in the Right Frame of Mind:

- Think positively! Congratulate yourself for being a serious student and put yourself in a positive frame of mind to enjoy what you are about to learn.
- **Organize all the materials and equipment you will need in advance** so you don't have to interrupt your thoughts to find them later.
- Look over your syllabus and any other instructions and know exactly what your assignment is and what you need to do.

- Review what you have already learned. Is there anything that you aren't sure about? Write it down as a formal question, then go back over previous materials and try to answer it yourself.
- If you haven't figured out the answer after a reasonable amount of time and effort, move on. The question will develop in your mind and the answer will probably present itself as you continue your studies. If not, at least the question is written down so you can discuss it later with your instructor.

Be Active with the Material:

- **Learning is reinforced by repetitive and relevant activity.** The more you interact with your study materials and the more physical things you do with them, the better! When studying, feel free to talk to yourself, scribble notes, draw pictures, pace out a problem, tap out a formula, create learning mnemonics for concepts, etc.
- Have highlighters, pencils, and note pads handy. Highlight important data, read it out loud, and make notes.
- If there is a concept you are having problems with, try pacing while you think it through. See the action(s) connected with the concept taking place in your mind.
- Perform internet research on difficult concepts; exposure to different perspectives may unlock your own understanding. However, make certain the sites you visit are reputable and sponsored by valid institutions, colleges, and scientific organizations. Do not rely on information from unmonitored public sites such as Wikipedia.
- Think about what you observed, try to connect it to other things you know or have seen, and consider how what you have learned applies to the real world.
- Throughout the day, try to recall things you have learned, incorporate them into your conversations, and teach them to friends. These activities will help to imprint the related information in your brain and move you from simple knowledge to true understanding of the subject matter.

Do the Work and Think about What You are Doing:

- **The things we really learn are the things we discover ourselves**. That is why we don't usually learn science concepts as effectively from lectures or when someone gives us the answers as we do through experiencing them though experimentation.
- Don't just go through the motions of your assignments and experiments. Enjoy your work, think about what you are doing, be curious, examine your results, and consider the real world implications of your findings.
- Learning to “think critically” will improve and enrich the learning process.

When an assignment is completed independently and thoroughly, you will have gained knowledge and can be proud of yourself because you have persevered.

How to Study Biology Independently

There is no denying that learning through any method of independent study is a lot different than learning through classes held in traditional classrooms. **A great deal of personal motivation and discipline is needed to succeed in a course of**

independent study where there are no instructors or fellow students to give you structure and feedback. But these problems are not insurmountable and meeting the challenges of independent study can provide a great deal of personal satisfaction. The key to successful independent study is having a **personal study plan** and the personal **discipline** to stick to that plan.

Properly Use Your Learning Tools: The basic tools all distance learning methods are similar and normally consist of computer hardware and software, textbooks, videos, and study guides. Double-check with your course administrator or syllabus to make sure you acquire all the materials you will need. These items are usually obtained from your campus bookstore or library or via the Internet. Course lectures and videos may even be broadcast on your area's public and educational television channels. If you choose to do your laboratory experimentation independently, you will need the special equipment and supplies described in this lab manual and contained in the companion BK-105 LabPaq. The LabPaq is purchased directly online at www.LabPaq.com.

For each study session, you should first work through the appropriate sections of your course materials. These basically serve as a substitute for classroom lectures and demonstrations. Take notes as you would in a regular classroom. Actively work with any computer and/or text materials, carefully review your study guide, and complete all related assignments. If you don't feel confident about the material covered, repeat these steps until you do. It's a good idea to perform internet research on difficult concepts and to review your previous work before proceeding to a new section. This reinforces what you previously learned and prepares you to absorb new information. Experimentation is the last thing done in each study session, and it will only be really meaningful if you have first absorbed the text materials that it demonstrates.

Schedule Your Time Wisely: The more often you review study materials the more likely you are to reinforce and retain the information. Thus, it is much better to study in several short blocks of time rather than in one long, mind-numbing session. Plan to schedule several study periods throughout the week, or even every day. Please do not try to do all of your study work on the weekends! You will burn out, won't really learn much, and will probably end up feeling miserable about yourself and biology. Wise scheduling can prevent that kind of unpleasantness and frustration. ***Some experiments take several days to complete. Read them in advance and plan accordingly.***

Choose the Right Place for Your Home Laboratory: If you are doing the LabPaq experiments at home, the best place to perform your micro- and small-scale biology experiments is in an uncluttered room that has these important features:

- a door that can be closed to keep out children and pets,
- a window or door that can be opened for fresh air ventilation and fume exhaust,
- a source of running water for cleanup or fire suppression
- a counter or table-top work surface, and
- a heat source such as a stove top or hot plate.

The kitchen usually meets all these requirements, but be sure to clean all work areas well both before and after experimentation. This will keep foodstuff from contaminating your experiment and your experiment materials from contaminating food. Sometimes a bathroom makes a good laboratory, but it can be rather cramped and subject to a lot of interruptions. Review the "Basic Safety" section of this manual to help you select the best location for your home-lab and to make sure it is adequately equipped

Organization of the Lab Manual

Before proceeding with the experiments, you need to know what is expected of you. To find out, please thoroughly read and understand all the various sections of this manual.

Laboratory Notes: As do all serious scientists, you will record formal notes detailing your activities, observations, and findings for each experiment. These notes will reinforce your learning experiences and knowledge of biology. Plus, they will give your instructional supervisor a basis for evaluating your work. The *Laboratory Notes* section of this manual explains exactly how lab notes should be organized and prepared.

Required Equipment and Supplies: The first page of each experiment contains a list of the equipment and supplies needed to perform it. Students performing these experiments in a non-lab setting must obtain the LabPaq designed to accompany this manual. It includes all the equipment, materials, and chemicals needed to perform these experiments, except for some items usually found in the average home or obtainable in local stores. At the beginning of each experiment, **read through the materials section** that states exactly which items the student provides and which items are found in the LabPaq. Review this list carefully to make sure you have all these items on hand before you begin the experiment. *It is assumed that campus-based students will have all the needed equipment and supplies in their laboratories and that the instructors will supply required materials and chemicals in the concentrations indicated.*

Laboratory Techniques: While these techniques primarily apply to full-scale experiments in formal laboratories, knowledge of them and their related equipment is helpful to the basic understanding of biology and may also be applicable to your work with micro- and small-scale experimentation.

Basic Safety and Micro-scale Safety Reinforcement: The use of this lab manual and the LabPaq, plus authorization to perform these experiments, is expressly conditioned upon the user reading, understanding and agreeing to fully abide by all the safety rules and precautions noted herein. Additional terms authorizing use of the LabPaq are contained in the purchase agreement at www.LabPaq.com. These safety sections are relevant to both laboratory and non-laboratory experimentation. They describe potential hazards as well as the basic safety equipment and safety procedures designed to avoid such hazards. **The Basic Safety and Micro-scale Safety Reinforcement sections are the most important sections of this lab manual and should <u>always</u> be reviewed before starting each new experiment.**

Experiments: All experimental materials and procedures are fully detailed in the laboratory manual for each experiment. Chemicals and supplies unique for a specific experiment are contained in a bag labeled with the experiment number.

How to Perform an Experiment

Although each experiment is different, the process for preparing, performing, and recording all the experiments is essentially the same.

Review Basic Safety: Before beginning each experiment or part of an experiment, reread the safety sections, try to foresee potential hazards, and take appropriate steps to prevent problems.

Read the Entire Experiment Before you Start: Knowing what you are going to do before you do it will help you to be more effective and efficient. Words printed in **bold** are important vocabulary words; make certain you understand their meanings. The time estimates provided are general averages for the amount of time required to complete each experiment. The time it takes you to perform each experiment will probably vary.

Organize Your Work Space, Equipment, and Materials: It is hard to organize your thoughts in a disorganized environment. Assemble all required equipment and supplies before you begin working. These steps will also facilitate safety.

Outline Your Lab Notes: Outline the information needed for your lab notes and set up required data tables. This makes it much easier to concentrate on your experiment. Then simply enter your observations and results as they occur.

Perform the Experiment According to Instructions: Follow exactly all directions in a step-by-step format. This is not the time to be creative. DO NOT attempt to improvise your own procedures!

Think About What You Are Doing: Stop and give yourself time to reflect on what has happened in your experiment. What changes occurred? Why? What do they mean? How do they relate to the real world and what I've been studying in my textbook? This step can be the most fun and often creates "light bulb" experiences of understanding.

Complete Your Lab Notes and Answer Required Questions: If you have properly followed all the above steps, this concluding step will be easy.

Clean-up: Blot any minute quantities of unused chemicals with a paper towel or flush them down the sink with generous amounts of water. Discard used pipets and other waste in your normal trash. Always clean your equipment immediately after use or residue may harden and be difficult to remove later. Return equipment and supplies to their proper place, and if working at home with a LabPaq, store always the LabPaq out of the reach of children and pets.

LABORATORY NOTES and LAB REPORTS

Normally two basic records are compiled during and from scientific experimentation. The first record is the Laboratory Notes that are recorded as the experiment is performed. Entries into the lab notebook are the basis for the second record, the Laboratory Report which formally summarizes the activities and findings of the experiment. Lab Reports are the document normally submitted to instructor for grading.

Scientists keep track of their experimental procedures and results through lab notes that are recorded in a journal-type notebook as they work. In laboratories these notebooks are often read by colleagues, directors and co-workers on a project. In some cases scientific notebooks have become evidence in court cases. Thus, lab notes must be intelligible to others and include sufficient information so the work can be replicated and there can be no doubt about the honesty and reliability of the data and the researcher.

Notebooks appropriate for data recording are bound and have numbered pages that cannot be removed. Entries normally include all of the scientist's observations, actions, calculations, and conclusions related to each experiment. Data is never entered onto pieces of scratch paper to later be transferred, but rather is always entered directly into the notebook. When erroneous data is recorded, a light diagonal line is drawn neatly through the error, followed by a brief explanation as to why the data was voided. Information learned from an error is also recorded. Mistakes can often be more useful than successes and knowledge gained from them is valuable to future experimentation.

As in campus-based science laboratories, independent study students are normally expected to keep a complete scientific notebook of their work that may or may not be periodically reviewed by their instructor. Paperbound 5x7 notebooks of graph paper usually work well as science lab notebooks. Since it is not practical to send complete notebooks back and forth between instructors and students for each experiment, independent study students instead usually submit only formal Lab Reports along with regular assignments via email, fax, or website upload.

Lab notes of experimental observations can be kept in many ways. Regardless of the procedure followed, the key question for deciding what kind of notes to keep is this: "Do I have a clear enough record so that I could pick up my lab notebook or read my Lab Report in a few months and still explain to myself or others exactly what I did?"

Here are some general rules for keeping a lab notebook on your science experiments:

- Leave the first 2 to 4 pages blank so you can later add a "Table of Contents" at the front of the notebook. Entries into the table of contents should include the experiment number and name plus the page number where it can be found.

- Your records should be neatly written without being fussy.

- The notebook should not contain a complete lab report of your experiment. Rather it should simply be a record of what you did, how you did it and what were your results. Your records need to be complete enough so any reasonably knowledgeable person familiar with the subject of your experiment, such as another student or your instructor, can read the entries, understand exactly what you did, and if necessary, repeat your experiment the way you performed it.
- Organize all numerical readings and measurements in appropriate data tables as shown in following section's sample Lab Report.
- Always identify the units for each set of data you record (i.e. centimeters (cm), kilograms (k), seconds, etc.).
- Always identify the equipment you are using so you can find or create it later if needed to recheck your work.
- In general, it is better to record more rather than less data. Even details that may seem to have little bearing on the experiment (such as the time data was observed, the temperature, and if temperature varied during that time) may turn out to be information that has great bearing on your future analysis of the results.
- If you have some reason to suspect that a particular data set may not be reliable (perhaps you had to make the reading very hurriedly) make a note of that fact.
- Never erase a reading. If you think an entry in your notes is in error draw a single line through it, but don't scratch it out completely or erase it. You may later find that it was significant after all.

Although experimental results may be in considerable error, there is never a "wrong" result in an experiment for even errors are important results to be considered. If your observations and measurements were carefully made then your result will be correct. Whatever happens in nature, including a laboratory assignment, cannot be wrong. Errors may have nothing to do with your investigation, or they may be mixed up with so many other events you did not expect that your report is not useful. Yet even errors and mistakes have merit and often lead to our greatest learning experiences. Thus, you must think carefully about the interpretation of all your results, including your errors.

Finally, the cardinal rule in a laboratory is to choose in favor of "getting your hands dirty" and fully carry out all phases of your experiments instead of "dry-labbing" or taking shortcuts. The Greek scientist, Archytas, summed this up pretty well in 380 B.C.:

> *In subjects of which one has no knowledge one must obtain knowledge either by learning from someone else or by discovering it for oneself. That which is learnt, therefore, comes from another and by outside help; that which is discovered comes by one's own efforts and independently. To discover without seeking is difficult and rare, but if one seeks it is frequent and easy; if, however, one does not know how to seek, discovery is impossible.*

Laboratory Report Format

The lab notes you make during experimentation will form the basis for your final Lab Report which is a formal summary of your lab procedures, observations, and outcomes.

Like lab notes, lab reports are evidence of your work and of your understanding of the concepts demonstrated in the experiments. They may also be the primary basis for your lab grade. Write neatly and try to express your thoughts clearly, concisely, and completely so that someone else could duplicate your results. Laboratory Reports should normally include the following components:

Student's Name _____ Date of Experiment

Date Report Submitted

Title: Should be the same as the experiment title from the laboratory manual.

Purpose: Write a brief statement about what the experiment is designed to determine or demonstrate.

Procedure: Briefly summarize what you actually did in performing this exercise. Do not simply copy the procedure statement from the lab manual.

Data Tables: Tables are an excellent way to organize observations and information. Where applicable, the "Procedures" section of the experiment often advises a table format for data recording. Always set up and draw out any tables before experimenting.

Observations: What did you observe, smell, hear, or otherwise measure? Remember, observations are often most easily recorded in table form.

Questions: Questions are frequently asked throughout and at the end of exercises. to help you think critically about the experiment you have performed. Answers to these questions should be woven into the body of the report.

Conclusions: What did you learn from the experiment? Your conclusions should be based on your observations during the exercise. Conclusions should be written in your best formal English, using complete sentences, paragraphs, and correct spelling.

LABORATORY DRAWINGS

Laboratory work often requires findings to be illustrated in representational drawings. Clear, well organized drawings are an excellent way to convey observations and are often more easily understood than long textual descriptions. The adage “a picture is worth a thousand words” really is true when referring to science laboratory notes.

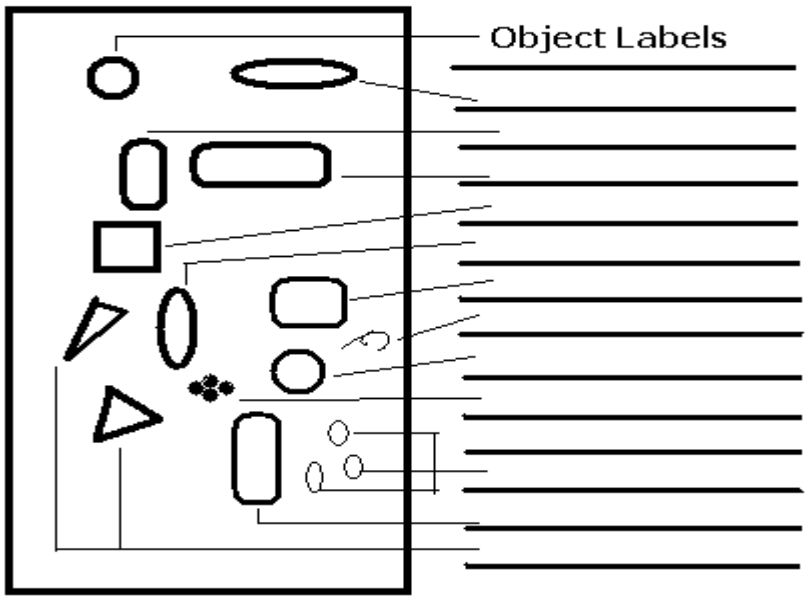
Students often say they can’t draw, but with a little care and practice, anyone can illustrate science lab observations. A trick most artists use is to place a mental grid over the object or scene and then approach their drawing from the standpoint of the grid areas. For instance, look at the diagram below and quickly make a free hand drawing of it. Then mentally divide the diagram into quarters and try drawing it again. In all likelihood, the second grid-based drawing will yield a better result.

Give yourself ample drawing space, and leave a white margin around the actual illustration, so it can be seen clearly. Also, leave a broad margin along one side of your drawing to insert labels for the objects in the drawing. Use a ruler to draw straight lines for the labels and as connecting lines between the objects and their related labels. The following is a good example of how your lab drawings should look when they are included in a formal lab report.

SOURCE OF DRAWING
Such as MUNG BEAN

Your Name
Date of Drawing

TITLE OF DRAWING
Such as CELL STRUCTURE



VISUAL PRESENTATION OF DATA

Learning to produce good graphs and tables is important because they can quickly and clearly communicate information visually. That is why graphs and tables are often used to represent or depict and compare data that have been collected. Graphs and tables should be constructed so they are able to "stand alone." That means all the information required to understand a graph or table must be included in it. A graph is composed of two basic elements: the graph itself and the graph **legend**. The legend adds the descriptive information needed to fully understand the graph.

Consider a simple graph plotting the "breathing rate of goldfish" versus the "water temperature." This graph consists of points plotted on a set of X and Y coordinates. The X-axis or **abscissa** runs horizontally, while the Y-axis or **ordinate** runs vertically. By convention, the X-axis is used for the **independent variable,** which is defined as a manipulated variable in an experiment or study whose presence or degree determines the change in the dependent variable. The Y-axis is used for the **dependent variable,** which is the variable affected by another variable or by a certain event. In our example, water temperature is the independent variable and should be plotted on the X-axis. The breathing rate is the dependent variable and should be plotted on the Y-axis since it may change with changes in the water temperature.

If we are dealing with two dependent variables, the choice of an X and Y variable is arbitrary. This would be the case, for example, if we collected data on pairs of mating frogs, and plotted male size vs. female size (or vice versa).

In all graphs, each axis is labeled, and the units of measurement are specified. When presenting a graph in a lab report, the variables, the scale, and the range of measurements should be clear. A legend for the above goldfish example might be something like: *The relationship between the breathing rate of goldfish (operculum movements per minute) and water temperature (^{o}C)*. Graphs are often the clearest and easiest way to depict patterns in data -- they give the reader a "*feel*" for all the data.

Graphs are often prepared using the graphing capabilities of spreadsheet programs like Excel®. A short Excel® graphing tutorial is presented in Appendix B to this manual.

The rules for constructing a table are similar. The important point is that the data are presented clearly and logically. The decision to present data in a table rather than a graph is often arbitrary. However, a table may be more appropriate than a graph when the data set is too small to warrant a graph, or is large and complex and not easily illustrated. Frequently a data table is provided to display the raw data, while a graph is then used to make visualization of the data easier.

REQUIRED EQUIPMENT AND SUPPLIES

BK-105 has been specifically assembled to allow students to conduct the experiments included in this manual in a non-laboratory setting. It includes the basic equipment described at the beginning of each experiment plus separate packages containing the required chemicals for many experiments. Independent study students will need to provide any items noted in the "Student supplies" section of required materials. These items are usually found in the average household, local pharmacy, or hardware store.

For campus-based students, all of the items and chemicals listed in the experiments should be provided by their instructor and available in their campus laboratory.

Equipment and supplies to be provided by student: You must first secure a safe and appropriate working environment. These experiments assume your home lab working area has adequate ventilation, running water, and a heat source such as a stovetop or hotplate. Review the materials list for each experiment and pre-assemble all required items before starting work. **Writing paper and pens to record notes plus paper towels, distilled water, and cleaning supplies are always needed but not always noted in the materials lists.** Other items you must provide at various times are listed below. Save time and frustration by making certain you have acquired and organized everything you need before you start each experiment.

Aluminum foil
Aspirin
Assistant (s) for several experiments
Baking soda (NaHCO3)
Blender, kitchen or food processor
Blue cheese
Candle, matches, or lighter
Celery stalks, fresh
Coffee cups
Cotton swabs (Q-tips®)
Crushed ice
Dish detergent
Distilled water, 1 gal.
Drinking glasses, short and tall
Egg, fresh raw
Flashlight
Flower, fresh and large
Glass jar, pint and quart sizes
91% Isopropyl (rubbing) alcohol
Lamp with 100W or higher bulb
Mixing bowls, small & medium
Mushroom, fresh
Old newspapers
Onion, fresh
Pan for boiling water
Pan/skillet for water bath
Paper, clean white sheets
Parsnip or carrot, fresh
Pea pod, fresh
Pie plate
Pond or stream water
Potato, small white
Rubber band
Salt, non-iodized
Scissors
Spoon, large
Strainer, small kitchen
Strawberries
Sugar
Tap water
Tape
Tent stakes, 4 or similar
Timer/clock with second hand
Toothpicks
Tree or shrub branch stem
Vegetable oil
Whisk or fork
White vinegar

Elodea or similar aquatic plant: The supply of Elodea and other aquatic plants is unpredictable. They are often, but not always, found at Wal-Mart and aquarium supply shops One on-line source for Elodea is http://www.aquaticplantdepot.com, but order early as shipping may require a week or more.

NOTE: Many items and chemicals are used multiple times and for several different experiments. Those that are used only for one individual laboratory experiment are often in a specific bag marked for that experiment. At the end of each experiment, always tightly reseal chemical bottles and clean all equipment used before returning them to the LabPaq box. Remember to safely store the LabPaq box and ensure it will be out of the reach of small children and pets!

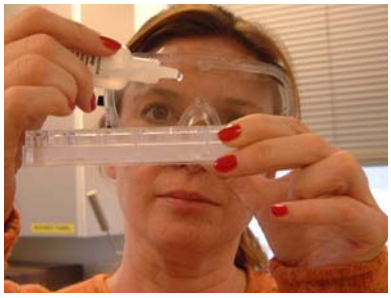
LABORATORY TECHNIQUES

Note: *Many of the techniques explained below are designed for use with full-scale laboratory equipment. Yet most are equally applicable to micro- and small-scale experimentation. Knowledge of these techniques will be of benefit should the student ever work with full-scale equipment in a formal laboratory.*

Dispensing Chemicals:
When pouring liquid chemicals from reagent (pronounced *re-agent*) bottles with glass stoppers, remove the stopper and hold it in between the top knuckles of your fingers while carefully pouring the liquid into the desired container. When pouring from a screw-cap bottle, set the cap upside down on its top so it does not contaminate or become contaminated. Be certain to put the correct cap on the bottle after using it. **NEVER POUR EXCESS CHEMICALS BACK INTO A REAGENT BOTTLE** since this may contaminate the reagents. If you spill any liquid or drip some on the side of the bottle, clean it up immediately.

To obtain samples of a powdered or crystalline solid from a container, it is best to first pour the approximate amount desired into a clean dry beaker or onto a small piece of clean paper that has been creased down the center. Creasing the paper makes it easier to contain and transfer the solids. Pour powders and crystals from their container by tilting it and, with a gentle shaking and rotating motion, work the solids up to the lip and allow them to fall out slowly. If you pour too much, leave the excess on the paper for later use. **NEVER PUT ANY SOLIDS BACK INTO THE JAR.** Also, never put spatulas, or paper into a jar of solid reagent, as they too may cause contamination.

Dropping Chemicals:
In micro-scale experimentation where only small drops of chemicals are used, it is extremely important that the drops are uniform in size and that they are carefully observed for accurate counting. To ensure uniformity of drop sizes, first cut off the tip of the pipets with scissors. Cut straight across the pipet as cutting at an angle will distort drop sizes. Then turn the bottle or pipet upside down so that the dispensing chamber behind the dropper is full of liquid. Finally, hold the dropper in front of your eyes so you can carefully observe and count the number of drops as you slowly squeeze the pipet or bottle to dispense drops.



Here you can see the wrong (left) and correct (right) way to dispense drops. The bottle or pipet should be held in a vertical position to ensure drops are uniform in size and to allow you to see and correctly count the number of drops dispensed.

Heating Chemicals: Heating Liquids in Test Tubes: When heating a liquid in a test tube, always use a test tube holder. The test tube should be carefully heated by moving it back and forth in the flame so that the contents are evenly heated. Heat the test tube near the top of the liquid first. Heating the test tube at the bottom may cause the liquid to suddenly boil and fly out.

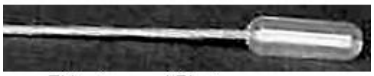

Test Tube Clamp (Holder)

Volume Measurement Equipment: To obtain accurate measurements from any volume measurement equipment, you must identify and correctly read a curved surface known as the **meniscus**. The meniscus of water and water-based solutions curves downward and is read at the very bottom of the curve. A mercury meniscus is convex and is read at the top of its curve.

The Graduated Cylinder: Graduated cylinders are available in a wide range of sizes. To read a volume in a graduated cylinder, hold the graduated cylinder up to eye level so the meniscus can be viewed directly and in a straight line. Looking at a meniscus from below or above will create parallax and cause a false reading. Always read any scale to the maximum degree possible, including an estimate of the last digit.


50 mL Graduated Cylinder

The Pipet: Pipets are small tube-type containers with openings at one (plastic) or both (glass) ends. They come in a range of volumes and are generally used to transfer specific amounts of liquids from one container to another. LabPaq chemicals are often packaged in thin stemmed plastic pipets. To use, snip off the end tip of the stem with scissors; make straight cut that is perfectly perpendicular to the stem and not at a slant.


Thin stemmed Pipet

Distilled Water or Deionized Water: Tap water frequently contains ions that may interfere with substances being studied. To avoid such interference, distilled or deionized water is used any time water is needed for dilution of concentration or for the preparation of experimental solutions. Glass or plastic containers should be washed with soap, rinsed with tap water, and then rinsed with distilled water.

Filtration Equipment: Gravity Filtration: Gravity filtration is used to remove solid precipitates or suspended solids from a mixture. This device works like a small funnel or strainer except that it is lined with fine conical filter paper to trap solids. After the mixture has been poured into the filter from a beaker, a spatula is used to scrape any remaining solids from the beaker wall into the conical filter paper. A wash bottle is then used to rinse residue from the beaker and spatula into the filter cone and to ensure that all the mixture's particles pass through the filter.

Heating Methods for Small-scale Techniques: For micro- and small-scale experimentation, the most commonly used heat sources are alcohol burners, candles, and chafing dish or burner fuel. Alcohol burners can be a problem because their flame is almost invisible and

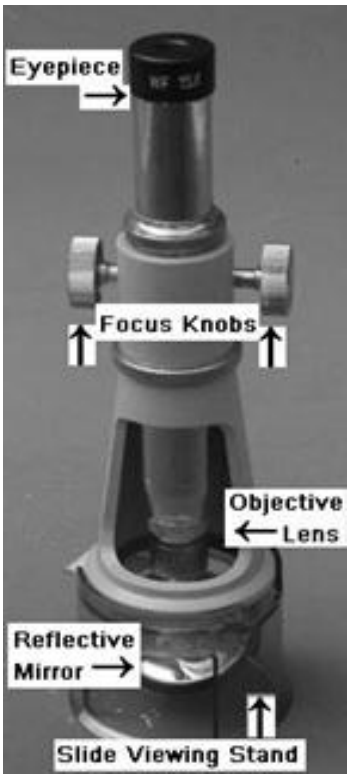
they cannot be refilled while hot. Candles tend to leave a sooty carbon residue on the container being heated that can obstruct observations. Chafing dish fuel is the best alternative. It has a visible flame, is easily extinguished, and does not leave flame residue. Remember, regardless of the heat source:

No ignited heat source should ever be left unattended!

USING THE FIELD MICROSCOPE

Your LabPaq includes a field microscope with a slide viewing stand housed inside a travel case. This field microscope is designed for a biologist or anyone needing portable magnification capability beyond that of a hand magnifier. It has the same optics as a typical table microscope but in a smaller and more portable frame. The objective lens is 10x (the x denotes power) and the eyepiece is 15x with a wide field for ease in viewing. Total magnification power is computed by multiplying the power of the objective lens by that of the eyepiece and is therefore 10x * 15x = 150x. The field microscope's lens mounts and focal lengths are those of a standard microscope; thus, the total magnification can be changed by purchasing a different power objective lens and/or eyepiece.

The field microscope is a vertical cylinder with knurled knobs on each side for single rack and pinion focus adjustment. This microscope is designed to view an object placed directly under it while its base rests upon the same surface as the object or upon a slide viewing stand.



To examine lichen growing on a rock, you would center the area to be viewed in the barrel of the microscope and rest the base on the rock. Begin by looking at the barrel of the microscope, move the objective lens assembly down with the focus knobs until the lens nearly touches the object. Then, looking through the eye piece, raise the objective with the focus adjustment knobs until the subject comes into focus. The procedure of first moving the objective lens down while viewing the lens from the side and only then raising the lens while looking through the eye piece, is extremely important when viewing glass slides as they are easily broken if the objective lens moves down too far or too fast.

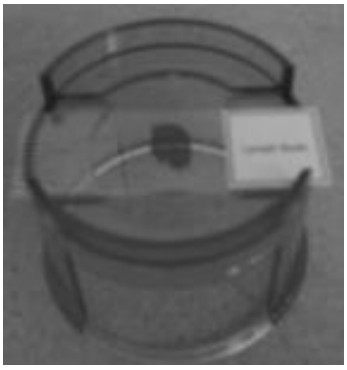
Try out the basic functions of the microscope by placing this open laboratory manual on a flat table; then place the microscope over an area of text and focus. You should see how an individual letter more than fills the field of view. At 150x magnification, you can see the tiny dots of ink that result from the printing process. Make a mental note of the objective lens position

when an individual letter is in focus. This position approximates the focal length necessary to view other objects.

It is important to have good lighting to view anything through a microscope. Sunlight is normally strong enough to view an object with the field microscope in the outdoors, but more light is usually needed indoors. Note the opening on the base of the field microscope which is specifically designed to admit light both indoors and out. A desk light, preferably one with a reflector and a focused bulb, can be used for stronger lighting; simply direct its light toward the scope's base

Light transmitted through a viewed object helps to make its details stand out in relief. Thus, bottom-up lighting is best for the proper viewing of slides. Regular laboratory microscopes have a reflecting mirror or built-in light source to reflect ample light upward through slides. This lighting from below the stage on which the slide rests allows the slide's contents to be clearly seen.

To view a prepared microscope slide with the field microscope, first move the objective lens upward to avoid breaking the slide. Place the microscope stand over a sheet of white paper or on a flat, light colored surface and then place the field scope securely onto the stand. Slip the prepared slide to be viewed into the stand's side opening to the slide stage area and position the slide so its contents are centered directly under the lens. You should be able to slightly move the slide around the stage platform to view different areas of the slide.



Bringing different areas of the slide into view requires delicate and carefully controlled movements plus a bit of practice to perfect your technique. Such manual manipulations would be very difficult if you were using much higher powers of magnification, but at 150x you should be able to quickly master using the field microscope.

Similar to the illuminator of a laboratory scope, the field microscope's clear stand allows natural light to reflect upward through the slide. For additional light and better viewing, position the desk lamp to shine onto the light colored paper or surface below the stand so that its light reflects directly up through the slide.

As you become comfortable with the use of the field microscope, you will enjoy viewing nature and other objects in the environment at 150 power magnification! Try looking at the textures of various things: a desktop, pieces of rocks or minerals, an eyelash, the surface of a cracker or a leaf. There is no end to the things you can discover through magnification. After enjoying magnified objects and the minutia of life, look at the following photo of the Sombrero galaxy and contemplate the huge gulf of scale between tiny microscopic objects and the huge vastness of the universe.

BASIC SAFETY

This section contains vital information that must be thoroughly read and completely understood before a student begins to perform experiments.

PREVENT INJURIES AND ACCIDENTS! Laboratory experimentation is fun. It can and should always be conducted safely. To do so requires students to learn and then always follow basic safety procedures. While this manual tries to include all relevant safety issues, not every potential danger can be foreseen. Each experiment involves slightly different safety considerations. Thus students must always act responsibly, learn to recognize potential dangers, and always take appropriate precautions. Regardless of whether a student will be working in a campus or home laboratory setting, it is extremely important that he or she knows how to anticipate and avoid possible hazards and is safety conscious at all times.

BASIC SAFETY PROCEDURES: Experimentation sometimes involves using toxic chemicals, flammable substances, sharp items, and potentially dangerous equipment. All of these things can cause injury and even death if not properly handled. These basic safety procedures apply when working in either a campus or a home laboratory.

Because eyes are very vulnerable to chemical spills and splashes,

- Students must always wear eye protecting glasses or safety goggles when using chemicals.

Because toxic chemicals may enter the body through digestion,

- Drinking and eating are always forbidden in laboratory areas, and

- Students must always wash their hands before leaving the laboratory.

Because toxic substances may enter the body through the skin and lungs,
- The laboratory area must always have adequate ventilation,
- Students must never "directly" inhale chemicals,
- Students should wear long-sleeved shirts, pants, and enclosed shoes when experimenting, and
- Students must wear gloves and aprons when appropriate.

Because hair, clothing, and jewelry can create hazards, cause spills and catch fire,
- Students should always tie or pin back long hair,
- Students should always wear snug fitting clothing (preferably old), and
- Students should never wear dangling jewelry or other objects.

Because a laboratory area contains various fire hazards,
- Smoking is always forbidden in laboratory areas.

Because chemical experimentation involves numerous potential hazards,
- Students must know how to locate and use basic safety equipment,
- Students must never leave a burning flame or reaction unattended,
- Students must specifically follow all safety instructions,
- Students must never perform any unauthorized experiments, and
- Students must always properly store equipment and supplies and ensure these are out of the reach of small children and pets.

BASIC SAFETY EQUIPMENT: The following pieces of basic safety equipment are found in all campus laboratories. Informal and home laboratories may not have or need all of these items, but simple substitutes can usually be made or found. Students should know their exact location and proper use.

SAFETY GOGGLES - There is no substitute for this important piece of safety equipment! Spills and splashes do occur, and eyes can very easily be damaged if they come in contact with laboratory chemicals. While normal eyeglasses do provide some protection, chemicals can still enter the eyes from a side splash. Safety goggles cup around all sides of the eyes to provide the most protection and can be worn over normal eyeglasses if required.

EYEWASH STATION - All laboratories should have safety equipment to wash chemicals from the eyes. A formal eyewash station looks like a water fountain with two faucets directed up at spaces to match the space between the eyes. In case of an accident, the victim's head is placed between the faucets while the eyelids are held open so the faucets can flush water into the eye sockets and wash away the chemicals. In an informal laboratory, a hand-held shower wand or kitchen sink spray nozzle can be substituted for an eyewash station. After the eyes are thoroughly washed, a physician should be promptly consulted.

FIRE EXTINGUISHER - There are several types of fire extinguishers, at least one of which should be found in all types of laboratories. Students should familiarize themselves with, and know how to use, the particular type of fire extinguisher in their laboratory. At a minimum, home laboratories should have a bucket of water and a large pot of sand or dirt available to smother fires.

FIRE BLANKET - This is a tightly woven fabric used to smother and extinguish a fire. It can cover a fire area or be wrapped around a victim whose clothing or hair has caught on fire.

SAFETY SHOWER - This shower is used in formal laboratories to put out fires or douse people who have suffered a large chemical spill. A hand-held shower wand or kitchen sink spray nozzle may be substituted for a safety shower in an informal or home laboratory.

FIRST-AID KIT - This kit of basic first-aid supplies is used for the emergency treatment of injuries and should be found in both formal and informal laboratories. It should always be well stocked and easily accessible.

SPILL CONTAINMENT KIT - This consists of absorbent material that can be ringed around a spilled chemical to keep it contained until the spill can be neutralized. The kit may simply be a bucket full of sand or other absorbent material such as kitty litter.

FUME HOOD - This is a hooded area containing an exhaust fan that expels noxious fumes from the laboratory. Experiments that might produce dangerous or unpleasant vapors are conducted under this hood. In an informal laboratory, such experiments should be conducted only with ample ventilation and near open windows or doors. If a kitchen is used for a home laboratory, the exhaust fan above the stove may substitute as a fume hood.

MSDS: MATERIAL SAFETY DATA SHEETS

A Material Safety Data Sheet (MSDS) is designed to provide chemical, physical, health, and safety information on reagents and supplies. An important skill in deciding on the safe use of chemicals is being able to read a MSDS. It provides information about how to handle store, transport, use and dispose of chemicals in a safe manner.

MSDS also provide workers and emergency personnel with the proper procedures for handling and working with chemical substances. While there is no standard format for an MSDS, they all provide basic information about physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid procedures, chemical reactivity, safe storage, safe disposal, protective equipment required, and spill cleanup procedures. An MSDS is required to be readily available at any business where any type of chemical is used. Even day-care centers and grocery stores need MSDS for their cleaning supplies.

It is important to know how to read and understand the MSDS. They are normally designed and written in the following sections:

Section 1 **Product Identification Data:** Name and Trade Names of Chemicals	**Section 2** **Hazardous Ingredients Data:** Components and Percentages of Chemicals	**Section 3** **Physical Data:** Boiling point, density, solubility in water, appearance, color, etc.
Section 4 **Fire and Explosion Data:** Flash point, extinguisher media, special fire fighting procedures, and unusual fire and explosion hazards	**Section 5** **Health Hazard Data:** Exposure limits, effects of overexposure, emergency and first aid procedures	**Section 6** **Reactivity Data:** Stability, conditions to avoid, incompatible materials, etc.
Section 7 **Spill or Leak Procedures:** Steps to take to control and clean up spills and leaks, and waste disposal methods	**Section 8** **Control Measures:** Respiratory protection, ventilation, protection for eyes or skin, or other needed protective equipment	**Section 9** **Special Precautions:** How to handle and store, steps to take in a spill, disposal methods, and other precautions

Summary: The MSDS is a tool that is available to employers and workers for making decisions about chemicals. The least hazardous chemical should be selected for use whenever possible, and procedures for storing, using, and disposing of chemicals should be written and communicated to workers.

MSDS information can be viewed at **http://www.hazard.com/msds/index.php**. Students can also find a link to MSDS information from **www.LabPaq.com** . If there is ever any problem or question about the proper handling of any chemical, the student should seek appropriate MSDS information.

POTENTIAL LABORATORY HAZARDS:

Recognizing and respecting potential hazards is the first step toward preventing accidents. Please appreciate the grave dangers the following laboratory hazards might represent. Work to avoid these dangers and consider how to respond properly in the event of an accident.

NEVER FORGET to make sure your LabPaq is ALWAYS stored out of the reach of children and pets!

FIRES: The open flame of a Bunsen burner or any heating source combined, even momentarily, with inattention, may result in a loose sleeve, loose hair, or some other unnoticed item catching fire. Other than water, most solvents including toluene, alcohols, acetones, ethers, and acetates are highly flammable and should never be used near an open flame. As a general rule, ***NEVER LEAVE AN OPEN FLAME OR REACTION UNATTENDED.*** In case of fire, use a fire extinguisher, fire blanket, and/or safety shower.

CHEMICAL SPILLS: Flesh burns may result if acids, bases, or other caustic chemicals are spilled and come in contact with skin. Simply flush the exposed skin with water for several minutes at a sink or safety shower. Acid spills should be neutralized with simple baking soda (sodium bicarbonate). If eye contact is involved, use the eyewash station or its substitute. Use the spill containment kit until the spill is neutralized. To better protect the body from chemical spills, wear long-sleeved shirts, full-length pants, and enclosed shoes (never sandals!) when in the laboratory.

ACID SPLATTER: When water is added to concentrated acid, the solution becomes very hot and may splatter acid on the user. Splattering is less likely to occur if instead the acid is slowly added to the water: Remember this ***AAA*** rule: ***A****lways* ***A****dd* ***A****cid to water, NEVER add water to acid.*

GLASS TUBING HAZARDS: Never force a piece of glass tubing into a stopper hole. The glass may snap and the jagged edges can cause a serious cut. Before inserting glass tubing into a rubber or cork stopper hole, be sure the hole is the proper size. Lubricate the end of the glass tubing with glycerol or soap, and then while grasping it with a heavy glove or towel, gently but firmly twist the tubing into the hole. Treat any cuts with appropriate first aid.

HEATED TEST TUBE SPLATTER: Splattering and eruptions can occur when solutions are heated in a test tube. Thus, you should never point a heated test tube towards anyone. To minimize this danger, direct the flame toward the top rather than the bottom of the solution in the tube. Gently agitate the tube over the flame to heat the contents evenly.

SHATTERED GLASSWARE: Graduated cylinders, volumetric flasks and certain other pieces of glassware are *NOT* designed to be heated. If heated, they are likely to shatter and cause injuries. Always ensure you are using heat-proof glass before applying it to a heat source.

INHALATION OF FUMES: To avoid inhaling dangerous fumes, partially fill your lungs with air and, while standing slightly back from the fumes, use your hand to waft the odors gently toward your nose, then lightly sniff the fumes in a controlled fashion. *NEVER INHALE FUMES DIRECTLY!* Treat inhalation problems with fresh air and consult a physician if the problem appears serious.

INGESTION OF CHEMICALS: Virtually all the chemicals found in a laboratory are potentially toxic. To avoid ingesting dangerous chemicals, never taste, eat or drink anything while in the laboratory. Home laboratories, especially those in kitchens, should always be thoroughly cleaned after experimentation to avoid this hazard. In the event of any chemical ingestion immediately consult a physician or call the **National Poison Control Center at 1-800-332-3073.**

HORSEPLAY: A laboratory full of potentially dangerous chemicals and equipment is a place for serious work, not for horseplay! Fooling around in a laboratory is an invitation for an accident.

VERY IMPORTANT CAUTION FOR WOMEN: If you are pregnant or could be pregnant, you should seek advice from your personal physician before doing any type of chemical or biological experimentation.

If you or anyone accidentally consumes or otherwise comes into contact with a chemical that might be toxic and cannot be easily washed away you should immediately contact a doctor or seek appropriate advice.

National Poison Control Center

1-800-332-3073

SCIENCE LAB SAFETY REINFORCEMENT AGREEMENT

While all experiments in this manual are micro-scale or small-scale, which reduces most potential risks, unforeseen risks may still exist. The need to prevent injuries and accidents cannot be over-emphasized!

Use of this lab manual and the LabPaq are expressly conditioned upon your agreeing to follow all safety precautions and accept full responsibility for your own actions. Thus it is prudent to review these basic safety rules and relevant safety precautions. You should study the safety section of the manual until you can honestly state the following:

__I know that except for water, most solvents such as toluene, alcohols, acetone, ethers, ethyl acetate, etc. are highly flammable and should never be used near an open flame.

__I know that the heat created when water is added to concentrated acids is sufficient to cause spattering. When preparing dilute acid solutions, I will always add the acid to the water (rather than the water to the acid) while slowly stirring the mixture.

__I know it is wise to wear rubber gloves when handling acids and other dangerous chemicals; that acid spills should be neutralized with sodium bicarbonate (baking soda); and that acid spilled on the skin or clothes should be washed off immediately with a lot of cold water.

__I know that many chemicals produce toxic fumes and that cautious procedures should be used when smelling any chemical. When I wish to smell a chemical, I will never hold it directly under my nose but instead will use my hand to waft vapors toward my nose. If I experiment at home I will keep a window or door open while performing experiments.

__I will always handle glassware with respect and promptly replace any defective glassware because even a small crack can cause glass to break when heated. To avoid cuts and injuries, I will immediately clean up and properly dispose of any broken glassware.

__I will avoid burns by testing glass and metal objects for heat before handling. I know that the preferred first aid for burns is to immediately hold the burned area under cold water for several minutes.

__I know that serious accidents can occur if the wrong chemical is used in an experiment. I will always carefully read the label before removing any chemical from its container.

__I will avoid the possibility of contamination and accidents by never returning an unused chemical to its original container. To avoid waste I will try to pour out only the approximate amount of chemicals required.

__I will select a work area that is inaccessible to pets and children while experiments are in progress. I will not leave experiments unattended and I will not leave my work area while chemical equipment is set up unless the room will be locked.

__To avoid the potential for accidents I will clear my home-lab workspace of all non-laboratory items before setting up my lab equipment and chemical experiments.

__Before beginning an experiment I will first read all directions and then assemble and organize all required equipment and supplies.

__I will wear approved safety glasses at all times while working on lab experiments involving chemicals, and if I ever spill any chemical on myself I will immediately flush the spill with a lot of water and then consult a doctor if required.

__To protect myself from potential hazards I will wear long pants, a long-sleeved shirt, and enclosed shoes and I will tie up any loose hair, clothing, or other materials when performing chemical experiments.

__I will never attempt an experiment until I fully understand it. If in doubt about any part of an experiment, I will speak with my instructor before proceeding.

__I will never eat, drink, or smoke while performing experiments.

__After completing all experiments I will clean up my work area, store the lab equipment in a safe place that is inaccessible to children and pets, and wash my hands to remove any chemicals.

__I will always conscientiously work in a reasonable and prudent manner so as to optimize my safety and the safety of others whenever and wherever I am involved with science experimentation and/or working with chemicals.

Permission to utilize a LabPaq is contingent upon you agreeing to follow all prescribed safety procedures. Please review this document several times until you are certain you understand it and then sign the agreement below. Your instructor may require you to send him/her a copy.

I am a responsible adult who has read, understood, and agree to fully abide by all safety precautions prescribed by my science lab manual for lab work and the use of LabPaqs. I recognize the inherent hazards potentially associated with science experimentation and I will always experiment in a safe and prudent manner. Thus, I unconditionally accept full and complete responsibility for any and all liability related to my purchase and/or use of a science LabPaq or other any science products or materials provided by Hands-On Labs, Inc. (HOL).

_____ _____

Student's Signature Date

Student's Printed Name: _____

EXPERIMENT 1: The Scientific Method

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow at least 90 minutes for this experiment.

Objectives: To systematically observe some properties of unknown substances and To use the Scientific Method to hypothesize about and identify unknown substances.

Materials:

Student Provides:
- Distilled water
- Paper, clean white sheets
- Paper towels

From the LabPaq:
- Goggles
- Hand magnifying lens
- Rubber stopper, #00 solid
- Spring scale, 10-g
- Small plastic weighing bag
- Graduated cylinder, 50-mL
- Test tube rack
- Test tubes, 6
- Test tube cleaning brush
- Wax pencil

Experiment Bag:
- Unknown substances, 6

Discussion and Review: To explain natural phenomena, scientists proceed in a carefully structured series of steps known as **The Scientific Method**. By conscientiously following these steps, scientists better ensure the validity of their work;:

1. **Observe:** Carefully observe the event or material. The observations may be made directly with the human senses or indirectly with instruments.

2. **Question:** Formulate questions concerning observations made. These questions usually include: What? How? When? Where? Why? Why is often the most difficult question to answer!

3. **Hypothesize:** Propose an answer to the questions. A hypothesis is an explanation of the observed event or material based upon personal knowledge and other knowledgeable sources, and it is often an educated guess. A hypothesis should be testable, if possible, in a manner that will provide evidence that either rejects or supports the hypothesis and that can be duplicated by others.

4. **Experiment:** Carry out an experiment in a set of controlled conditions to attempt to provide evidence to support the hypothesis. A **controlled experiment** is a type of experiment consisting of two events or procedures that are identical in all but one aspect. The control group is used as a basis of comparison to the experimental group from which it differs by only the one variable. Experiments need to be replicable in order to produce sets of data that are meaningful in analysis.

5. **Compile Data:** Gather **empirical data** through experimentation and observation and then compile and analyze it in order to determine if it supports or rejects the hypothesis. The two basic types of data are:

   - **Quantitative:** This is **objective data** that contains numbers and can be analyzed statistically. Quantitative data is considered the superior data type because it is not easily biased.

   - **Qualitative:** This is **subjective data** that includes attitudes, descriptions, and explanations of the phenomena studied per the perspective of the observer. Qualitative data is vulnerable to bias since it is cannot be precisely measured and does not contain verifiable numbers to be accurately analyzed by statistics.

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6. **Draw Conclusions:** Review, evaluate and analyze the data gathered to arrive at a conclusion that accepts or rejects the hypothesis. Acceptance may be within a range of confidence, rather than absolute acceptance or rejection. If the hypothesis is rejected, or if it is not accepted with a high level of confidence, one may repeat the steps of observation, questioning, hypothesizing, experimentation, and data collection to arrive at a new conclusion.

7. **Publish:** A scientist must make his/her observations, empirical data, and conclusions available to other scientists for their review and acceptance or rejection. Submitting your laboratory report to your instructor is publishing, an important reason why the report should be done with care.

8. **Form a Theory:** A scientific theory is formed when a majority of the scientific community supports and has confidence in the conclusion drawn from a hypothesis.

9. **Establish a Law:** A scientific law is established when a hypothesis is accepted as a uniform and constant aspect of nature and there is no empirical evidence to prove otherwise.

PROCEDURES: In this experiment, you will apply the steps and logical framework of the Scientific Method. Through systematic observation, drawing of a hypothesis, and deliberate experimentation, you will try to identify the six unknown substances in the experiment bag marked, Experiment 1. The unknowns are all substances often found in a typical home and none are toxic or dangerous. However, any unknown substance

should be treated as potentially dangerous and handled accordingly until completely proven otherwise. While taste would be a good indicator for an unknown, scientist NEVER use a taste test on any unknown and neither should you!

1. Begin by setting up a data table as shown below to record your work observations.

Unknown	A	B	C	D	E	F
Hypothesis						
Color						
Texture						
Shape						
Smell						
Solubility						
Density						
Conclusion						

2. Observe each of the containers of materials carefully. You may open the top of the container to observe the unknown more closely, but be careful not to spill or remove any of the unknown from the container at this time. Reseal the container when you have finished your observation.

3. Your question for each unknown is “what is the material?”

4. Form a hypothesis about the identity of each unknown. Consider the general appearance of the unknown and hypothesize what household product it might be. Record your hypothesis and your principal reasons for the hypothesis in the table.

5. Decide on a protocol for the sequence of steps you will use to determine the identity of the unknown items. For example, in this experiment, density should be determined first because you will (1) use the full container of material and *(2)* you will know the volume of material in the container plus the weight of the container. Further, you should determined solubility last because it requires trying to dissolve the material in water. It

will be difficult to continue to experiment with the material after it is mixed with water, especially in your limited laboratory facilities.

6. Set up an experiment in which you examine each unknown for the following characteristics:

   a. Density
   b. Color
   c. Smell
   d. Texture
   e. Particle shape
   f. Solubility

   Record quantitative data whenever possible and qualitative data when quantitative data is not possible. It is acceptable to provide both quantitative and qualitative data.

7. Work with only one unknown at a time and perform all of the following tests for each one. Record your observations and then draw conclusions about the unknown's identity. Complete the full series of tests for one unknown before starting to work on another. If you are still in doubt after performing all of the tests, read through the list of possible unknowns at the end of this experiment. Each of the unknowns is in that list.

8. Examine each unknown for each of the following characteristics:

   Density: Each unknown is packaged in a plastic container that, when full as each should be, contains exactly 1.5 cc (cubic centimeters) of material. Place the container of unknown in the small plastic bag provided and suspend it from the scale. Hold the scale steady at eye level until it stops moving and read and record the weight. *Note:* Be sure to calibrate the scale before using it. See calibration instructions on the box. Then calibrate the scale plus weighing bag before continuing.

   Fold a 10-cm square of clean white paper in half and open it so that it has a crease down the middle. Open the container and pour the contents of the container of unknown onto the center of the paper. Tap the container sharply on the paper to ensure that all of the contents are emptied. Place the empty container back in the plastic bag and weight it as before. Subtract the weight of the empty container and bag from the weight of the full container and bag. The difference between the two weights is the weight of the unknown in grams.

   Divide the weight of the unknown in grams by the known volume of the container, 1.5 cc. The quotient represents the density of the unknown in g/cc (grams per cubic centimeter). This density measure should fall somewhere in the range of 0.5 to 1.5 g/cc. Recall that the density of water is 1 gm/cc, and think about how dense these materials are relative to water.

   Color: Note the color and related visual aspects of the unknown. Is it shiny or dull in reflection or does it have any luminescence or color shift as does a hologram?

Texture: Take a pinch of the material and rub it between your fingers. Observe how it feels to the touch. Is it gritty or smooth, dry or oily, etc.? Note: if you had not been told the substances were non-toxic, you would wear gloves to test the texture.

Particle shape: Observe the material on the paper with the hand lens. If the unknown is a powder or granular, observe the shape of the particles. Note if the individual particles are too small to see or, if they are visible, are they smooth or sharp edged, regular or irregular, crystalline or amorphous?

Smell: Prepare to smell the unknown the way a scientist does. To avoid inhaling potentially dangerous fumes or powders, partially fill your lungs with air, and, while holding the paper in front of you, use your hand to waft the odors gently toward your nose. Then lightly sniff the fumes in a controlled fashion. *NEVER INHALE FUMES DIRECTLY!* Observe if the unknown smells sweet or sour, chemical or organic, or is in some other way, known to you. Can you hypothesize the identity of the material from the smell?

Solubility in water: Place approximately half of the unknown material in a clean, dry test tube. Measure out 5 cc of distilled water into the graduated cylinder and pour it into the test tube. Stopper the test tube with a clean dry rubber stopper and shake vigorously.

- **Soluble** material will disappear after shaking and the water will be clear, not cloudy. If the water does clear, observe if it has any color.

- **Insoluble** material will not disappear in the water and the water will have a cloudy appearance after being shaken. If the unknown goes into suspension, the water will remain cloudy after several minutes. If it is **not in suspension**, the material will settle to the bottom and the water will clear.

After observing, use the wax pencil to write the letter of the unknown's on the test tube before placing it in the test tube rack for further observations. Remove, wash, and dry the rubber stopper before testing the next unknown.

9. Repeat the above procedures for each of the five remaining unknowns. Record your observations for each in the data table you prepared at the beginning of this lab.

10. Allow the test tubes to sit undisturbed in the rack at least one hour but preferably longer or over night. Record your observations. Has anything happened to change your mind about previous observations and/or conclusions?

Questions:

A. Which of the six measures in the experiment give you quantified data?

B. For which of the unknowns was your conclusion the same as your hypothesis?

C. Were any of your conclusions different from your original hypothesis? If so, what particular factor(s) changed your mind?

D. What is your level of confidence (0 – 100%) in your conclusion for each unknown?

Cleanup: Although not hereafter repeated, clean up for this and all other experiments is as follows:

- Carefully blot up with disposable paper towels any remaining chemicals or materials or pour the chemical mixtures down the sink and rinse with a lot of water.

- For any remaining chemicals that will not be further used, squeeze their pipets or containers and allow the chemicals to flow down a sink drain; then flush the drain with lots of water. Throw the empty chemical containers in the garbage.

- If you wish to reseal any pipets for later use - though not applicable to this experiment - do so with a heated knife blade. Heat the blade of an old knife over a heat source until red hot. Then briefly press the pipet tip against the side of the blade while turning the pipet back and forth between your fingers. **NEVER USE A DIRECT FLAME TO SEAL A PIPET OF CHEMICALS!**

- Thoroughly clean all beakers, test tubes, and other equipment with the test tube cleaning brush and soapy water. **Use distilled water for a final rinse** and dry with paper towels. Properly pack and safely store your LabPaq and all equipment away from the reach of children and pets.

- **Wash your hands with soap and water** and similarly clean your work area.

List of Possible Unknowns:

Alka Seltzer®	Flour	Salt
Baby powder	Jell-O® powder	Sand
Baking soda	Plant fertilizer	Scouring powder
Cornstarch	Potting soil	Powdered Sugar
Cat litter	Powdered milk Powdered	Tooth powder
Coffee	Sugar	Unrefined sugar

EXPERIMENT 2: The Microbiome

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Part 1 takes 3 to 4 days to incubate. Allow 30-45 minutes for slides and drawings in each part.

Objectives: To observe and identify the organisms found in bean water
To observe and identify the organisms found in a pond or stream
To observe if each organism is motile or sessile
To identify each organism as a producer or a consumer
To hypothesize the relationship between producers and consumers

Materials:

Student Provides:
- Tap water
- Toothpick
- Coffee cup or glass with water
- Timer or clock with second hand
- Heat source: Candle, matches, or lighter
- Pond or stream water
- Glass jar, pint-size or larger

From the LabPaq:
- Beaker, 50-mL
- Eye Dropper from dissection kit
- Blank microscope slides
- Microscope slide covers
- Test tube clamp
- Crystal violet stain from Gram stain bag
- Staining tray
- Microscope
- Plastic funnel
- Disposable gloves
- Lab apron
- Coffee filters -2

Experiment Bag:
- Beans

Discussion and Review: When a biological community is described, it is often in terms of trees, plants, birds, insects, rodents, etc. These easily viewable and describable organisms are **macro organisms** and make up the **macrobiome**. But there are many more organisms in the community, most of which are observable only with a magnifying glass, and even more observable only with the magnification of a microscope. These are **microorganisms** and they make up the **microbiome**. These microorganisms usually escape notice, but with a microscope they become visible in their myriad complexity. There are far more species of microorganisms than of macro organisms,

far too many to identify or catalog in this laboratory exercise, but it is possible for you to identify some categories of microorganism.

Microorganisms are often divided along two broad dimensions: (1) motile versus sessile and (2) producers versus consumers. **Motile**, also called mobile, microorganisms are able to move, often with the aid of fine hair like flagella or other appendages, and are therefore able to move toward or away from light, heat or chemicals, according to their survival reactions or instincts. **Sessile** microorganisms have no means of locomotion and remain stationary, moving only with the medium in which they exist.

Producers are generally plants and are able to synthesize organic nutrients directly from inorganic chemicals and gases in their environment. Some producer organisms use sunlight via photosynthesis to produce sugars. Other microorganisms, such as those able to use chemicals as an energy source, live in places where there is no light as at the bottom of the ocean or in a cave, but still produce their own food. Still other microorganisms live in non-liquid environments. These include fungi in cheese, powdery mildew on plant leaves, and many others.

Producer microorganisms often contain colored pigments that are the chemicals (such as chlorophyll) that allow the production of nutrient sugars. The pigments can vary widely, ranging from yellow to green to blue-green. These pigments are most often seen in microorganisms that live where they can capture light, such as photosynthetic euglenoids (Euglena) and algae. **Consumers** lack those chemicals and are usually colorless. They obtain nourishment by ingesting and breaking down other organisms.

These sites contain excellent presentations of small and microscopic fresh water organisms: http://www.microscopy-uk.org.uk/fullmenu.html and
http://www.microscopy-uk.org.uk/pond/

PROCEDURES:

Note: Bean water incubation requires at least 3 days. Plan accordingly.

Part 1 - A Bean Water Microcommunity:

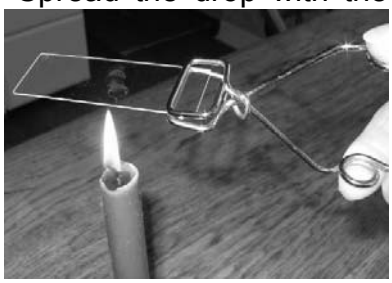
You will need: Table shown below to record observations, six beans, 50 mL beaker, tap water. After incubation, a clean slide, eye dropper from dissection kit, drop of bean water, toothpick, heat source, crystal violet stain, staining tray, glass of water, work area protected from staining and microscope.

1. Set up the following table to record observations for Part 1 and Part 2.

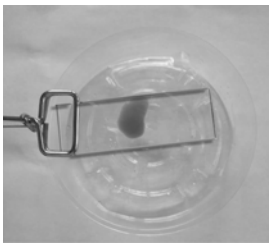
Organisms in Microcommunities				
Organism	**Bean water**	**Pond water**	**Motile or sessile (reason)**	**Producer or consumer (reason)**

2. Place six beans from the experiment bag in the 50-mL beaker and half-fill it with tap water. Allow the beaker to stand in a warm place for 3 to 4 days. Good locations include the kitchen near the stove and a utility room near a hot water heater.

3. Prepare and stain a slide of the bean water. To do this, after the beans have been in the water for several days, dip the clean eyedropper into the bean water and place a small drop of bean water on a blank microscope slide. Spread the drop with the toothpick until it is about 2 mm in diameter.



4. Light a candle or ignite a long kitchen match or lighter to dry the bean-water slide. Use the test tube clamp to hold the slide by one end in a horizontal position with the spot of bean water on top. Gently pass the slide through the flame several times. This process is called "fixing" the specimen and sticks the cells in the bean water to the slide. Do not heat the slide after the water has evaporated or pass it through the flame so slowly that soot can accumulate on the bottom of the slide. But if the latter happens, you can clean the bottom of the slide with a damp tissue.



5. Continue to hold the hot slide with the test tube clamp. Have a glass with clean water by the sink or basin where you're working. Also have a clock or watch with a second hand nearby. Hold the slide over the staining tray with the test tube clamp. Place a few drops of crystal violet stain on the dried bean residue on the slide. Be careful not to spill the crystal violet outside the staining tray as it is a very strong stain. Staining the slide will make the microorganisms from the bean water have more contrast and easier to see.

6. After one minute, rinse the excess stain off of the slide by dipping it into the glass of water. Gently shake excess water from the slide into a staining tray, and allow the slide to air-dry.

7. View the dry slide directly with your microscope; no slide cover is required. Draw what you see. Use the chart of microorganisms at the end of the experiment to identify what you draw. List the organisms you observe in the table you've made. Explain your reasoning for each identification.

1. Next, make a wet-mount slide of the bean water. Place a tiny drop of the bean water on a blank slide and carefully add a cover slip by touching it to the edge of the drop and lowering the cover slip slowly. Check carefully for organisms. If none seem visible, put a drop of crystal violet on the tip of a toothpick. Shake most of the crystal violet off the toothpick into the staining tray. Gently lift the cover slip and place the toothpick tip covered with crystal violet into the bean water on the slide. Such a small amount won't color the water but it will stain the organisms and help you see them more easily.

2. View the wet-mount slide with your microscope and draw what you see. Use the following chart of microorganisms to identify organisms. List the organisms you observe in the table. Explain your reasoning for each identification.

3. Determine if each organism is motile or sessile.

4. Determine if each organism is a producer or a consumer.

Part 2: A Pond or Stream Water Microcommunity

You will need: Plastic funnel, disposable gloves, lab apron, coffee filter, blank microscope slides, microscope slide covers, crystal violet stain, toothpick, pond or stream water, glass jar- pint-size or larger, safety goggles and microscope.

1. Wear your laboratory apron and disposable rubber gloves to collect a sample of water from a pond or slow moving stream in a pint-size or larger glass jar. The source does not need to be of drinkable quality. In fact, a still or even stagnant source may have a richer micro-community provided there are no contaminants in the water such as oil or chemical pollutants that might kill the microorganisms. You should wear your safety goggles when handling this pond water sample since you don't know what it contains.

2. If you cannot find any surface water, fill a quart or larger container or a bucket with distilled water or well water, not water that contains chlorine, add a handful of rich natural soil, some leaves or other natural materials you find in your yard, stir, let it sit for a week, and draw your sample from that water.

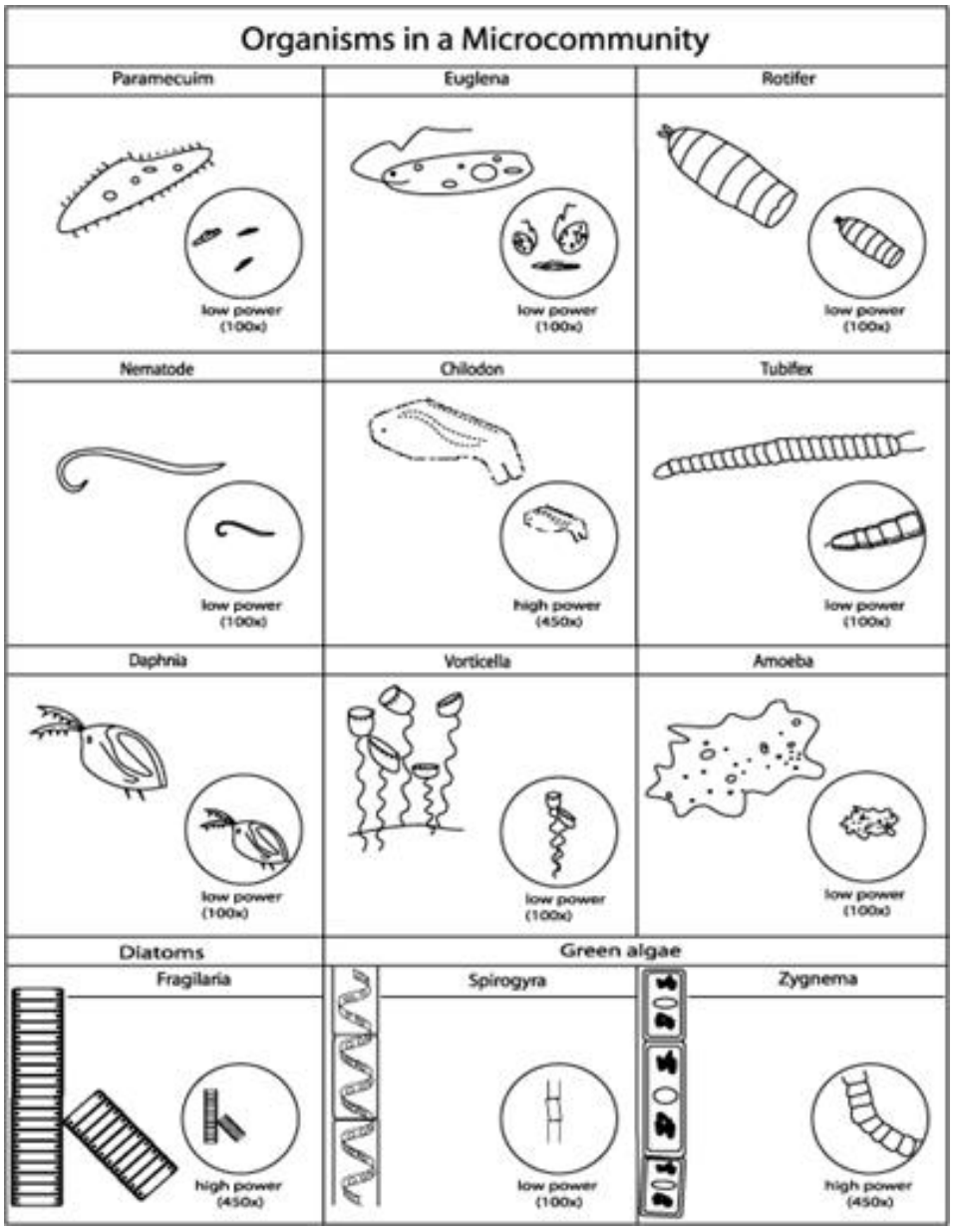
3. Place a coffee filter inside the mouth of the funnel. Place the funnel in a small clean glass that will suspend the funnel and keep it from falling over but is large enough to hold half a cup of liquid below the funnel mouth,

4. Fill the 50-mL graduated cylinder with the collected pond water and slowly pour the water sample through the filter. Repeat so that at least 100 mL of pond water passes through the filter.

5. Remove the coffee filter from the funnel after all the water sample has drained through it. Observe if there are any organisms large enough to see without magnification on the filter paper. Quickly draw them for later identification. Turn the coffee filter inside out and touch the moist tip area with the most collected residue from the pond water to a clean glass slide. If the slide is dry, add a drop of pond water. Carefully apply a cover slip.

6. Place the wet mount slide under the microscope. Draw what you see. Use the chart of microorganisms at the end of this experiment to identify organisms. List the organisms you observe in the table. Explain your reasoning for each identification. If organisms are difficult to see, refer to step #6 in Part 1 and use a tiny amount of crystal violet on a toothpick to stain the organisms so they'll show up more clearly.

7. Determine if each organism is motile or sessile, and if each organism is a producer or a consumer.



Questions:

A. What are possible sources of the microorganisms in the bean water?

B. Explain the color differences between the producers and the consumers.

C. Are most organisms in the bean water producers or consumers? Are most organisms in the pond water producers or consumers?

D. Are most producers motile or sessile? Are most consumers motile or sessile?

E. Explain the overall differences between the two environments.

EXPERIMENT 3: Mitosis

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Note: Allow 45 to 60 minutes for completion of this lab

Objectives: To understand and be able to recognize the phases of mitosis.
To understand the cell cycle necessary for the growth of plants and animals
To learn to understand the computations and uses for mitotic indexes

Materials: None Required

Discussion and Review: Two different types of cell division occur in **eukaryotic cells**. One process is called **mitosis**, the other **meiosis**. Cells formed by mitosis contain the same number of chromosomes as the original cell. Cells formed by meiosis contain only half the number of chromosomes as the original cell.

Therefore, mitosis is the process by which a cell divides and replicates its DNA into two identical halves each containing the same amount of nuclear material as the original cell. In most plants and animals, cells contain two sets of chromosomes (homologous pairs) and are said to be **diploid (2n).**

In the other form of cell division, called **meiosis,** the cells formed have only half the number of chromosomes as the parent cell. This is the process by which sperms and eggs are produced and involves two separate nuclear divisions. In this process, when only one set of chromosomes is present, the cell is said to be **haploid (n). Meiotic** cell division can occur only in diploid cells, but **mitotic** cell division may occur in both haploid and diploid cells. The table right compares and differentiates characteristics of mitosis and meiosis.

	Mitosis	Meiosis
Chromosome number of parent cells	2n	2n
Number of DNA replications	1	1
Number of divisions	1	2
Number of daughter cells produced	2	4
Chromosome number of daughter cells	2n	n
Purpose	Growth and repair	Gamete and spore production

Haploid and diploid are terms referring to the number of sets of chromosomes in a cell. Diploid organisms are those with two (di) sets of chromosomes. The cells of human beings (except for their gametes), most animals, and many plants are diploid which is abbreviated as 2n. Haploid organisms' cells have only one set of chromosomes,

abbreviated as n. Organisms with more than two sets of chromosomes are termed **polyploid**. Chromosomes that carry the same genes are termed **homologous chromosomes**. Organisms normally receive one set of homologous chromosomes from each parent

During mitotic cell division, cells replicate themselves while maintaining the same number of **homologous chromosome** pairs. In unscientific layperson terms, mitosis involves the duplication of a cell's chromosomes via the unzipping of the chromosomes of the original **parent cell** and the orderly re-zipping of the old chromosomes and newly replicated chromosomes into two exact copies of the original cell.

Mitosis is one part of the continuously reoccurring **cell cycle** responsible for growing, replacing, and renewing cells in plants and animals. The mitotic process of replicating, dividing, then re-pairing the chromosomes is immediately followed by **cytokinesis**. During cytokinesis the cell divides the **cytoplasm**, the watery substance that contains other cellular components important to the functioning of the cell, and forms a dividing membrane that completes the formation of the two new cells. In animals this process is accomplished through the formation of a **cleavage furrow**, followed by the pinching off of the new cells. In plants, the dividing membrane is called the **cell plate.**

The process of mitosis, though divided into stages is actually a continuous process happening at different stages in specific parts of the plant or animal. The nucleus of a cell contains all the genetic information or DNA the cell needs to reproduce. However, the process of mitosis is complex. The sequence of basic events is divided into the phases identified in the following table and illustrated with both very high power electron microscope photos and illustrative graphic.

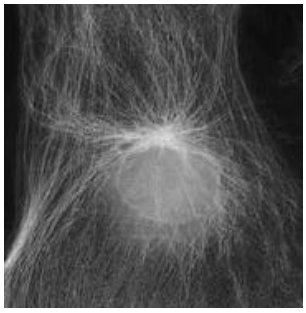
Cell populations grow larger when cells pass through interphase and mitosis to complete the cell cycle. As they mature, many cells lose the capacity to divide or divide only rarely while other cells are able to rapidly divide.

It is possible to quantify the rate of a dividing cell population and then examine and evaluate the differences in various cells' capability to divide. This quantification allows the dynamics of cell division to be evaluated in different and/or adjacent cell areas of a plant.

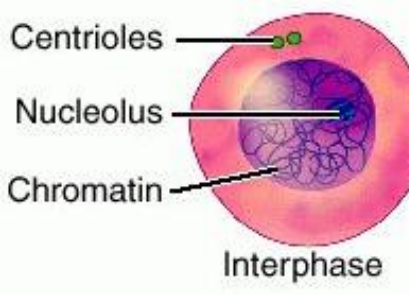
If a group of cells rarely completes the cell cycle, a high percentage of the cells can be assumed to be in the resting stage of the cell cycle. When a cell population is rapidly dividing, a high percentage of cells can be assumed to be in the stage of mitosis. Cell division is quantified by the **mitotic index** which is a simple whole number percentage derived by dividing the number of cells in mitosis to the total number of cells:

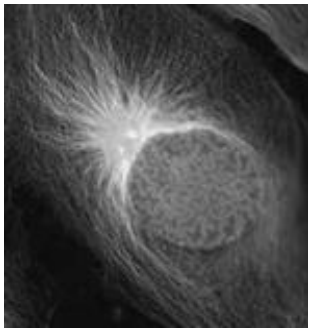
$$\frac{\text{number of cells in mitosis}}{\text{total number of cells}}$$

The Phases Associated with Mitosis

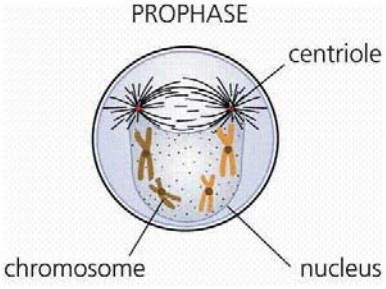


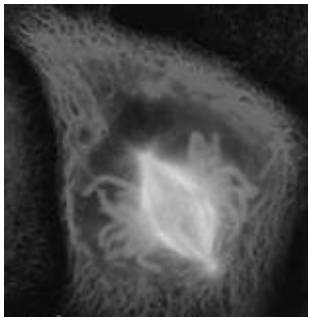
Interphase: Before the process of actual cell division begins, the cell first goes through this preliminary phase during which it grows, duplicates chromosomes, grows again, and finally begins to divide. This is a metabolically active non-dividing stage of the cell cycle.



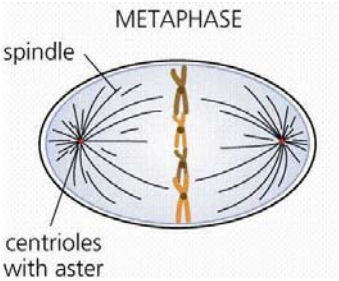


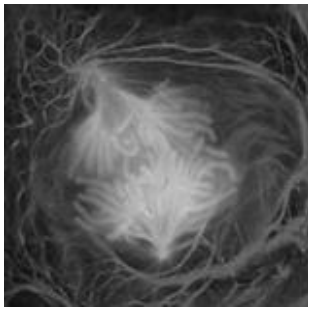
Prophase: This is the first stage of actual mitosis during which the chromosomes condense and become visible, the nuclear membrane breaks down, spindles begin forming at opposite poles of the cell, and the chromatid pairs of two sister chromatids become short and thick.



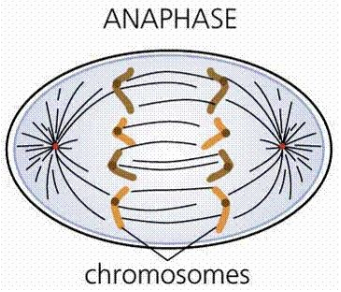


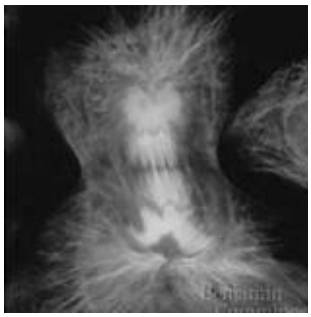
Metaphase: At this stage in mitosis (and meiosis) spindle fibers attach centrioles to the chromatid pairs and the chromosomes become aligned along the equatorial plane of the cell called the metaphase plate.



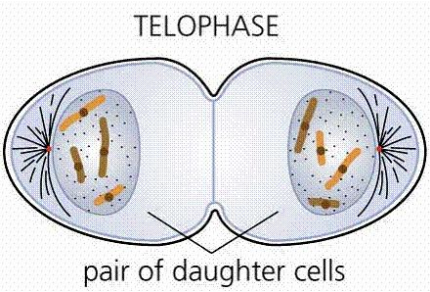


Anaphase: During this stage in mitosis and meiosis chromosomes begin moving to opposite ends (poles) of the cell where sister chromatids separate and daughter chromosomes migrate to opposite poles of the cell.





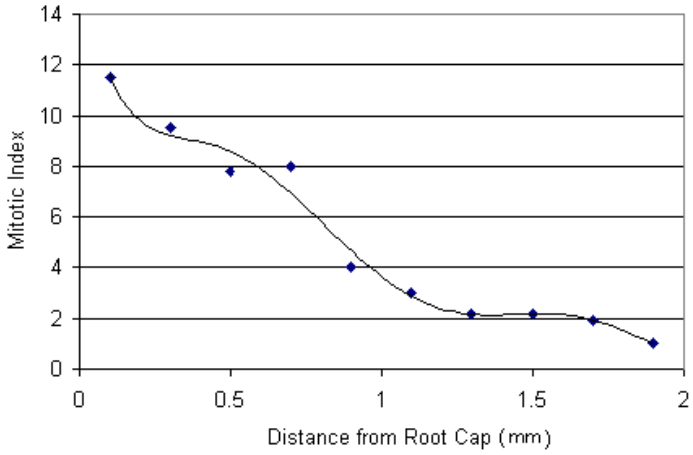
Telophase: In the final stage of mitosis the two sets of separated chromosomes de-condense and become enclosed by nuclear envelopes as the chromosomes of daughter cells are grouped in new nuclei.



Microscope slides by the University of Miami
Graphic illustrations by Academy Artworks

The most rapid cell division is usually observed near the tip of a plant root, in the **apical meristem.** This fact can be confirmed by mitotic index calculations. A **root cap** is a thimble-shaped mass of cells that cover and protect the growing tip of a root. As a root cap detects the pull of gravity, it directs the rapid growth of cells near the plant tip to push the root through the soil.

The graph at right was derived from mitotic index measurements taken from cell areas at various distances from the root cap. It reflects a general fact that the mitotic index decreases as the distance from the root cap increases and that mitotic activity increases toward root tips.



Mitotic index comparisons are often used to evaluate the impact of environmental factors on plants. For example plants grown in the microgravity of space have a higher mitotic index than control plants grown on the ground. Mitotic index measurements are also very valuable in medical research and are a key factor in evaluating the growth of various cancers

PROCEDURES:

1. Carefully study the previous table of photos, illustrations, and descriptions of the phases associated with mitosis. Also, perform Internet research to observe other examples of mitosis and reinforce your understanding of the mitotic processes. There are numerous exceptional mitosis links including several with videos of actual plant mitosis in process. One excellent site may be found at:
   http://www.phschool.com/science/biology_place/labbench/lab3/mitfilm.html

2. When you feel comfortable about your knowledge of mitosis, closely examine the six photos at the end of this lab which depict cells from the apical meristem of an onion root in various stages of mitosis. Answer the questions asked about each photo.

3. Pretend the six slides of mitosis when combined represent one area of a plant root. Considering only complete cells from the six photos, not those cut by photo edges, categorize and count them to compute the mitotic index of the total root area.

Questions:

A. What were your responses to the questions related to the six mitosis slides?

B. What is the purpose of mitosis?

C. Why is the cell cycle important for human beings

D. What role does mitosis play in the growth of an embryo?

E. How many chromosomes are there in a cell produced by mitosis?

F. What is one main difference in the mitotic processes between plant and animal cells?

G. If the cell that has undergone mitosis has the diploid (2n) number of chromosomes, then what number of chromosomes will the nuclei in the two new cells have?

H. Can mitosis ever be used for reproduction?

I. What parts of plants are most impacted by mitosis? Why would mitosis be easy to study in the tip of the root? Where else in a plant might you find a large amount of mitotic cell division occurring?

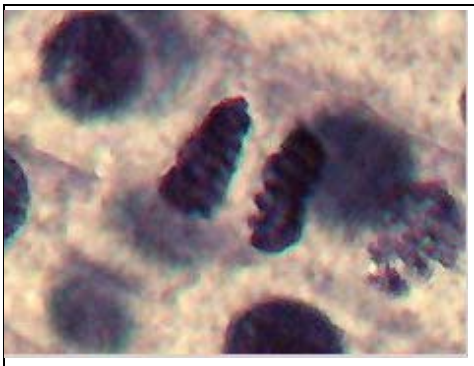
J. Why does zero-gravity increase mitosis?

K. How might the mitotic index be applied in agriculture?

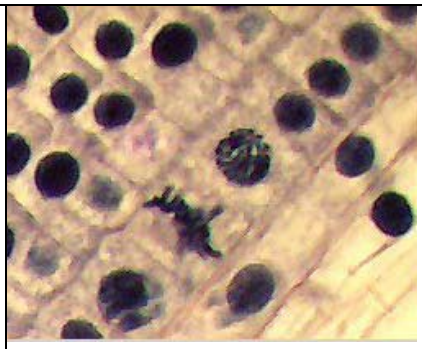
L. What did you compute to be the mitotic index of the combined six slides? In comparison to the indexes reflected in the earlier graph, from approximately which area of a root was the sample taken?

Mitosis Worksheet

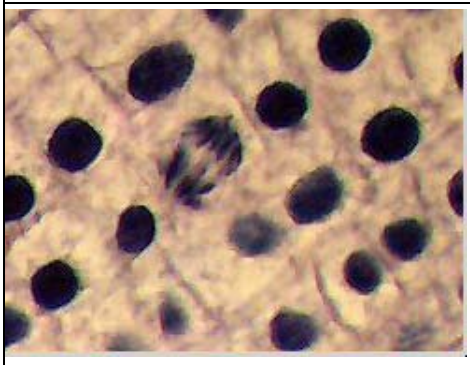
Onion root tip photos at 600X and 1000X taken by Stan Carpenter of Hands-On Labs, Inc.



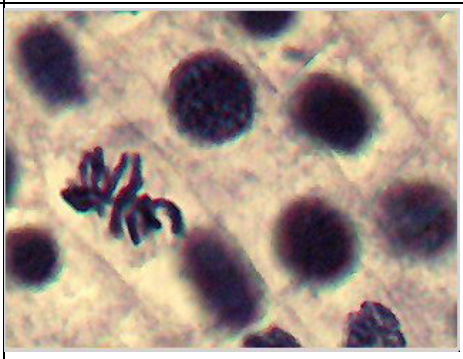
1. What phase is this cell displaying? What's about to happen?



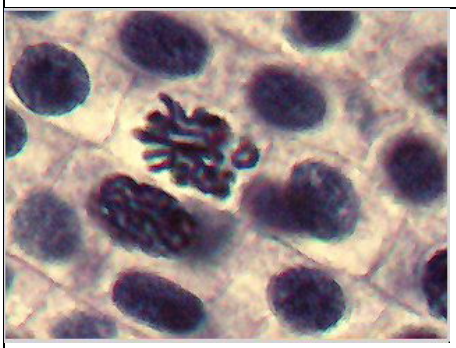
2. The two center side-by-side cells are displaying two different phases of mitosis. What are they? What diagnostic features can help you with your decision?



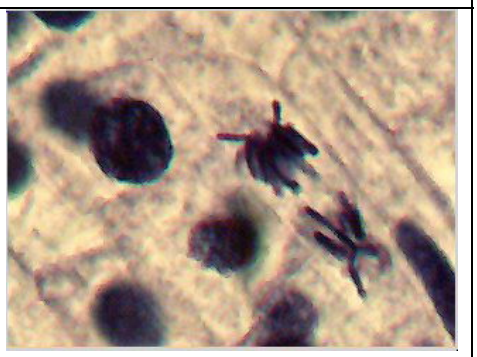
3. What phase is this cell displaying? How can you tell?



4. This cell has clearly visible chromatids. What's the next phase this cell will undergo? Where will the chromatids go in the coming phase?



5. The cell in the lower center is exhibiting signs of what phase of mitosis? What phase does it appear the cell above it is undergoing?



6. Explain what happens during anaphase. When the chromatids separate, what is each called?

EXPERIMENT 4: Macromolecules of Life

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Each section, except Part 5, takes 30 - 45 minutes. Plan accordingly

Objective: To introduce the basic molecules of life and to test for their presence.

Materials:

Student Provides:
- Egg, fresh raw
- Coffee cups, 2
- Distilled water
- Onion, small
- Whisk or fork
- Mixing bowl or blender jar
- Sugar
- Sauce pan or deep skillet
- Clock or timer
- Potato, small white
- Vegetable oil
- White Vinegar

From the LabPaq:
- Safety goggles
- Test tube rack
- Test tubes, 4
- Test tube cleaning brush
- Wax Marker pencil
- Microscope
- Microscope slides, 4
- Microscope slide covers, 4
- Mortar and pestle
- Pepsin powder in dropper bottle
- Biuret reagent in pipet
- Glucose powder in dropper bottle
- Benedict's reagent in dropper bottle
- Potato starch solution in dropper bottle
- IKI Solution in glass vial
- Long stem pipet, empty, labeled *IKI Solution*
- Sudan III solution
- Scalpel from dissection kit

Discussion and Review: There are four types of molecules that are essential to the biology of life. In this lab you will become familiar with these molecules and with the bonding that occurs among them. We talk about molecules as commonplace items in biology and chemistry although we can not see or easily study them. However, by

performing chemical tests, we can learn about the composition and attributes of molecules even though they are not easily visible.

Foods such as grains, milk, and meat are not single substances but complex mixtures of chemical compounds found in nature. Processed foods and other food items purchased at stores are also often a complex mixture of natural compounds plus food coloring, preservatives, artificial flavoring, and even chemical compounds from residual pesticides.

Four generalized types of molecules are the basis for most of the more complex molecules of life. These generalized molecules are: **amino acids, carbohydrates, lipids,** and **nitrogenous bases**. This laboratory exercise is designed as an introduction to these molecules. After doing the simple chemical tests and answering the questions, you will be familiar with these four molecules and some of their characteristics. You will also learn how molecules are the building blocks for some of the chemicals of life and will be able to conduct simple chemical tests to detect them.

Bonding is the way molecules are held together and is very important in studying the biology of living organisms. Most bonding occurs between the electrons of different atoms of the molecules, but sometimes there is bonding between molecules, such as in hydrogen bonding. Protein structure, membrane characteristics, acidity, solubility, and even chemical secretions of various organs are influenced by chemical bonds of molecules.

This experiment has five parts. The parts may be done on separate days, but you should fully complete one part before beginning the next. Throughout this exercise you will observe reactions between organic substances and test reagents. You will also use controls by testing reagents in distilled water to compare the reaction with the test of organic substances. Record all observations and test results.

PROCEDURES:

Part 1 - Testing for Amino Acids:

You will need: Raw egg white, graduated cylinder, distilled water, pepsin solution, pinch of sugar, Biuret reagent, test tubes, test tube stand, wax marker, ruler. Note: Making the pepsin solution and egg white solution before you begin speeds the process.

Amino acids are the building blocks of proteins. Proteins are polymers formed from sub units (**monomers**) that are in turn formed from one or more of 20 amino acids. Proteins are found in many locations, including cell membranes, chromosomes, and in the cytoplasm of cells. Proteins have a critical role in cell-to-cell recognition, determining blood type, and controlling the transport of materials in and out of a cell. Antibodies, enzymes, blood, and hormones are made of proteins, and muscle tissue, hair, nails, and skin are high in protein. Proteins vary widely in structure and shape.

In this exercise you will identify the presence of amino acids by using Biuret reagent as an indicator for the presence of protein. Biuret reagent is composed of sodium hydroxide and copper sulfate; it is pale blue in the absence of protein, but it changes to violet in the presence of proteins. You will add Biuret reagent to test tubes containing egg white, pepsin, sugar, and distilled water. Before beginning the experiment, state your hypothesis as to which tubes will change color and how strong the color change, indicating the amount of protein present, will be for each substance.

1. With the wax marker pencil, label four test tubes #1, #2, #3, and #4. Place a mark on each test tube 2 cm from the bottom.

2. Separate a raw egg by cracking it over a small clean cup and allowing only the egg white (albumen) to flow into the cup. Save the yellow egg yoke in a second cup for later use.

3. Add 30 mL of distilled water to the albumin/ egg white. . With a whisk or fork, stir the mixture until the egg white is completely liquefied.

4. Prepare a 1% pepsin solution: With clean fingers, wobble, loosen, and remove the dropper tip to the bottle that contains 0.1 g of pepsin powder. Add 10 mL of distilled water to the pepsin powder. Replace the dropper tip and lid. Shake well and allow the pepsin to completely dissolve before proceeding. At the end of this experiment, keep the remaining pepsin solution as it will be used in a future experiment.

5. Fill test tube #1 to the 2-cm mark with the albumen (egg white) solution.

6. Fill test tube #2 to the 2-cm mark with the pepsin solution.

7. Add a pinch of household sugar to test tube #3, fill it to the 2-cm mark with distilled water, and swirl to dissolve.

8. Fill test tube #4 to the 2-cm mark with distilled water.

9. Cut off the end tip of the thin stem from the pipet of Biuret reagent; the cut should be perfectly straight, not at a slant. Leave as long a stem as possible. Biuret reagent contains a strong alkali, so use caution. If you accidentally spill any on your skin, immediately rinse it off with lots of water. When not using the Biuret reagent pipet, place it upright in the test tube rack.

10. Add Biuret reagent to test tube #1, drop by drop - counting the drops, just until a color change occurs. Do not use more drops than necessary for an initial color change or the excess Biuret reagent will obliterate your results. Record your observations and the quantity of drops used.

11. Add the exact same number of drops of Biuret reagent previously used to each of the other test tubes: #2, #3, and #4. Record your observations of test tube colors and color changes.

12. Pour the test tube contents down the sink and flush with lots of water. Rinse the test tubes with distilled water and dry them for a later experiment.

Questions:

A. What is the purpose of this experiment, and for what substance are you testing?

B. Which of the test tubes is the control?

C. Which test tube did you find to contain the most of the test substance?

D. Beside the control, which test tube contained the least of the test substance?

E. What can you conclude from your observations?

F. How might you use the Biuret reagent test in a practical real-life situation?

G. What do you feel you have learned in doing this experiment?

Part 2 - Testing for Sugars:

You will need: Hot water bath about 2 inches deep, prepared glucose solution, prepared potato starch solution, onion juice, Benedict's reagent, distilled water, test tubes, test tube stand, wax marker, ruler

Carbohydrates are monomers derived from sugars. Common carbohydrates include:

- Glucose, one of many ***monosaccharides*** and a major energy source for living organisms;
- Sucrose, common table sugar, one of many ***disaccharides*** consisting of two linked monosaccharides;
- Starch, a plant nutrient storage molecule that is a ***polysaccharide*** consisting of many monosaccharides bonded together;
- Cellulose, a structural molecule found in the cell walls of plants that is also a **polysaccharide** consisting of many monosaccharides bonded together; and
- **Glycogen**, a storage molecule of animals that is also a polysaccharide consisting of many monosaccharides bonded together. Glycogen in animals is similar to starch and cellulose in plants.

The test for sugar uses a blue solution, Benedict's reagent, containing copper sulfate.

When Benedict's reagent is added to a solution containing a reducing sugar such as glucose, and the resulting solution is heated, the solution changes color from blue to green to brick red. If no sugar is present, the solution will remain blue. Benedict's reagent changes color only in the presence of reducing sugars. These are sugars with active **aldehyde** groups, COH, which are reduced by copper ions in the reagent.

Not all sugars are reducing sugars, so the use of the Benedict's reagent is not a universal test for sugar. It will not detect sucrose. However, it does work with all monosaccharides and with some disaccharides like maltose.

When heat is applied to a sample solution with the Benedict's reagent present, the amount of reducing sugar present determines the final color of the solution according to the following scale:

Low ----------amounts of reducing sugars present----------High

green	orange	red	brown

In this exercise you will compare the relative amounts of reducing sugars present in several samples including glucose solution, potato starch solution, pulverized onion, and distilled water. State your hypothesis as to how much color change will occur, i.e. how much reducing sugar is present in each of the test solutions when the Benedict's reagent is added.

1. To make a hot-water bath, fill a small saucepan or skillet with about 2 inches of tap water, place it on your home lab's heat source (kitchen stovetop or a hot plate), and bring it to a boil. Turn the heat down to its lowest setting so that the water merely simmers instead of boils rapidly. Only a few small air bubbles should be coming to the top of the water. If necessary you can add a little cool water to quickly reduce the temperature from boiling to just below boiling.

2. While the water is heating, use the wax marker pencil to label four test tubes #1, #2, #3, and #4. Then mark each test tube at ½ cm and at 2 cm from the bottom.

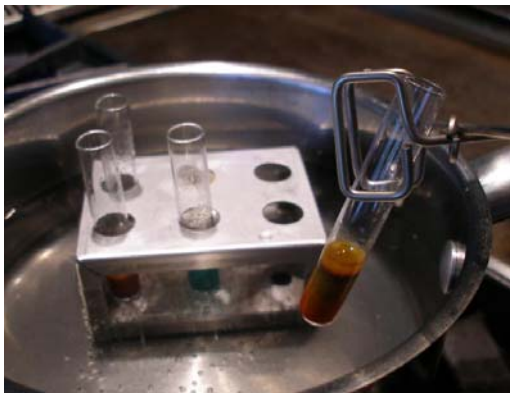
3. Remove the dropper tip of the glucose powder bottle and prepare a 1%-glucose solution by following the instructions on the dropper bottle to add a specific quantity of distilled water and shake well. At the end of this experiment, keep the remaining glucose solution in the refrigerator for use in a future experiment.

4. Fill test tube #1 to the lower ½-cm mark with glucose solution and add Benedict's reagent to bring the level in the tube to the second 2-cm mark. Place the test tube in the test tube rack.

5. Fill test tube #2 to the lower ½-cm mark with potato starch solution and add

Benedict's reagent to bring the level in the tube to the second 2-cm mark. Place the test tube in the test tube rack.

6. Grind a quarter section of a small onion with a mortar and pestle until you can pour ¼ to ½ cm of clear onion juices into test tube #3. If your onion juice yield is below the ½-cm mark, use a clean dropper to add distilled water, drop by drop, to reach the ½-cm mark. Add Benedict's reagent to bring the level to the 2-cm mark. Place the test tube in the test tube rack.



7. Fill test tube #4 to the lower ½-cm mark with distilled water. Add Benedict's reagent to bring the level in the tube to the 2-cm mark. Place the test tube in the test tube rack.

8. Place the test tube rack with the four test tubes in the hot water bath. After five minutes of heating in simmering water, turn off the heat under the water bath and use the test tube holder to carefully lift and examine each test tube's contents. Remember, the contents are hot! Replace the test tube into the rack and record your observations.

9. Allow the water and test tube contents to cool before removing them from the water bath. Pour the test tube contents down the sink and flush with lots of water. Rinse the test tubes well with tap and then distilled water, then dry them well for the next experiment.

Questions:

A. What is the purpose of this experiment, and for what substance are you testing?

B. Which of the test tubes is the control?

C. Which test tube did you find to contain the most of the test substance?

D. Beside the control, which test tube contained the least of the test substance?

E. If the color changes are not as you expected what are possible explanations?

F. What can you conclude from your observations?

G. How might you use the Benedict's reagent test in a practical real-life situation?

H. What do you feel you have learned in doing this experiment?

Part 3 - Testing for Starch:

You will need: IKI solution, small white potato, small onion, clean slides, slide covers, scalpel. Note: scalpel blades may be in a pocket of the dissecting kit behind the instruments. Open the blade packet carefully and, holding the blade away from you, insert the nib of the blade into the slot in the scalpel and push up firmly until the blade snaps in place. BE CAREFUL. Scalpel blades are very sharp!

The carbohydrate group of organic molecules contains both starch and sugars. Animals eat the starch produced by plants and digest it with the enzymes in their systems to produce sugars that then produce energy.

The test for starch uses the reagent called Lugol's solution or IKI, which stands for Iodine Potassium Iodide; K is the chemical symbol for potassium. IKI is yellow-brown to dark red-brown in color, depending on its concentration. When IKI is placed in a solution containing starch, the solution changes color from yellow-reddish brown to blue-black. If no starch is present the solution remains yellow-reddish brown.

1. Cut open a white potato and use the scalpel to slice as thin a layer of cells as you can from the inside of the potato. Trim the slice to about 1-cm square and place it on a microscope slide. Add a drop of distilled water and then cover it with a microscope slide cover slip.

2. Place the slide under the microscope and observe. Draw what you see. Try to identify a potato cell, the cell wall and the grains of starch within the cell. Label these parts in your drawing. If you can't identify cells, proceeding to the next step will help.

3. Leave the cover slip in place on the potato slide. Use the dropper pipet labeled IKI solution to add two drops of IKI stain at one edge of the cover slip. Then hold a small piece of paper towel against the opposite edge of the cover slip so capillary action will move the IKI stain under the cover and into the potato slice.

4. Place the slide under the microscope. Observe the cells closest to the edge under the cover slip where you originally added the IKI stain. Count the number of stained oval bodies in a few cells. Observe the color that the IKI has turned the potato cells. Record your observations.

5. Repeat steps 1 through 4 above using a very thin slice of onion in place of potato.

Questions:

A. What is purpose of this experiment and for what substance are you testing?

B. Which vegetable has the higher content of the test substance?

C. How many oval bodies are there in potato cells as compared to onion cells?

D. What do you conclude that the oval bodies are made of?

E. What can you conclude from your observations?

F. If the color changes are not as you expected, what are possible explanations?

G. How might you use the IKI reagent test in a practical real-life situation?

H. What do you feel you have learned in doing this experiment?

Part 4 - Visual Demonstration of Lipids:

1. Place a small drop of vegetable oil on a clean microscope slide.

2. Place a drop of Sudan III solution on top of the oil. Observe and record what happens.

3. Carefully place a slide cover slip over the specimen by touching the cover slip to one edge of the Sudan III drop and then lowering it down onto the slide.

4. Place the slide under the microscope and observe. Draw what you see and record your observations. Be careful not to mistake tiny air bubbles that may be trapped under the cover slip for cells or other structures.

5. Use the scalpel to again slice a thin layer of cells from the inside of a white potato and trim it to fit under a slide cover slip. Place the slice on a microscope slide, add a drop of Sudan Red III, blot to remove most of the stain and add a cover slip.

6. Place the slide under the microscope and observe. Draw a potato cell. Label the cell wall and any details of cell content that you see.

7. Repeat the above steps using a very thin slice of onion instead of potato.

Questions:

A. What is the purpose of this experiment, and for what substance are you testing?

B. What are the stained areas made of?

C. Which slide has a higher content of the test substance?

D. What can you conclude from your observations?

E. What do you feel you have learned in doing this experiment?

Part 5 - Chemical Bonds:

In this experiment you will observe chemical bonding by making mayonnaise – and yes, you will be able to eat it when you are finished. The vinegar is a compound with polar bonds, whereas the oil has non-polar bonds. Normally the two will not mix. However, the lecithin in the egg yolk acts like a detergent and allows the two to mix together.

1. Into a mixing bowl or blender jar, place the egg yoke you saved in Part 1 above. Add 25 mL of white vinegar and mix.

2. V-e-r-y slowly add vegetable oil to either the bowl as you whip the mixture vigorously with a whisk or through the top of the blender jar with the blender running on high speed. Stop mixing when you reach the right consistency for mayonnaise. It normally takes about one cup of vegetable oil to make this recipe. Add a pinch or two of salt to taste and other seasonings, such as garlic, if desired. Store the mayonnaise in your refrigerator and enjoy a practical results of chemical bonding!

Questions:

A. If you were given a piece of hot dog and a piece of carrot, using what you learned in this exercise, how would you analyze the composition of these materials?

B. What classes of compounds are present in living organisms that are not tested for in this exercise? Can you suggest how you would show how much of a specific compound is present in a given sample?

EXPERIMENT 5: Cell Membrane Transport

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow 1 1/2 hours for Part 1, 45 minutes for Part 2, and 90 minutes for Part 3.

Objective: To understand the transport of molecules across cell membranes.

Materials:

Student Provides:
- Glass or glass jar, pint size
- Distilled water
- Pan or deep skillet for water bath
- Rubber band
- Crushed ice
- Table salt
- Fresh potato
- Coffee cups or small glasses, 3
- Timer or clock
- Paper towels

From the LabPaq:
- Safety goggles
- Disposable gloves
- Test tubes, 4
- Test tube rack
- Test tube cleaning brush
- Thermometer
- Graduated cylinder
- Graduated pipet
- Marker pencil
- IKI solution
- IKI dropping pipet
- Benedict's reagent
- Spring Scale, 10 gm
- Glass stirring rod
- Ruler and bulb dropper from dissection kit
- Glucose solution
- Starch solution
- Potassium permanganate crystals
- Dialysis tubing

Discussion and Review: The movement of materials in and out of the cells of living organisms is vitally important. Many different terms are used to describe this specialized movement of materials in biological systems: **osmosis, diffusion, active transport, facilitated diffusion, endocytosis**, etc.

Materials must be able to move in and out of cells in order to fuel the cell functions and to remove wastes if cells are to survive. A plasma membrane surrounding the cell protects it from the outside environment, but that membrane must also selectively allow materials the cell needs to pass into its interior and other materials, such as waste products, to pass out of the cell. The property of the cell membrane that allows some materials to pass through while blocking the passage of others is called **selective permeability**.

The cell membrane is sometimes characterized as a bilayer of **phospholipids** embedded with proteins and carbohydrates and best characterized by the **fluid mosaic model.** This means that the cell membrane regulates what enters and leaves cells.

Molecules move from an area of high molecular concentration into an area of low molecular concentration in a process called **diffusion**. The speed of this process is a function of temperature since molecules move or vibrate faster when they are warmer. Diffusion is a **passive process** as far as the cell membrane is concerned, since it generally does not require any energy from the cell. Examples include the diffusion of oxygen and carbon dioxide and the osmosis of water. **Active transport**, however, does require cellular energy, usually in the form of **adenosine triphosphate (ATP)** which is essential cellular processes.

During this experiment we will observe passive transport in cells and across artificial membranes (model cells). Unfortunately, active transport is not readily observable and requires sophisticated equipment and methods to demonstrate.

PROCEDURES:

Part 1 - Diffusion through an Artificial Membrane

You will need: Safety goggles, disposable gloves, dialysis tubing, distilled water, small pan to make a hot water bath, graduated cylinder, clean glass or jar, IKI solution, IKI pipet, glass stirring rod, string or rubber band, glucose solution, starch solution, wax pencil, Benedict's reagent, thermometer, test tube, test tube rack, test tube holder.

The plasma membrane surrounding a cell is described as **differentially permeable** because it discriminates between the molecules on either side of the membrane and only allows molecules with certain characteristics to pass through the membrane. In this exercise you will demonstrate differential permeability in a model cell. Dialysis tubing, a synthetic material that is differentially permeable, will represent the cell membrane.

Remember from previous experiments that IKI solution reacts in the presence of starch to turn from yellow-brown to blue-black, and that Benedict's reagent reacts in the presence of reducing sugars to turn from blue-green to orange, red, and eventually brown.

1. Soak the piece of dialysis tubing in distilled water until it becomes soft and pliable.

2. Make a hot-water bath by filling a small saucepan or skillet with about 2 inches of tap water and placing it on your home lab's heat source (kitchen stovetop or a hot plate). Allow the water to heat to boiling while you prepare the rest of the lab. Once the water has boiled, turn the heat down to its lowest setting so the water merely simmers instead of boils. There should be only a few small air bubbles coming to the top of the water. If necessary you can add a little cool water to quickly reduce the temperature from boiling to simmering. The bath will be used later in the experiment.

3. Use the graduated cylinder to measure and pour 150 mL of distilled water into a clean glass or glass jar.

4. Use the IKI dropping pipet to slowly add, drop by drop, IKI solution to the water in the jar until the water is the color of strong tea. Use the glass stirring rod to mix the solution while you are adding drops. Record the color intensity, dark or light, of the jar's contents when finished.

5. Securely seal one end of the dialysis tubing by tying a knot in it or by turning up and binding the end with a rubber band or string. Test the seal by adding a small amount of distilled water to the dialysis bag. If it leaks, tighten the knot, rubber band, or string and test again. Empty and discard the distilled water before proceeding.

6. Use a clean graduated cylinder to measure out 4 mL of glucose solution and pour it into the membrane bag. Note: It will be easier to add the solutions if you stand the tubing in a clean, empty glass with the top of the tubing folded over the glass's edge until you pour in the solutions. Remember that glucose is a reducing sugar. Rinse the graduated cylinder well, then use it to measure out 4 mL of starch solution and pour it too into the membrane bag with the glucose solution.

7. While holding the mouth of the dialysis bag closed, knead the outside of the bag to mix the contents together. Rinse the outside of the bag with tap water, making sure water does not enter the mouth of the bag. Record the color of the bag's contents.

8. Place the dialysis bag containing the sugar and starch mixture into the jar containing the tea colored IKI solution. Fold the open end of the tubing over the side of the jar and secure it to the jar by wrapping a rubber band around the mouth of the jar. The dialysis tubing should be well closed so that the contents cannot spill into the jar.

9. Record the color of the water in the jar and of the contents of the membrane bag.

10. Allow the dialysis tubing to stay in the jar until you see a definite color change. This could take 30 to 45 minutes. Record again the color of the water in the jar and the contents of the membrane bag.

11. Remove the dialysis tubing from the water in the jar. Save the water in the jar, but wash the contents of the dialysis tubing down the sink and throw the used dialysis tubing in the trash.

12. Use the wax marker pencil to mark a test tube at 1 cm and 3 cm from the bottom.

13. Fill the test tube to the lower (1-cm) mark with water from the glass jar. Fill to the second (3-cm) mark with Benedict's reagent.

14. Use the thermometer to confirm that the water in the bath is just below or at boiling (100º C, or less at higher altitudes).

16. Place the test tube rack in the hot water bath and place the test tube of water and Benedict's reagent in the rack. Take care that the hot water does not flow into the test tube. Maintain the temperature of the bath and leave the test tube in the hot water bath for 10 minutes.

17. Use the test tube clamp to remove the test tube from the rack. Observe and record any color change in the water in the test tube and record your observations.

18. Carefully pour the hot solution from the test tube down the drain and wash it away with plenty of water. Wash, rinse and dry the test tube for future use.

Questions:

A. What is the purpose of this experiment? For what are you testing?

B. What color change did you observe in the membrane bag and what does that change indicate?

C. What color change did you observe in the water from the jar before heating, and what does that change indicate?

D. What color change did you observe in the water from the jar after heating, and what does that change indicate?

E. What does the Benedict's reagent detect?

F. What does the IKI solution detect?

G. What substance in the dialysis tubing or in the jar water DID NOT diffuse across the dialysis bag membrane and why?

H. In what way is a cell membrane similar to the model cell made of dialysis tubing?

I. Is the transport mechanism in the model cell passive or active, and why?

Part 2 - Diffusion at Different Temperatures:
This brief experiment will demonstrate the effect of temperature on the rate of diffusion. Be sure the water in each test tube is at the required temperature and is completely still before adding the potassium permanganate crystals

Caution: The potassium permanganate crystals used in this experiment make a very powerful dye that can create permanent stains. Do not spill!

1. Select three cups or small glasses that are narrow enough to support a test tube so it leans only slightly and will not fall over. When you place solutions and then test tubes into these containers, the tube will tend to float which is normal. However, it is important that no water from the cups or glasses enters the tubes. Thus, before proceeding, ensure the glasses or cups used are narrow enough to hold the test tubes upright. Fill a practice container and test tube with tap water and place the tube into the baths BEFORE adding any crystals. If the test tube falls over or water enters it, devise a way to make the test tube stands upright before proceeding.

2. Fill one cup or glass to a level of about 5 cm high with crushed ice with a bit of water. Respectively fill the second and third cups or glasses with room temperature tap water and with very hot tap water. Measure the temperature of each of the three water bath solutions and record. Temperatures of the baths should be near 0°C, 25°C and 40° C.

3. Put three dry test tubes into the test tube rack. Use tweezers to transfer a few grains of potassium permanganate from the vial into each of the three test tubes. Place equal quantities of crystals into the test tubes.

4. For all three test tubes, but working with one at a time:

    a. Hold the test tube of crystals at a slight angle and use the graduated pipet to measure and very slowly introduce 7mL of distilled water down the test tube's side. There will be some diffusion when the water contacts the crystals, but you will be measuring diffusion from the mass of dark crystals in the bottom, so DO NOT STIR or agitate the test tube contents.

    b. Very gently place the test tube in one of the water solutions. Make certain its base is fully in the solution and that no water can enter the test tube. Do not agitate or move the test tubes once they are in the water.

5. After five minutes, gently lift each test tube from its cup or glass and measure the level of any color rise up into the distilled water from the mass of potassium

permanganate crystals in the bottom. Gently return each test tube to its cup or glass. Record the measurement for each test tube.

6. Repeat measuring and recording the rise of color from the bottoms of the test tubes at five minute intervals for four more measurements. You will take five measurements over a 25 minute period of diffusion.

7. After taking five color rise measurements, pour the potassium permanganate solutions down the sink and flush with lots of water.

8. Prepare a line graph for the rise of color in each test tube; plot the color rise for each test tube on the vertical axis and the time on the horizontal axis. Label the lines "I" for ice bath, "R" for room temperature bath, and "H" for the hot water bath.

Questions:

A. How does temperature affect the rate of diffusion?

B. State a general hypothesis to cover all three elements of the experiment.

Part 3 - Tonicity and Diffusion: **Note: Requires one hour of observation time.**

You will need: 10% sodium solution you prepare from distilled water and table salt, graduated cylinder, distilled water, fresh potato, scalpel, test tubes, and test tube rack.

A substance dissolved in a solution is called a **solute**. The concentration of a solute outside of a cell as compared to the concentration of the solute inside the cell is referred to as the **tonicity**. **Osmosis** is the process by which water moves across a differentially permeable membrane. **Diffusion** occurs through osmosis. When the concentration of a solute in water is equal on both the inside and outside of a cell membrane, the cell is said to be in an **isotonic** solution or environment.

If the concentration of the solution, osmotic pressure, on the outside of the cell is less than the concentration on the inside, the cell environment is **hypotonic**. Where the opposite is true and there is a greater concentration of solute outside the cell than inside, the cell is in a **hypertonic** environment. The following exercise will demonstrate these differences.

1. Prepare a 10%-sodium chloride (NaCl) solution by adding 1 gram of table salt to a graduated cylinder containing 10 mL of distilled water. Mix well to dissolve the salt grains completely.

2. Mark two test tubes #1 and #2 with the marker pencil.

3. Fill test tube #1 with distilled water.

4. Fill test tube #2 with the 10% sodium chloride (NaCl) solution made in Step 1.

5. Cut two strips as thin as possible from the interior of a fresh white potato. The strips should be approximately 1 cm wide and 7 cm long.

6. Place one potato strip in each test tube; immerse them completely it in the liquids.

7. Place both test tubes in a rack and allow them to sit for approximately one hour.

8. Remove the potato strips from the test tubes and place them on a piece of paper towel. Keep track of which strip came from which test tube. Observe their respective conditions and record your observations.

Questions:

A. What is the condition of each potato strip after sitting in the test tubes for an hour? Which one is limp and which one is crisp?

B. How would you explain the difference using concepts of tonicity?

C. What was the tonicity of the fresh water solution with respect to the potato cells?

D. What was the tonicity of the salt water solution with respect to the potato cells?

E. How do the changes in the condition of the potato strips relate to the wilting of vegetables?

F. How does putting vegetables in the refrigerator slow down the wilting process? Note: wilting is characterized by drooping and shriveling.

EXPERIMENT 6: Photosynthesis & Respiration

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow at least 90 minutes for each Exercise.

Objectives: To observe the effect of photosynthesis in capturing and storing energy from the sun,
To observe the effect of respiration in the production of energy, and
To understand how photosynthesis and respiration are complementary processes.

Materials:

Student Provides:
- Lamp with 100-watt or stronger bulb
- Baking soda ($NaHCO_3$)
- Dish detergent, liquid
- Rubber bands, 2
- Timer or clock with second hand
- Distilled water
- Aluminum foil
- Tall drinking glass
- Glass jar, quart size or larger
- Elodea or similar broad leaf aquatic plant

From the LabPaq:
- Safety goggles
- Graduated cylinder, 50-mL
- Test tube, 25x125-mm
- Test tube cleaning brush
- Respirometer tube with stopper
- Wax marker pencil
- Glass stirring rod
- Bromthymol Blue solution, 30 mL
- Scalpel and ruler from dissection kit
- Straw
- Spring scale, 10 gm.
- Small weighing bag

Discussion and Review: Photosynthesis and respiration are the two principal sources for energy in plants and animals. Both animals and plants **respire** or breathe, but only plants can capture energy directly from sunlight during photosynthesis. In **photosynthesis**, plants create foods in the form of proteins, carbohydrates, and fats while absorbing carbon dioxide (CO_2,) and producing oxygen (O_2). In **respiration**, plants and animals break down stored food while producing CO_2.

The atmosphere of earth is 0.035% CO_2 and 21% O_2, and the remainder is mainly nitrogen. The oceans absorb CO_2, complementing the action of plants. Together, plants and the oceans are normally able to absorb enough of the CO_2 produced by respiration, wild fires, and the burning of hydrocarbon fuels to maintain a relatively steady ratio between the O_2 and CO_2 in our atmosphere.

Photosynthesizing plants convert about 2% of the incoming solar radiation into energy storing food, and animals acquire this energy by eating plants, either directly or by consuming other animals that eat plants. This experiment will allow you to observe the energy transfer processes responsible for all life, including your own.

Exercise 1 – Photosynthesis:

You will need: The large 25 mm X 125 mm test tube, respirometer assembly (bent glass tube and #4 stopper), large drinking glass, rubber bands, baking soda, distilled water, 10 gm spring scale, weighing bag, glass stirring rod, water plant, marker pencil, ruler, scalpel, glass jar with tap water, strong light.

When sunlight strikes a leaf, energy in the form of **photons** excites the electrons of the chlorophyll in the **chloroplasts** of plant cells. The energy captured by the electrons allows the plant cells to produce carbohydrates (CH_2O), water (H_2O) and, oxygen (O_2), from carbon dioxide (CO_2) and water (H_2O).

The oxygen and water produced in photosynthesis are then expelled through the plant leaves adding both oxygen and moisture to the atmosphere. In this exercise we will measure the rate of oxygen production from photosynthesis.

This experiment works best with a sprig of Elodea, a fresh water plant usually found in aquarium stores. If you are unable to find Elodea, substitute any other leafy fresh water aquarium plant. However, if no fresh water aquarium plants are available, the next best substitutes are fresh leaves from plants growing in streams or lakes and then fresh leaves of lettuce, parsley, cilantro, or similar plants from the supermarket. All live plants will exhibit photosynthesis and respiration, but the reaction will proceed more rapidly with fresh Elodea or similar broad leaf aquatic plant than with most other plants.

PROCEDURES:

1. Prepare a data table similar to the one below:

Photosynthesis	
Rates	**mm of travel**
Net Photosynthesis	
Respiration	
Gross Photosynthesis (Net Photosynthesis + Respiration)*	
Rate of Photosynthesis (Gross Photosynthesis ÷ 10 minutes)	

2. Make a respirometer using the large 25-mm x 125-mm test tube, the #4 rubber stopper, and the bent length of glass tubing. (**This may have been pre-assembled for you. If so, use these instructions to adjust it if necessary**.) Wear safety goggles and be careful as you assemble the respirometer. If the assembly is tight and you can not push the glass tube through the hole in the stopper, rub a few drops of dish detergent and water around the SHORT end of the glass tube so it will slip easily into the rubber stopper's hole. Rinse all soap from the tube and stopper with tap water, followed by a rinse of distilled water. If the glass tube has easy movement once it is in the rubber stopper you can put some Vaseline around the junction of the glass tube and the stopper to prevent gasses from escaping. Carefully insert the SHORT arm of the tube into the wide top section of the rubber stopper until the bend prevents further insertion and the tubing sticks out past the stopper's narrower bottom.

3. Use two rubber bands to attach the top and bottom of the large test tube to the outside of a drinking glass. If needed, pour tap water into the glass for stability. The objective is to securely hold the test tube in an upright position. Test inserting the rubber stopper of the respirometer assembly into the mouth of the large test tube. When you do, the long section of the tube should be horizontal and the short end should protrude into the test tube. Remove the respirometer assembly.

4. Prepare a 3% solution of sodium bicarbonate (baking soda, $NaHCO_3$): Use the spring scale and weighing bag to measure 1.5 gm of sodium bicarbonate, it to a graduated cylinder containing 50 mL of distilled water, and stir the solution with the glass stirring rod until dissolved.

5. Fill the large test tube about ¾-full with the sodium bicarbonate solution.

6. Trim the stalk end of the Elodea or other water plant used with a scalpel or sharp knife so that it has a clean cut end. Submerge the plant, with its cut-end up, into the sodium bicarbonate solution.

7. Insert the rubber stopper and tubing assembly into the large test tube. This should cause the solution to rise into the tubing and move past its bend; but the solution should migrate only slightly into the long horizontal section of tubing. Add or subtract solution until you have the perfect liquid level. After the level has been set remove the stopper apparatus and dry the stopper and inside edge of the glass test tube off and then reinsert the stopper into the test tube. DO NOT put pressure on the stopper assembly or you will create erroneous readings.

8. Use the wax marker pencil to carefully mark the solution's starting position on the horizontal section of tube.

9. Fill a large clear glass jar with water and seal it with a lid so it cannot spill.

10. Direct a strong light toward the respirometer test tube. The bulb should be bare, not covered by a shade, but will preferably have a reflector like that of a desk lamp. The bulb should be at least 100 watts, 150 watts if possible. Place the light fixture on the same level as the respirometer. Make sure it is stable and cannot burn anything and that it can easily be turned on and off. Place jar of water in line between the light and the respirometer. This will stop the light bulb's heat from warming the respirometer while still providing it with ample light.

11. Turn the light on and closely observe the respirometer. Begin timing the experiment when the solution in the horizontal tube begins to move toward the tube's end.

12. After 10 minutes, turn off the light. Mark the finishing position of the solution on the horizontal tube. With a ruler, measure in mm the distance the solution moved; this is the net photosynthesis rate. Record this measurement in the data table.

13. Completely cover the respirometer test tube assembly with an aluminum foil tent. Gently crimp it around the sides to exclude all light from the plant. Keep the test tube upright and be careful not to disturb the rubber stopper-bent tube assembly.

14. After 10 minutes, carefully remove the aluminum foil and mark the finishing position of the solution in the horizontal tube. Measure the distance that the solution has moved in the tube back toward the rubber stopper. This distance in mm is the respiration rate. Record this measurement in the data table.

    Remember, respiration was occurring during photosynthesis. To obtain total or gross photosynthesis rate (the total O_2) that would have been generated in the absence of respiration, the O_2 that was consumed by plant respiration during the 10 minutes of darkness must be added back.

15. Remove the Elodea or other plant from the respirometer, rinse and place it in water to prevent it from drying out. It will be required again in the next part of the lab.

16. Rinse the test tube's solution down the sink; thoroughly clean the test tube and respirator apparatus, removing all measurement marks. Leave the bent tubing in the rubber stopper since it will be used again, but flush the inside of this assembly with water and then rinse well with distilled water.

QUESTIONS:

A. What molecule did the plant produce when exposed to light?

B. What resources were consumed to produce the molecule?

C. What part did the light play in the reaction?

D. What molecule was consumed by the plant while it was in the dark?

E. What molecule was produced by the plant while it was in the dark?

F. Where did the molecules produced in the dark go?

G. What critical service do plants provide to us?

H. Would animal life on earth be possible without plants?

I. Would any life on earth be possible without sunlight?

Exercise 2 – Respiration:

You will need: Respirometer assembly from Exercise 1, large drinking glass, rubber bands, bromthymol blue, distilled water, a straw, water plant, scalpel, wax marker pencil, ruler, glass jar with tap water, strong light.

The complementary reaction to photosynthesis is respiration. Respiration in plants and animals supports cellular function. The process consumes carbohydrates and oxygen and it releases carbon dioxide and energy in the form of adenosine triphosphate (ATP), essential to all energy requiring cellular processes such as active transport, cell division, and muscle contraction.

Carbohydrates are broken down and combine with oxygen during respiration. When this reaction is incomplete it is called **fermentation** and the end product is an alcohol and carbon dioxide. Fermentation also causes the buildup of lactic acid in the muscles when we exercise because the circulatory system cannot carry fresh oxygen to the muscles rapidly enough and the muscle cells become depleted of oxygen. The result is incomplete respiration, and recovery requires the input of more oxygen.

When carbon dioxide is dissolved in water, it forms carbonic acid, H_2CO_3, a weak acid. During photosynthesis, a plant consumes carbon dioxide and water to produce oxygen and carbohydrates. The carbonic acid dissociates and decreases in the solution to satisfy the imbalance caused by the removal of the carbon dioxide, decreasing the acidity of the solution and causing the pH of the solution to be closer to neutral.

Removing the carbon dioxide from a solution of carbonic acid will drive the equilibrium toward the left, as indicated by the longer arrow pointing left below. Adding carbon dioxide to the solution drives the reaction to the right, making it more acidic. Photosynthesis, in which carbon dioxide is consumed by the plant, causes a solution to become less acidic.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \text{ (weak acid)}$$

This experiment explores the cycle of exchange between oxygen and carbon dioxide.

PROCEDURES:

1. Support the respirometer tube with a glass and rubber bands as described in Exercise 1.

2. Bromthymol Blue solution is a pH indicator. It is yellow in an acidic solution (pH ~ below 7) and blue in a neutral or basic solution (pH ~ 7 or greater). Pour about 80% of the Bromthymol Blue solution from of the 30 mL bottle into the respirometer tube. The color should be blue.

3. Unless you have several straws and will not need to re-use this one, mark the end of the straw you're putting into your mouth, then gently breathe bubbles into the Bromthymol Blue solution. *Do not blow so hard you splash the solution out of the tube!* Continue blowing bubbles into the solution until it becomes a light yellow color. Keep the solution in the respirometer tube.

4. Rinse the Elodea or other plant from Exercise 1 well with distilled water or use a well rinsed new plant. Again, submerge the plant in the respirometer solution with the stalk's cut-end at the top of the test tube.

5. Assemble the respirometer as in Exercise 1. Insert the rubber stopper with tubing into the large test tube. This should cause the pH indicator solution to rise into the tubing, moving past the bend, but the solution should migrate only slightly into the horizontal section of tubing. If there is not enough solution to reach the respirometer tube, add distilled water as needed. Add or subtract solution until you have the perfect liquid level. After the level has been set DO NOT touch the stopper. Putting any pressure on the stopper after you start the experiment will result in erroneous readings.

6. Mark the starting position of the solution in the horizontal tube.

7. Arrange the light source as in Exercise 1 with sealed a jar of water placed between the light and the respirometer to absorb the light's heat.

8. Turn on the lamp. *Start timing when the solution in the horizontal tube begins to move toward the tube's end.*

9. Continue to watch the respirometer closely until you see the color of the solution in the respirometer start to change. Note the end time.

10. Remove the plant from the respirometer and place it in the trash. Add a bit more Bromthymol Blue solution to restore the solution to its previous level.

11. Again use the small straw to gently breathe bubbles into the Bromthymol Blue solution until it becomes a light yellow to golden yellow color.

12. Repeat Steps 5 through 9 above without a plant in the respirometer.

QUESTIONS:

A. When you blew through the straw into the Bromthymol Blue solution what gas did you add? What color change did you observe in the solution and why?

B. What color change occurred after you placed the plant into the respirometer and turned on the light?

C. What was happening when the solution in the horizontal tube started to move?

D. How long did it take after the solution started to move for the color change to occur?

E. Why did the color change?

F. When you repeated the experiment without a plant in the respirometer, what happened and why?

Now that you have completed this week's lab make sure you read the lab for next week this will help you plan your time better. Take some time and highlight anything you will need to prepare in advance. As you read the lab write out a hypothesis for each exercise.

EXPERIMENT 7: DNA & Protein Synthesis

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Cutting out the models takes time and makes your hand tired. Allow ample time for this chore.

Objectives: To introduce the structure of the DNA molecule and how it functions, and
To model the globin B molecule.

Materials: Student Provides: Scissors
Tape

From the LabPaq: Nothing Required

Discussion and Review: The initials DNA stand for **deoxyribonucleic** acid. The DNA molecule is vital to life and has two primary functions.

1. First and most importantly, DNA is the material of genetics. Offspring generally have the same characteristics as their parents because they receive DNA from both parents.

2. Second, DNA is the master template for coding the structure of specific proteins. This is important for the ever occurring chemical reactions in cells or cellular metabolism.

Chemically, chromosomes, and therefore genes, are made up of DNA. Chromosomes are found in each cell of all living organisms. Each chromosome is composed of a series of genes. Each gene directs the formation of a specific protein.

Proteins are large organic molecules that play many essential and complicated roles in living organisms. Proteins are composed of varying combinations of 20 different kinds of amino acids. Because protein molecules are large, often containing several hundred amino acids, the number of amino acid sequences and therefore the possible variety of protein molecules, is enormous.

DNA resembles a twisted ladder and is composed of a series of **nucleotides**. Each nucleotide is made up of three components: a sugar (deoxyribose), a phosphate group, and a nitrogenous base.

The nucleotides are named according to which of the four bases they contain. There are four different types of nucleotides in DNA because there are four possible bases: **adenine (A), guanine (G), cytosine (C), and thymine (T).** The sides of the ladder contain only sugar-phosphate molecules while the rungs are two bases bonded to each

other. *A is always paired with T. C is always paired with G.* Adenine and Guanine are purines and Cytosine and Thymine are pyrimadines. A purine structure is composed of two carbon rings while a pyrimadine is made up on one carbon ring structure. When the DNA bases are paired up having a puriine pair with a pyrimadine will maintain the even double helix structure.

Exercise 1 – Modeling DNA:

PROCEDURES:

1. In this lab you will need to print out and then cut apart the individual DNA nucleotides found in figures 1 and 2. You will use these to create double stranded DNA molecules. You should have six of each of the 4 different nucleotides. As you pair them together make note that a pyrimidine can only pair with a purine, this pairing relationship helps to maintain the even structure of the DNA molecule.

2. List the four bases which are found in DNA.

3. Fit any six nucleotides together to form a row, then list the six nucleotides in the order you used them.

4. Work with your model pieces and try fitting the bases together to make a double strand as shown in the illustration above. Which nucleotides form pairs?

5. The following list indicates the bases on one strand of a DNA molecule. Write in the names of the bases that could pair with each to make the complimentary strand.

cytosine	_____	adenine	_____
adenine	_____	guanine	_____
cytosine	_____	thymine	_____
cytosine	_____	guanine	_____

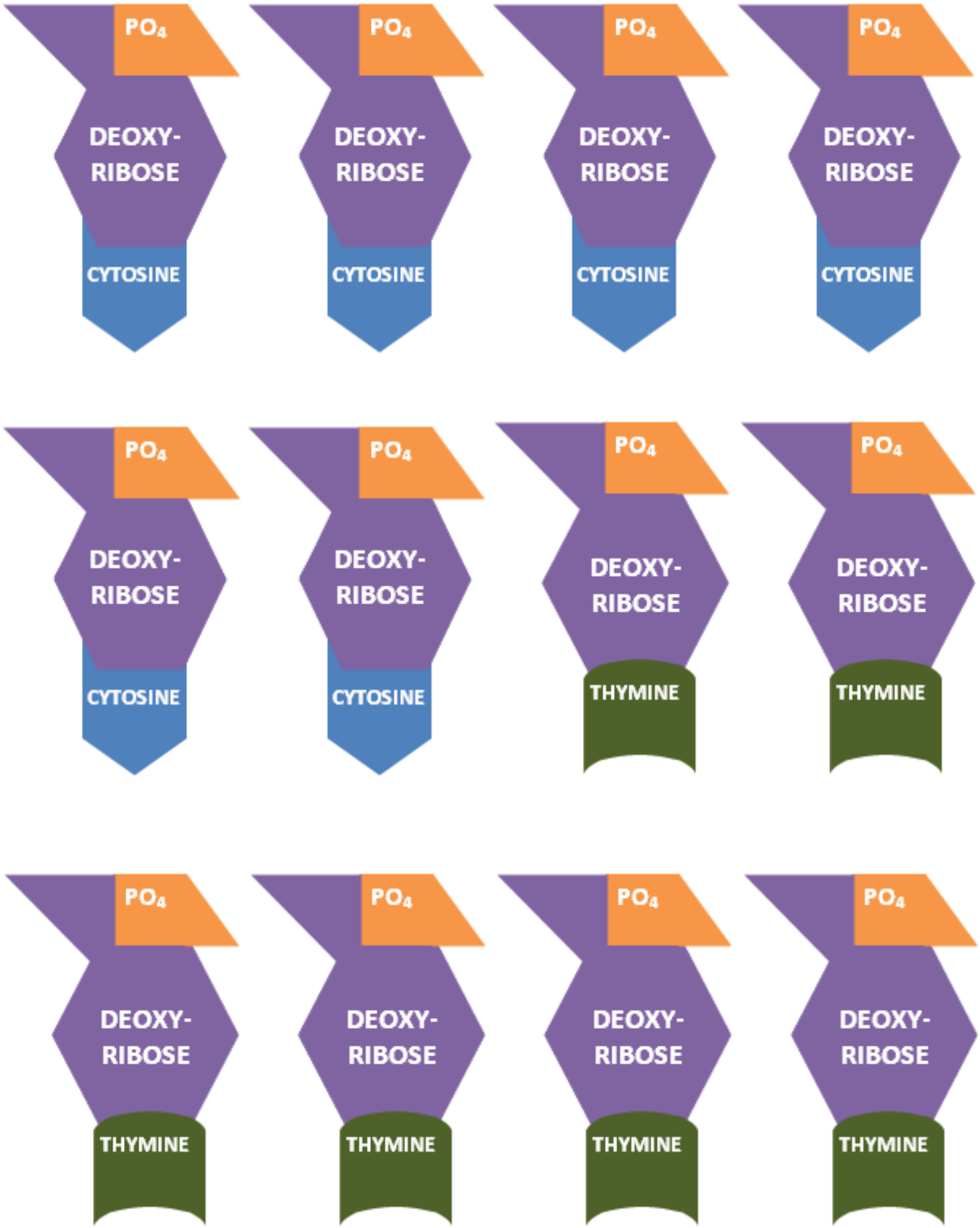
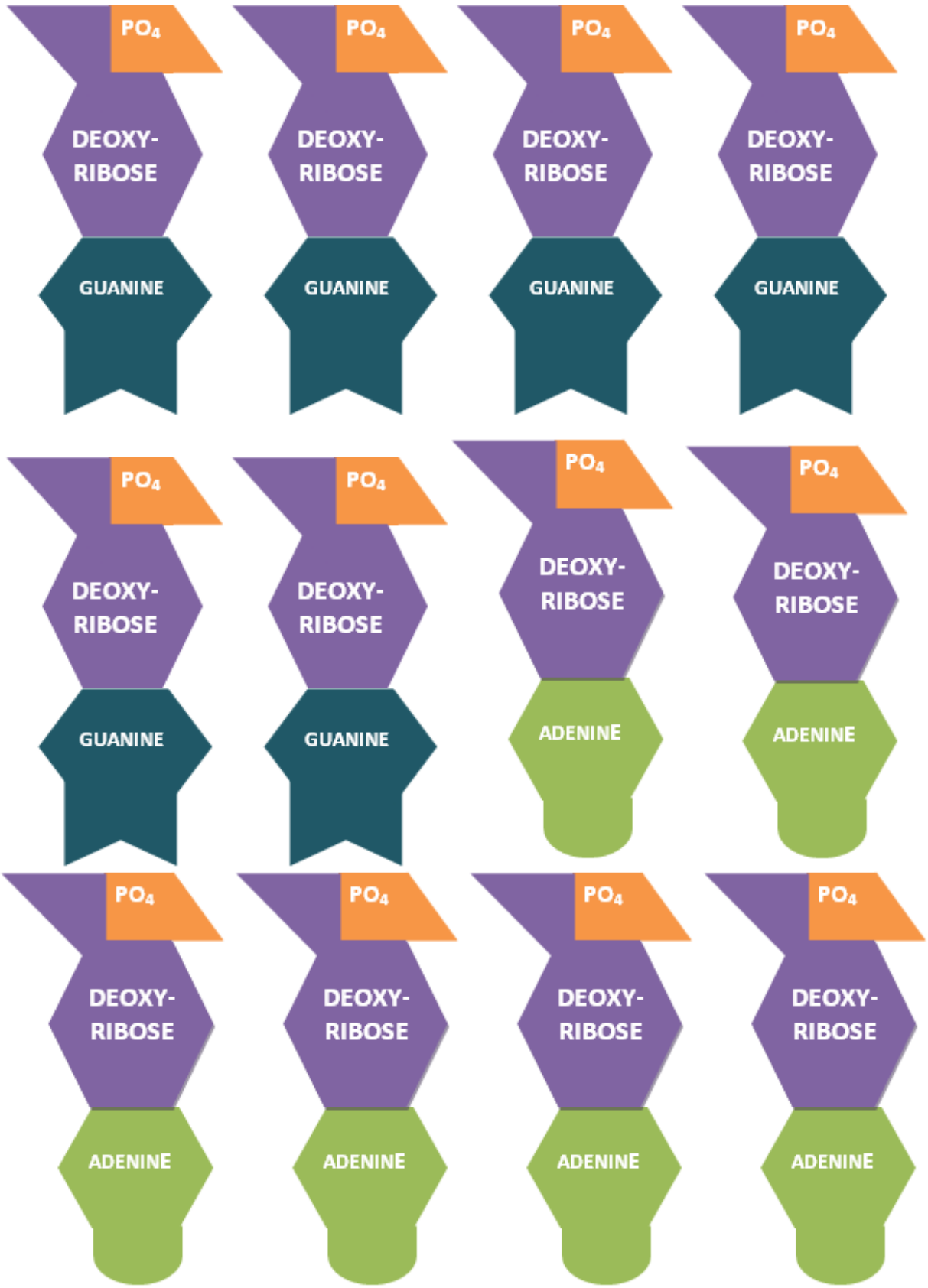


Figure 1: DNA Pyrimadines this page, Figure 2: DNA Purines next page.


PO4
DEOXY-RIBOSE
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ADENINE

QUESTION:

A. What are the three components of a DNA nucleotide?

Exercise 2 - The Globin Gene:

The **globin** molecule is made up of 146 amino acids. The nucleotides that code for the 146 amino acids of the globin protein are called the globin gene. Each gene codes for a particular protein. Globin is the red blood cell protein that is responsible for oxygen transport. The amino acid sequence for a portion of the globin B protein is:

--proline --glutamic acid --glutamic acid –lysine—

PROCEDURES:

1. Use the following table to write the nucleotide sequence that codes for these four amino acids that are part of the globin molecule. Write the code from left to right. Directly below this coding strand sequence indicate the bases that could form the other side of the DNA ladder.

Amino Acid	DNA Code
alanine	CGT
glutamic acid	CTC
lysine	TTC
proline	GGA
Valine	CAC

Coding Strand:
Complementary Strand:

2. Draw the DNA coding strand for this portion of the globin gene, using the letters that represent the four proteins.

QUESTIONS:

A. How many nucleotides would it take to construct the coding strand of the globin molecule?

B. How many nucleotides would it take to model this part of the DNA molecule?

C. Do you think this is a complete DNA molecule (chromosome) or part of one? Explain.

Now that you have completed this week's lab make sure you read the lab for next week this will help you plan your time better. Take some time and highlight anything you will need to prepare in advance. As you read the lab write out a hypothesis for each exercise.

EXPERIMENT 8: Extraction of DNA

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Place the 91% Isopropyl alcohol in freezer the night before. Allow 1 hour for this experiment.

Objective: To extract and observe the characteristics of DNA.

Materials:

Student Provides:
- 91% Isopropyl (rubbing) alcohol
- Strawberry, fresh or frozen
- Non-iodized table salt
- Liquid dishwashing detergent
- 2 Toothpicks
- 3 Glasses, small, clear drinking
- Distilled water
- Tissue or paper towel

From the LabPaq:
- Microscope
- Blank slides
- Slide cover slips
- Gram stain Iodine
- 50-mL Graduated cylinder
- Mortar and Pestle
- Coffee filter
- Glass stirring rod
- Large test tube
- Spring scale
- Funnel

Discussion and Review: This lab is designed to introduce you to the structure of the DNA molecule and how it functions. As you should know by now, DNA stands for deoxyribonucleic acid and it is important to living organisms in two primary ways:

1. First: DNA is the material of genetics. Offspring generally have much the same DNA characteristics as their parents.

2. Second: DNA is the master template for coding the structure of specific proteins your body needs for the ever occurring chemical reactions in cells or cellular metabolism.

Chemically, chromosomes and the genes they carry are made up of DNA. Chromosomes are found in each cell of all living organisms. Each chromosome is composed of a series of genes. Each gene directs the formation of a specific protein.

Wild strawberries, like humans, are diploids, meaning they have two sets of chromosomes. But cultivated strawberries, the ones found in most supermarkets, are octoploids, meaning they have eight sets of genes in each genome. This means strawberries have large amounts of available DNA as well as enzymes (pectinases and cellulases) that help break down cell walls, making them ideal subjects for a DNA extraction experiment.

PROCEDURES:

You will need: 1 strawberry (fresh or frozen), mortar & pestle, non-iodized salt, spring scale, dish detergent, 3 small glasses, large test tube, 91% Isopropyl alcohol, graduated cylinder, coffee filter, gram stain, glass stirring rod, glass microscope slide, slide cover, microscope.

NOTE: You will be using the graduated cylinder multiple times in this experiment. It is important that you thoroughly rinse and dry the graduated cylinder between each use.

1. The night before beginning this experiment, place a sealed container with at least 25 mL of 91% Isopropyl alcohol in the freezer in order to chill it to freezing temperature. The entire bottle can be chilled without harming it. If no freezer is available, place the alcohol in a container packed completely in ice at least three hours before beginning the experiment.

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2. Pour 90 mL of distilled water into a small clean glass. Add 3 grams of non-iodized table salt and stir with the glass stirring rod until the salt is completely dissolved.

3. Measure exactly 5 mL of dish detergent with the graduated cylinder and add it to the glass of salt water. Stir gently yet thoroughly with the glass stirring rod, trying not to create bubbles.

4. If the strawberry has leaves, remove them. Put the strawberry in the mortar and use the pestle to crush it. Spend a minute or so making the mixture very mushy.

5. Measure 10 mL of the salt/soap mixture with the graduated cylinder and add it to the crushed strawberry in the mortar. Blend the two mixtures gently but thoroughly with the pestle, again trying to not make soap bubbles. The detergent and salt break down the cell phospholipid membrane, releasing DNA from the nuclei inside the strawberry cells. The dish detergent blended into the strawberry and salt mixture dissolves the lipids in the plasma membranes of the strawberry cells so that the contents of the cells can flow into the solution.

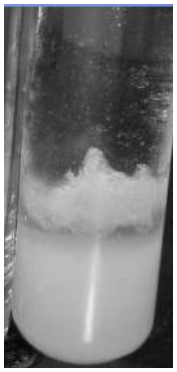
6. Place the coffee filter inside the funnel and hold this assembly over a clean glass. Pour the strawberry mixture from the mortar into the filter and let the filtrate run into the glass until at least half of the filtrate has run through.

7. Measure 5 mL of the filtrate mixture in a clean graduated cylinder and pour it into the large 25x125-mm test tube. Stand the test tube in the third small glass to give you

two free hands for the next step. Working quickly, measure 11 mL of ice cold 91% Isopropyl alcohol into a clean graduated cylinder.

8. Pick up the test tube and hold it at an angle. Slowly pour the ice cold alcohol down the test tube's side so the alcohol forms a layer on top of the filtrate.

9. Watch for small oxygen bubbles and clumps of DNA to form where the two layers meet. The test tube on the right shows strawberry DNA forming clumps of DNA threads at the junction of the icy cold alcohol and the strawberry filtrate.
10. After 15 to 30 seconds, use the glass rod to gently stir the alcohol mixture. Allow the DNA threads to gather, then pull them out of the test tube with the stirring rod and put them on a slide. Pull the DNA strands apart with 2 toothpicks.
11. Look at the DNA strands through the field microscope. At this low magnification you *will not be able to see the double helix formations* or even just one DNA thread as that takes a much more powerful microscope. Draw what you actually can see.
12. Add a drop of Iodine from the Gram stain kit to the top of the DNA material on the slide. Carefully blot excess stain from the side of the slide with a tissue or paper towel.



13. Place a cover slip over the stained DNA strands and carefully observe the slide of strawberry DNA with the microscope. Draw what you see.
14. If you have access to a stronger power microscope, use it to observe your DNA slide, and describe the similarities and differences observed in you lab report.
15. If you have time, for fun, try extracting DNA from other fruit and things around the house such as kiwis, oranges and even oatmeal. Be sure to use very cold isopropyl alcohol. The photo at right shows a test tube with clumps of DNA treads from an orange.

Questions:

A. Describe in your report what you saw under the microscope.

B. Is this what you expected to see?

C. How is it like or different from your expectations?

D. What did the detergent do?

E. Why is it important to keep the Isopropyl alcohol very, very cold?

EXPERIMENT 9: Plant Genetics

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow 30 minutes for Part 1. Timing for Part 2 varies. Part 3 takes at least 5 days.

Objectives: To understand the genetic information that can be inferred from the phenotype of seeds, and
To learn how to subject the data from observations to statistical analysis for validity.

Materials: Student Provides: Calculator (optional)
Planting soil or garden location

From the LabPaq: 100^{+} F2 Corn seed

Discussion and Review: Modern theories of heredity find their origins in experiments originally conducted by Gregor Mendel, on the **crossbreeding** or **hybridization** of garden peas. Like humans, these are diploid organisms, containing pairs of **alleles** or **genes** for each trait, located on chromosomes. Characteristics that are inherited are called traits, and Mendel was the first person to accurately predict how and in what relative frequency traits would be transferred from one generation to another.

Like the corn seed with which you will work in this experiment, Mendel started with true breeding plants in which each generation that self-pollinated looked exactly like the previous generation. They differed in only one characteristic. This is a **monohybrid cross** because the plants being crossbred differ in only one trait. In Mendel's experiments the difference was height. With the corn seed in your experiment, the difference is color.

In the parent or true breeding generation of the corn, both alleles are the same in each plant - either both green or both albino in color. In the first generation of the cross or F1 generation, all seeds contain two different alleles, one green and one albino. Since the green allele is dominant, the seeds will all appear green. In the next generation of cross, the F2 generation, there are four possible combinations of alleles. Thus, since it is possible some seed may contain two recessive alleles for albino, it is possible for these recessive alleles or genes to determine the phenotype or appearance of the seed.

Through his experiments, Mendel stated the **rule of dominance** that says certain alleles are dominant and others are recessive. When both the dominant and the recessive alleles are present, the dominant one will be observed and the recessive one will disappear, as far as the appearance or phenotype is concerned.

Mendel also stated the **law of segregation**. For each trait, each plant in the F1 generation carries one dominant allele and one recessive allele, having received one from each of its parent plants. Because each F1 plant has two different alleles, it can produce two different types of gametes, each having the dominant or the recessive allele. During fertilization, these gametes randomly pair to produce four different combinations of alleles. This pairing should be statistically predictable.

PROCEDURES: Part 1 - The Punnett Square

Reginald Punnett, an English biologist, devised a shorthand way to determine the expected proportions of possible genotypes in the offspring of cross pollination. This method is called a Punnett square.

For a **monohybrid** which differs in only one trait, the Punnett square is two boxes tall and two boxes wide because each parent can contribute two different types of alleles for the trait. The two types of gametes from one parent that correspond to the two possible alleles are shown across the top of the square, one above each box. The two types of gametes from the other parent are shown down the side of the square, one beside each box.

In each box, the resulting combination of alleles is shown according to the intersection of the information shown outside the box. This information is the genotype (the genetic makeup of the organism), and from it you can predict the phenotype or actual appearance of the organism. The combinations shown in the Punnett square boxes are all the possible combinations of alleles and, following the law of segregation, the combinations in each box will occur in equal numbers.

In a monohybrid for corn in which color is the only variable, the dominant allele is written as “G” (for green) and the recessive allele for albino color is written as “g”. The F1 generation of a monohybrid cross for green-albino corn is **heterozygous**, each containing the allele for green and the allele for albino. As the G allele is dominant, all specimen plants of the F1 generation appear green. A Punnett square for the second or F2 generation would look as follows:

	G	**g**
G	GG	Gg
g	Gg	gg

1. Looking at the Punnett square, hypothesize the phenotype or appearance of a plant resulting from each combination of alleles. On the basis of your hypothesis, predict the proportions of seed that you would expect to have the phenotype of green versus those with the phenotype of albino and record this that as a proportion (i.e. 2 green**:** 2 albino). Calculate the percentage of the total plants you expect to appear green.

2. Open the envelope of corn seed and spread them on a piece of white paper. Some of the seed will appear an orange color and others will have a lighter yellow color.

The technical terms green and albino are used to describe these differences, but they refer to the plants that will grow for the corn, not the actual color of the kernels. Look at the corn seed until you begin to see a difference in the two seed colors.

3. Separate the corn seeds into two groups, the "green" and the "albino". Some may be difficult to group but just move quickly and let your first reaction make the decision. It will probably be more accurate than worrying about it.

4. Count the seeds in each group. Calculate the percentage of the total seeds that are "green" and will produce green plants.

Questions:

A. What ratio of green to albino did you hypothesize from the Punnett square?

B. What percentage of green in the total did you predict?

C. What was the actual percentage of green in the total?

D. What are possible explanations for any difference in the percentages?

Part 2 – Analysis of Data Using Chi-square

In your hypothesis, you may have predicted that the trait of green versus albino would appear in a certain ratio, such as 3:1, but the actual count of seed may have turned out different from your hypothesis. You need a statistical tool to tell you if this difference is an acceptable variation possibly due to your sample being small and not taken from a great number of seed or if the difference is so great that your hypothesis may be invalid. The Chi-square or X^2 value will allow you to make that evaluation.

If, for example, you are given 100 corn seed that are the offspring of a monohybrid cross, you would expect the phenotype of the offspring to be in a ratio of 3:1, that is 75 showing the dominant allele for the trait and 25 showing the recessive trait. If, however, the offspring turn out to be in the ratio of 1:1 you may have reason to question your hypothesis or the conditions of the cross.

To calculate X^2, study the following table:

Trait	**Specimens Observed (o)**	**Specimens Expected (e)**	**Difference d=o-e**	**d^2/e**
Dominant	80	75	5 = 80-75	25/75, or 0.333
Recessive	20	25	5 = 20-25	25/25, or 1.0

The Trait is the phenotype or expression of the trait under consideration which in this case is dominant versus recessive alleles. The number of Specimens Observed, 80

dominant and 20 recessive, is placed in the second column. The number of Specimens Expected, 75 dominant and 25 recessive, is placed in the third column. The absolute Difference between the number observed and the number expected, disregarding any resulting negative sign, is placed in the fourth column.

The X^2 is the sum of the values of d^2/e, the numbers calculated by the equation in the last column. This is written as $X^2 = \sum d^2/e$.

In this case, the value of X^2 is the sum of 0.333 and 1.0, or 1.333

Chi-square is a reflection of three factors:

- The amount of the deviation,
- The size of the sample, and
- The number of opportunities in which deviation can occur.

Our hypothesis is that the color of the corn seed will be distributed in the ratio 3:1 in an F1 monohybrid cross. We have counted the number of each color in a presumably random sample. In order to evaluate the significance of the deviation from the expected values and validate our hypothesis, we must ask two questions:

- How probable is it that our values would have occurred simply by chance?
- Based on the values and the number of samples, should we accept or reject our hypothesis?

The first question can be answered by comparing the X^2 value (1.333) with those in the following standard table of values for chi-square.

Less Reason to Doubt Hypothesis←----------→More Reason to Doubt Hypothesis										
P	.99	.95	.80	.50	.30	.20	.10	.05	.02	.01
C-1										
1	.00016	.0039	.064	.455	1.074	1.642	2.706	3.841	5.412	6.635
2	.0201	.103	.446	1.386	2.408	3.219	4.605	5.991	7.824	9.210
3	.115	.352	1.005	2.366	3.665	4.642	6.251	7.815	9.837	11.341
4	.297	.711	1.649	3.357	4.878	5.989	7.779	9.488	11.668	13.277
5	.554	1.145	2.343	4.351	6.064	7.289	9.236	11.070	13.388	15.086

This table shows the maximum X^2 value allowable if the deviation found is to be considered due to chance alone. The vertical column C-1 (classes minus 1) shows the number of data classes under consideration, minus one, a number usually called the degrees of freedom. In this example there are 2 classes, dominant versus recessive for one trait, so the degrees of freedom are 2 minus 1, or 1.

The P value expresses the probability that deviations yielding the indicated X^2 value are due to chance alone, given the relevant degrees of freedom. Our example has one degree of freedom and an X^2 value of 1.333. This X^2 value lies between the numbers 1.074 and 1.642 on the 1 degree of freedom row, so the probability of this difference or variation from the expected values being due solely to chance is between the corresponding probabilities of .20 and .30, or about .25. This means that if the experiment was repeated, a deviation as great or greater than observed will be due solely to chance 25% of the time.

You must now decide on an acceptable value for P in order to answer the second question of whether to should accept or reject the hypothesis as valid. A high value for X^2 indicates that the experimental data values deviate greatly from the predicted results. The greater the difference between the predicted and the actual values, the greater is the probability that the hypothesis upon which the predictions were based is incorrect.

However, some deviation is acceptable without rejecting a hypothesis, as deviation can occur for reasons other than an incorrect hypothesis. Serious doubt about a hypothesis would be caused by a statistically significant deviation where the probability of it having occurred by other than chance alone is 0.05 or less. In other words, any X^2 value large enough to yield a P value as low as 0.05 is considered to reflect a statistically significant deviation, one that warrants reconsideration of the hypothesis.

In our example, the number of corn seed observed with the dominant trait deviates from the predicted value by a number that yielded an X^2 value of 1.33, corresponding for one degree of freedom to a P value of .25, not a low enough probability to cause us to reject our hypothesis.

1. The corn seed provided in the LabPaq are F2 monohybrids of green and albino corn. Draw a Punnett square for the F1 cross showing what the offspring would be in the F2 generation.

2. Form a hypothesis for the ratio of green phenotype to albino phenotype that you expect to find in the F2 corn seed.

3. Based on your hypothesis of the ratio of green to albino, calculate the number you would expect to find of green versus albino phenotypes.

4. You should have already counted the numbers of green and albino seed in Part one. If not, separate the seed into green and albino phenotypes now and count the actual numbers of green versus albino phenotypes.

5. Set up a chi-square table as previously shown. Fill in the values predicted and actually counted, and calculate the value of X^2. From the table of values for X^2 identify the P value for your sample.

Questions:

A. Do your results confirm your hypothesis or does the value of P cause you to reconsider your hypothesis?

B. What are possible causes for deviation from the predicted ratio of green to albino seed?

Part 3 – Confirming Your Hypothesis

1. In Part 2 above, there are really two hypotheses. One hypothesis is that the ratio of green to albino seeds in an F-1 monohybrid cross is 3:1. The other hypothesis is that the color of the seeds reflects the genotype of the seed. You have carried out a chi-square computation to test that hypothesis and the results may or may not have confirmed the combined hypotheses. If not, it may be because the hypothesis that the seed color reflects the genotype is not valid, but that the hypothesis of ratio of genotypes is still valid. The only way to resolve this is to confirm the genotypes by growing the seed.

2. To aid sprouting of seeds, soak in water overnight before planting. Plant all of the seeds in a bed of good soil in a sunny outdoor location if the weather is warm. If not, plant them indoors in trays filled with potting soil and placed in a sunny location. Keep the seeds and soil moist but not wet. The seeds should sprout and be visible in about five days. The plants two recessive or albino alleles will appear very pale, almost white in color. Those with one or more dominant alleles will be green.

3. Count the albino versus green sprouts of corn plants and repeat the chi-square computation.

Questions:

A. Do your results confirm your hypothesis or does the value of P cause you to reconsider your hypothesis?

B. What are possible causes for deviation from the predicted ratio of green to albino seed?

EXPERIMENT 10: Phenotype and Genotype

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow *40 to 60 minutes for this experiment*

Objectives: To understand phenotype and genotype, and
To understand why you have certain characteristics

Materials: Student Provides: Self
Mirror

From the LabPaq: PTC taste paper

Discussion and Review: Who you are is a product of both your physical nature and your nurturing or training. Genetics is the study of your nature. By looking at some of your physical features you can understand some of your genetics and why you have certain physical features.

The study of genetics is one of the hottest areas of study in biology today. The recent completion of the genome project, combined with growing interest in cloning and gene replacement therapy, has generated great interest in genetics. In biology, genetics can be divided into classical genetics (heredity) and molecular genetics. Heredity is generally thought of as a study of parents and offspring and how their traits or characteristics are related.

Two organisms can look alike but have different underlying gene combinations. The way an organism looks and behaves is called its *phenotype*. The combination of genes or ***alleles*** an organism contains for a particular trait is called its ***genotype***. You cannot always know an organism's genotype simply by its appearance.

Mendel's law of segregation explains the results of crosses between parents with similar characteristics or phenotypes but whose offspring may have different characteristics. He concluded that the two alleles for each trait must separate when gametes are formed, and that a parent passes on at random only one allele for each trait to each offspring. Each offspring therefore has two alleles, but one will dominate and determine the phenotype. An organism is *homozygous* if its two alleles for a trait are identical, and it is *heterozygous* for a trait if its two alleles for the trait differ from each other.

In this experiment, you will look at several human traits in order to observe Mendel's law of segregation. These traits are controlled by a single gene with two alleles and each allele, if it is dominant in the pair, produces a distinct phenotype.

PROCEDURES: For each of the following traits determine your phenotype and if possible your genotype. If you have a dominant phenotype, you may have either a recessive allele and a dominant allele or two dominant alleles, whereas if your phenotype is recessive, your genotype is recessive homozygous. Record your observations in a table.

1. **Dimpled chin:** A cleft in the chin is a dominant trait.

2. **Free ear lobe:** For ear lobes to hang free is dependent on a dominant allele. If two recessive alleles are present the ear lobes are directly attached to the head.

3. **Ability to taste PTC:** Some people have the dominant gene that allows them to detect the bitter taste of PTC while others do not. Put a piece of PTC impregnated paper on your tongue for about five seconds. If you are a taster you will know it.

4. **Interlocking fingers:** When the fingers are interlocked, some people place the right thumb on top of the left (dominant allele); others place the left thumb over the right (recessive allele).

5. **Mid-digital hair:** Some people have hair on the second (middle) joint of one or more of the fingers, while others do not. The presence of hair is due to a dominant allele and the absence of hair from all fingers is due to a recessive allele.

6. **Bent little finger:** A dominant allele causes the last joint of the little finger to bend inward toward the fourth finger.

7. **Widow's peak:** The dominant allele produces a hairline with a point in the center of the forehead (widow's peak). The recessive allele gives a continuous hairline.

8. **Hitchhiker's thumb:** When you can bend the tip of the thumb back 90 degrees in relation to the rest of the thumb you are homozygous recessive for this allele.

9. **Pigmented irises:** When there is no pigment in the front part of the eyes and a blue layer at the back of the iris shows through you are homozygous for the recessive allele allowing blue eyes.

10. **Long palmar muscle:** Clench your fists tightly and examine the tendons running over the inside of the wrists. If there are three tendons you have the recessive alleles for the long palmar muscle, if there are only two tendons you do not.

Exercise: Refer to the previous experiment and construct a Punnett square showing both the genotype and phenotype ratios possible if two heterozygous brown-eyed individuals with dimpled chins were to have children. Your Punnett square will be 4 x 4 squares. Assume both independent assortment and segregation are occurring.

EXPERIMENT 11: Classification of Species

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow at least 1 hour for Exercises 1, and 2 to 3 hours for Exercise 2.

Objectives: To understand the concept of the classification of species, and
To practice using dichotomous keys for classification

Materials: Student Supplies: Scissors
Tape
Paper

From the LabPaq: Nothing Required

Discussion and review: Organism names are unique for each species and reflect the relationship of the species to related organisms. The hard part of naming a species is figuring out exactly what a species is. Some authors have 16 species of a certain group of organisms while another author might list 10 species of the same group of organisms. This lab will introduce you to the species concept and the use of dichotomous keys.

One of the most striking aspects of life is its extreme diversity. Everywhere we look we see a bewildering array of different organisms, yet the fossil record indicates that approximately 99% of all organisms that have ever existed are now extinct. Even so, biologists believe that there are some 5 to 10 billion species of organisms alive today. Of these, many have not yet been discovered or described. To make sense out of this huge and bewildering array of life, we attempt to categorize organisms into groups based on evolutionary relationships.

Taxonomy and **systematics** are biological fields of study that attempt to classify all living and fossil organisms into groups and subgroups. The results are useful because: (1) they reflect the evolution of organisms, (2) they reduce the millions of organisms into a lesser number of groups that are easier to understand and use, and (3) they provide a system for naming organisms. The taxonomic groups are arranged hierarchically as shown below and are named from the largest **taxon** to the smallest:

Domain
Kingdom
Division (for Plants) or Phylum (for Animals)
Class
Order
Family
Genus
Species

An easy way to remember these group titles is by a mnemonic device such as: "**D**ignified **K**ings **P**lay **C**hess **O**n **F**ine **G**reen **S**ilk -
D. K. P. C. O. F. G. S.

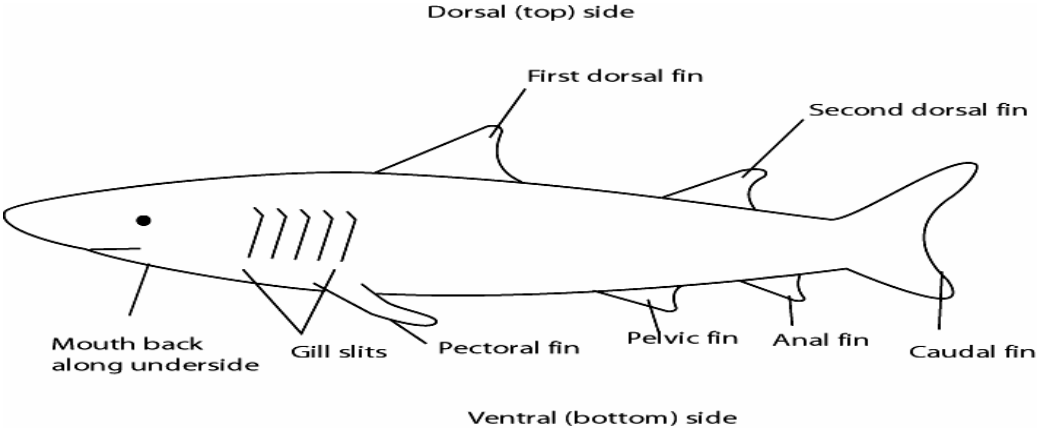
Exercise 1: Classification of Sharks:

PROCEDURES:

Classification is a way of separating members of a group of similar and related organisms into smaller subgroups. The scientific names of organisms are based on such a classification system. To identify and name a particular organism, scientists often use a key based on a listing of characteristics such as the structure and the behavior of the organism. The key is organized in such a way that an organism can be identified through a series of **dichotomous** questions. Dichotomous means dividing into two parts or having two possible answers hence the name dichotomous key.

1. Study the body characteristics of the shark in Figure 1 below. You will use this example to help determine the proper family name classifications for various sharks.
2. Print out the images of the sharks (Figure 2) from your DVD. Cut them apart so that you can more easily compare them to one another as you work each one through the key.

Figure 1:



3. Set up a table as shown below to list your classification of family names for the 14 sharks shown in Figure 2:

Classification of Shark Family Specimens

Specimen #	Family Name
1	
2	
3	
4, 5, 6...14	

4. Carefully examine the drawing of shark specimen #1 shown in Figure 2.

5. Read statement 1. a and 1. b of the classification key for sharks that follows the shark drawings. They describe a characteristic of sharks that can be used to separate the members of the order into two major groups. Follow the directions in these and successive statements until you determine a family name for shark #1. Record the family name in your table.

6. Repeat Steps 4 and 5 for each of the 14 specimens of sharks shown.

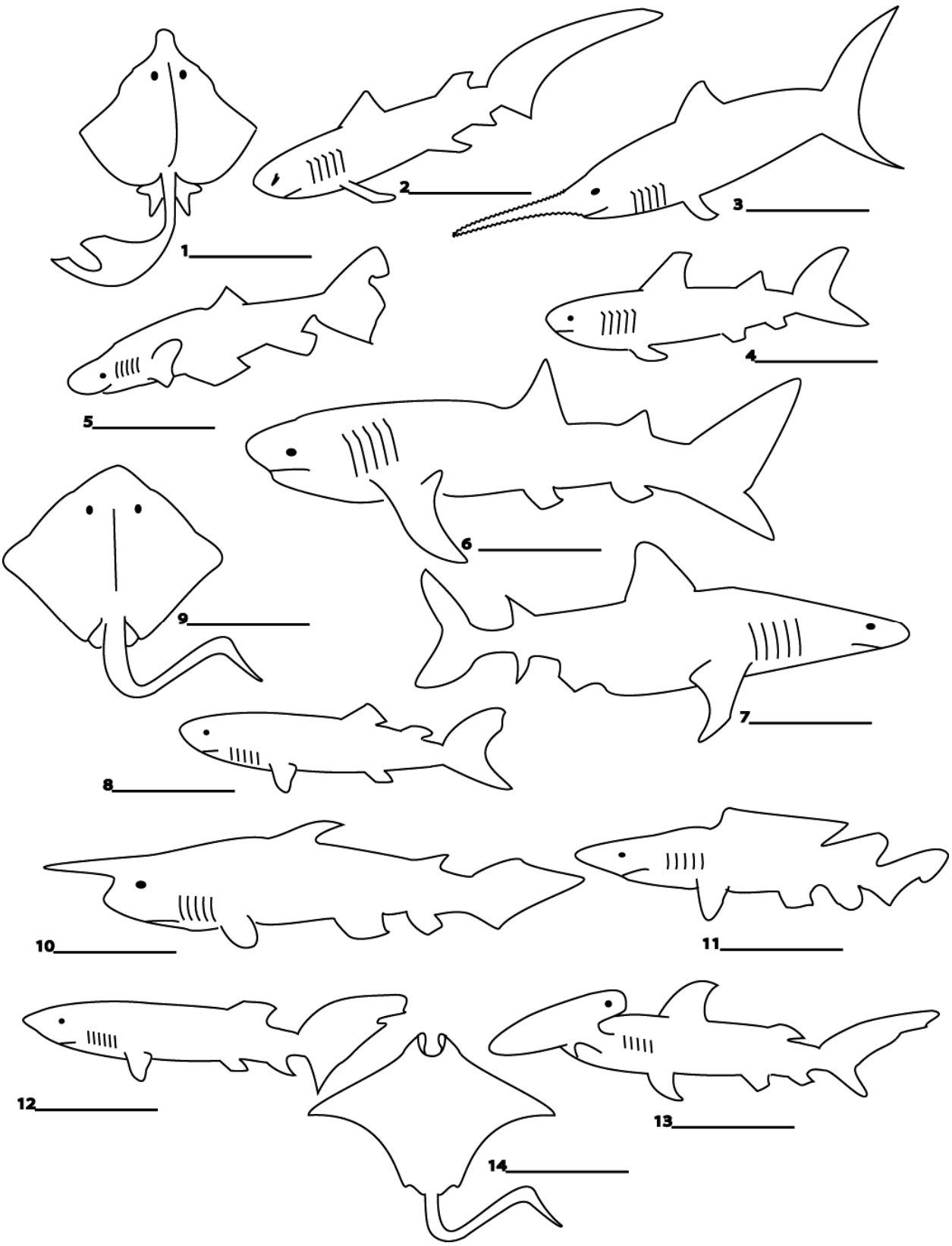


Figure 2

KEY TO THE CLASSIFICATION OF SHARKS BY FAMILY

1. a. The body is roughly diamond shaped if viewed from above----go to **12.**
b. The body is not diamond shaped if viewed from above----go to **2.**

2. a. There is no pelvic fin and the nose is saw-like-----Family Pristophoridea
b. There is a pelvic fin----go to **3.**

3. a. There are six gill slits on a side-----Family Hexanchidae
b. There are five gill slits on a side-----go to **4.**

4. a. There is only one dorsal fin-----Family Scyliorhinidae
b. There are two dorsal fins-----go to **5.**

5. a. The mouth is at the front of the head-----Family Rhinocodontidae
b. The mouth is along the underside of the head-----go to **6.**

6. a. The head is expanded laterally and the eyes are on the end of the extensions-----Family Sphyrnidea
b. The head is not expanded laterally-----go to **7.**

7. a. The top half of the caudal fin is the same size and shape as the bottom half-----Family Isuridae
b. The top half of the caudal fin different in size and shape from the bottom half-----go to **8.**

8. a. The first dorsal fin is almost half the total length of the body—Family Pseudotriakidae
b. The first dorsal fin is much, much less than half the total length of the body-----go to **9.**

9. a. The caudal fin is almost as long as the entire body-----Family Alopiidae
b. The caudal fin is much less than the length of the body-----go to **10.**

10. a. The nose has a long needle-like point on the end----Family Scapanorhynchidae
b. The nose does not have a needle-like point----go to **11.**

11. a. There is no anal fin-----Family Squalidae
b. There is an anal fin-----Family Carcharhinidae

12. a. There is a small dorsal fin near the tip of the tail-----Family Rajidae
b. There is no dorsal fin near the tip of the tail-----go to **13.**

13. a. There are horn-like appendages at the front of the body-----Family Mobulidae
b. There are no horn-like appendages at the front of the body-----Family Dasyatidae

QUESTIONS:

A. How might you arrange the sequence of the classification key differently while still achieving the same results?

B. What are eight characteristics used to identify the family of a shark (e.g., the shape of the body, etc.)?

C. What is the main characteristic that distinguishes shark Number 12 from shark Number 7?

Exercise 2 – Classification of a Fictional New Family:

Pretend you are a taxonomist presented with a mixture of living and fossil organisms in a ***mythical family named Crawla***. Your objective is to identify the members of the family to the genus and species level. Modern taxonomists have many aids for sorting and classifying organisms, but you have only your eyes and a dichotomous key.

Living crawlas were discovered on an island in the South Pacific by a **paleobiologist** who was excavating fossil crawlas. Crawlas are characterized by:

- A flexible, one piece **carapace** made of **chitin** that extends over the head,
- Two external nares (nostrils) penetrating the carapace, and
- Three pairs of jointed legs.

These characteristics taken together differentiate crawlas from all other organisms and place them in their own separate phylum named Crawla. Crawlas range from 5 to 10.0 centimeters in overall length, including antennae and tail.

Note: Genus names are capitalized and underlined or italicized. Species names are usually not capitalized but are also underlined or italicized.

PROCEDURES:

1. Print out the sheet of fossil members of the Crawlas phylum (Figure 3) and cut them apart. Arrange them on a sheet of paper to create a phylogenic tree showing the possible evolutionary history of the organisms. The fossil members of the family were found in different levels of rock from different geological periods, as noted under their drawings. Refer to the geological time scale in your text book to arrange the fossil members of the phylum in the phylogenic tree. Use tape to hold the specimens on the paper in their correct position.

2. Print out the specimens of the living phylum of Crawlas (Figure 4). Cut them apart and then identify each specimen as to the correct genus and species, based on structural similarities and differences. *For each specimen, follow the options presented in the dichotomous key at the end of the experiment*. Proceed down the key to the numbers indicated and continue making choices until all choices end.

3. On a sheet of paper, set up a table similar to the one below with rows for 15 specimens. Use this table to record the genus and species names for the specimens of the Crawla family you will examine.

Classification of Specimens from the Crawla Family

Specimen #	Genus Name:	Species Name
1		
2		
3		
4		
15		

QUESTIONS:

A. Were the characteristics of all of the living specimens able to fit in the key, or were there specimens that you had difficulty placing?

B. Were there some of the living Crawlas that were lumped together in the same species? If so do you think there were significant similarities to lump them together or should they be separated into different species? Defend your answer.

C. Were the characteristics of all of the fossil specimens able to fit in the key, or were there specimens that you had difficulty placing?

D. Can you hypothesize evolutionary links between fossil and living specimens based on the classification system?

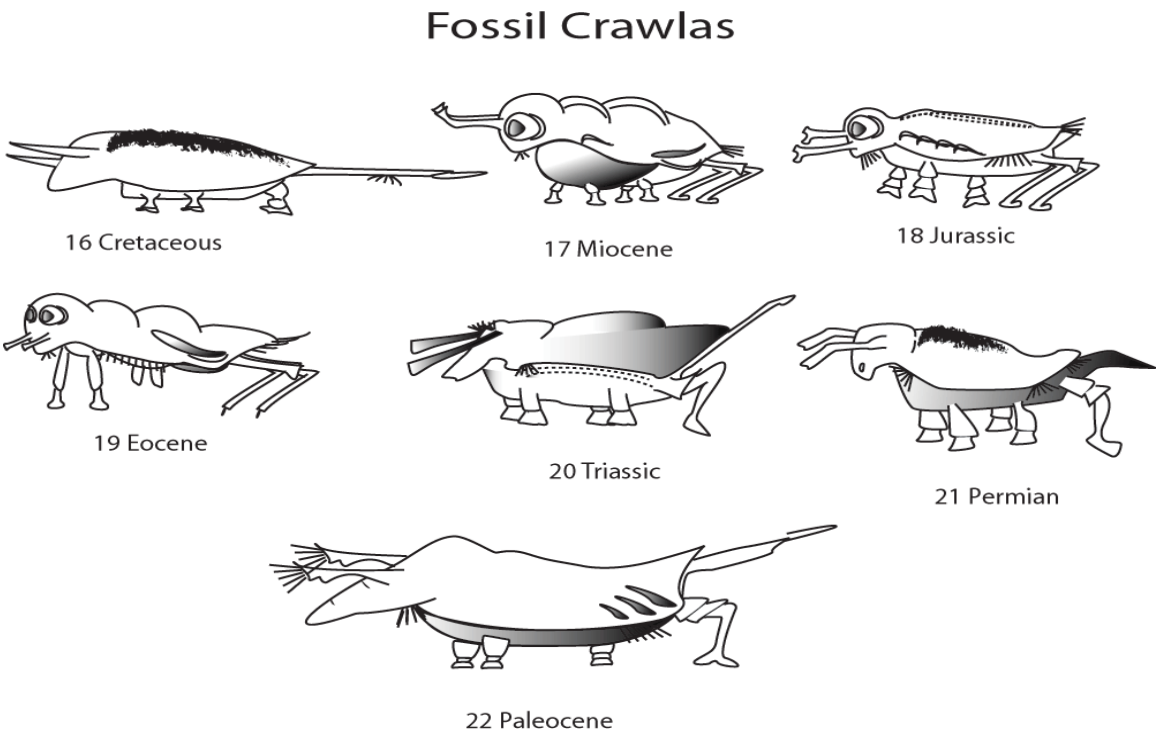

Fossil Crawlas
16 Cretaceous
17 Miocene
18 Jurassic
19 Eocene
20 Triassic
21 Permian
22 Paleocene

Figure 3

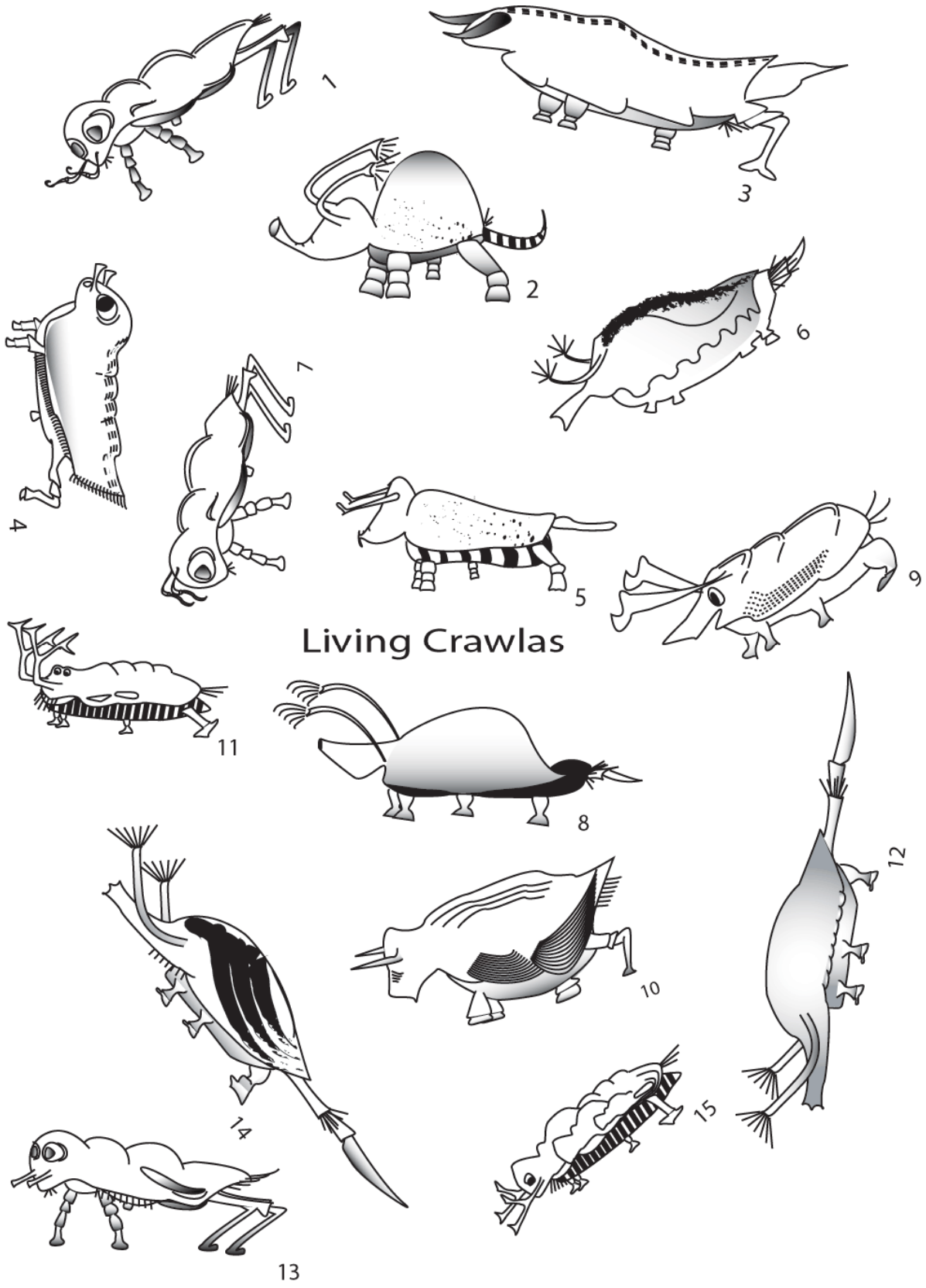


Figure 4

KEY TO THE GENUS AND SPECIES CLASSIFICATION OF THE FAMILY CRAWLA

1. a. The organism has well developed eyes, → go to **2**.
b. The organism is without eyes, or reduced eyes (smaller than 4 mm). → go to **5**.

2. a. The front legs show 3-4 segments → go to **3**.
b. The front legs show no segmentation, the carapace has no setae along the margin antennae are long and thicken at the distal end into a hook shape.
Genus, *Rhinopus*
One species, *hookei*

3. a. Front legs have 4 segments, → go to **4**.
b. Front legs have 3 segments, carapace has setae along the margin and perforations along the dorsal side. Antennae short and unbranched.
Genus, *Grasshopperus*
One species, *hairus*

4. a. Carapace shows four segments including head. Antennae attached above the eyes. Genus, *Fatopteris*
One species, *superious*
b. Antennae attached below eyes.
Genus, *Ratopteris*
Two species, Curved antennae, *curli*
Straight and short antennae, *straightus*

5. a. Organism has no eyes, →go to **6.**
b. Organism has small reduced eyes (smaller than 4 mm) and antennae are branched.
Genus, *Forbatensis*
One species, *curlabranchi*

6. a. Antennae are elongated and have distal setae tufts. → go to **7.**
b. Antennae have no distal setae, go to **9.**

7. a. Tail more than 2 cm long, with setae and segmentation,
Genus, *Molus*
One species, *blinda* note: some variation in carapace coloration and banding occur.
b. Tail less than 2 cm long generally only two segments seen some without segments, → go to **8.**

8 a. Tail unsegmented but banded, head with a long proboscis.
Genus, *Elephantus*
One species, bandei
b. Tail segmented, carapace varies between species.
Genus, *Sluggus*
Two species, smooth carapace, *smoothi*
Carapace hairy and margin notched, *notchilus*

9. a. Antennae entire not branched, go to → **10.**
b. Antennae branched carapace perforated,
Genus, *Bandus*
One species, *jailei*

10. a. Tail absent, setae at rear of abdomen, carapace lined.
Genus, *Torrus*
One species, *pointillus*
b. Tail present, antennae slightly curved. Carapace has perforations along the dorsal side.
Genus, *Bullanthus*
One species, *bullei*

Now that you have completed this week's lab make sure you read the lab for next week this will help you plan your time better. Take some time and highlight anything you will need to prepare in advance. As you read the lab write out a hypothesis for each exercise.

EXPERIMENT 12: Microbes Everywhere

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Part 1 takes 3 to 6 days to incubate. Allow 15-20 minutes per slide for Part 2.

Objective: To demonstrate the growth and characteristics of microorganisms.

Materials:

Student Provides:
- Paper towels
- Timer or clock
- Distilled water
- Candle, matches, or lighter
- Clear tape

From the LabPaq:
- Safety goggles
- Clinical face mask
- Disposable gloves
- Test tube clamp
- Thermometer
- Wax marker pencil
- Microscope slides, 5
- Field microscope
- Hand magnifying lens
- Metal spatula
- Staining tray

Experiment Bag:
- Sterile slant tubes of agar, 6
- Sterile swabs, 6

Gram Stain Kit:
- Crystal violet solution
- Gram Iodine stain
- Safranine stain
- Decolorizer

Discussions and review: Microbes are microorganisms such as bacteria, molds, and yeasts. They are around us all the time, and no matter how hard we try to clean and disinfect our environment, they always come back. Some microbes are carried in the air and then multiply when they find a hospitable surface. **Aerobic** microbes need oxygen to live, **anaerobic** microbes do not. Microbes tend to be found more often in some places than in others. Most microbes, especially those found around the home, grow best in warm areas like the kitchen and bathroom and the warm folds of the human body. They also grow best where there are ample nutrients for them such as body oils or food residue and dirt that accumulate from frequent handling and insufficient cleaning.

Since microbes can cause infectious diseases, it is important to know where they are most often found and to work to limit their numbers. In this laboratory exercise you will discover and compare the types of microbes found in your personal environment.

PROCEDURES: Part 1 - Finding Microbes

You will need: 6 sterile agar slant tubes, 6 sterile swabs, safe, warm incubation area. Note: Cultures need 3 to 6 days incubation period. Plan accordingly.

Because this experiment involves culturing microorganisms from a human environment, it is possible that unknown microbes may become incorporated into the sample. Any culture that may contain an unknown organism should be treated as potentially pathogenic. Therefore, be certain to wear the gloves and mask provided when handling the cultures to protect yourself from unintended exposure. Handle the cultures carefully and maintain an organized, clutter free work space to prevent spills. As with all materials in your biology LabPaq, use and store the cultures out of the reach of children and pets. **DO NOT CULTURE YOUR OWN THROAT OR OTHER BODY FLUIDS AS THEY MAY CONTAIN BACTERIA THAT WHEN CULTURED COULD INFECT SOMEONE ELSE.**

1. Consider where might be the best conditions in your home for microbes to thrive. Make a list and prioritize it based upon the above information about conditions favorable to microbe growth. Some places to consider are:

   - electric light switches
   - door knobs
   - residue around a bathtub or shower
   - inside the end of a water faucet
   - exhaust hood over the kitchen range
   - drain pan under the refrigerator
   - inside the garbage disposal (be sure to turn it off first!)
   - anywhere around the kitchen where food might spill

2. Select six sources from your prioritized list that are from different areas of your home. Set up a data table similar to the one shown below to number your microbes and record information and observations about them for Part 1 and Part 2 activities.

Location Of Microbes			Colony Growth Notes				Gram Stain Notes				
#	Description	Temp	Shape	Color	Size	Type	Shape	Color	Size	Type	Observations
1											
2											
3											
6											

3. Select a place where you can incubate cultures of the microbes you will collect. Chose a warm spot where the cultures will not be disturbed and cannot be reached by small children and pets. An ideal site is often the top of a hot water heater. If you choose to use your hot water heater, first place a multi-folded bath towel on top of the heater surface, and then place the incubation tubes on the towel. The towel will keep the tubes from rolling off plus provide insulation to keep them from overheating.

   Electrical equipment tends to generate heat and makes another potential incubation site. Consider areas around electronics that run most of the time such as a transformer, computer, or stereo. (As far as we know noise does not bother microbes.) There are usually lots of warm spots around a kitchen. However, if you incubate cultures in your kitchen, NEVER open the tubes around where food is prepared or eaten. Rather, the culture tubes should be sealed when you take them into the kitchen and not opened until you leave the kitchen.

4. Mark the six sterile slant tubes of agar with a wax marker pencil, numbered 1 through 6. These numbers will correspond to the microbes you will collect from the sources you numbered 1 through 6 in Step B above. ***Do not open the agar incubation tubes until you are ready to place specimens inside them.***

5. To collect smears of your six microbe sources, carefully follow these instructions for each one. Systematically collect only one microbe source at a time.

   a. First measure and record the temperature at the collection site.

   b. Open the cap of the correspondingly numbered sterile agar slant incubation tube for the source number to be tested. Let the opened cap rest over the mouth of the tube until you are ready to use it. Your objective is to have the sterile tube ready to accept a smear while at the same time limiting its exposure to contaminants

   c. Unwrap a sterile swab and tightly grip its stem in your dominant hand, usually the right hand. Wipe only the <u>end tip</u> of the swab firmly across a small area of the selected surface. Do not swirl the swab or touch anything else with it. You should perform this and the next step fairly quickly, yet very carefully.

   d. Remove the cap of the incubation tube and hold the open tube at eye level in the hand opposite the one grasping the swab. Very, very carefully insert the swab into the incubation tube and firmly touch the contaminated end of the swab tip to the center of the agar slant tube. Ensure the swab tip contacts nothing but this designated site on the agar.

   e. Recap the incubation tube; place the used swab in the trash, and repeat the above steps a. through d. for the next of your five microbe sources.

6. Place the six tubes containing microbe sources in the site previously selected for incubation. Make sure everyone in your home knows what the incubation tubes are and where they are located ***and that they should never be disturbed and especially that they should not be opened***. Leave a note stating "Experiment in Progress – Do NOT Disturb" with the tubes while they incubate for three full days.

7. Keep the culture tubes closed! Use the hand magnifying lens to observe the growths of colonies within the tubes. Note their shape, size, color, and anything else distinguishing. Bacteria will grow in small circular colonies, whereas molds will spread out more and may look fuzzy. Yeasts tend to grow initially in tight, compact colonies and their color is somewhat darker than that of bacteria. Record what you see in the data table. Draw what you see for each. Try to deduce the types of microbes each cultured colony contains: bacteria, mold, or yeast. Some cultures may contain more than one type of microbe.

8. If some of the cultures are not yet well developed let <u>all</u> of the cultures continue to incubate for up to three additional days until you see visible growth in them all. Record final observations for each tube..

Questions:

A. From which samples did you observe the most and least microbial growth?

B. Why do you think there were differences in the number and types of microbes at the different sampling sites?

C. Did microbes from warmer or cooler sites multiply faster? Hypothesize why.

D. How could the information gained in this experiment be useful in your home?

Part 2 - A Closer Look

You will need: safety goggles, disposable gloves, face mask, paper towels, microscope slides, metal spatula, gram stain experiment bag containing crystal violet stain (#1), PVP iodine stain (#2), decolorizer (#3) and safranine stain (#4), staining tray, clean water to rinse slides, candle or matches.

Most of the microbes that you have cultured are very small, much smaller than you can see individually with a hand lens and even a 150-power field microscope. However, at 150-power magnification you will be able to see the culture's structure and possibly some individual microbes. That is why you will now prepare microscope slides and take a closer look at your home's microbes.

To make the microorganisms stand out in relief from the background light it is necessary to stain them. A common way of doing this is with Gram stain, a process using a sequence of stains that divide all microorganisms into two categories, either gram-positive or gram-negative. **Gram-positive** microorganisms will hold the *blue color* of

the first stain in the sequence and will appear blue at the end of the process. **Gram-negative** microorganisms will lose the blue color of the first stain in the decolorizing step, but will hold the color of a subsequent stain, and will finally appear *red* in color.

Past research has identified certain bacteria or pathogens as gram-positive or gram-negative; thus gram staining is often used in medical laboratories as a first step in diagnosis. The color results from gram staining quickly narrows down the possibilities during the microbe identification process. For example, the Streptobacillus from which we get "strep throat" is a gram-negative microorganism. When the medical laboratory cultures and stains the swab from your throat and the final color of the stain is blue, they know that you do not have strep throat.

Important: *Wear a clinical face mask, disposable gloves, and safety goggles at all times during this part of the experiment.* *Some microbes can become airborne and you do not want to breathe them in. Do not touch your face, especially your mouth and eyes, during this part of the lab exercise. At the end of the experiment, place the face mask and gloves in the trash and wash your hands and equipment thoroughly. It is wise to perform this experiment in an area where food is not prepared or eaten. While most of the cultures will be harmless, it is always possible to produce one that is contagious, so don't take chances!* ***Put on your mask, gloves, and goggles!***

1. Select four of the six cultured microbes from which to prepare microscope slides. Ideally you should choose the culture with the largest growth plus, assuming they exist, one each of the cultures that appears to be bacteria, yeast, and mold per your earlier identification. In your data table, note the four culture selections next to their corresponding numbers, source names, and suspected types. Label four microscope slides with the corresponding numbers of the four sources selected.

2. As detailed below, successively process the four selected cultures onto separate microscope slides. These instructions are not complex, but they are long with many steps in this process. Read the instructions and fully understand all the steps before you begin. Complete all work for each slide before going on to the next culture.

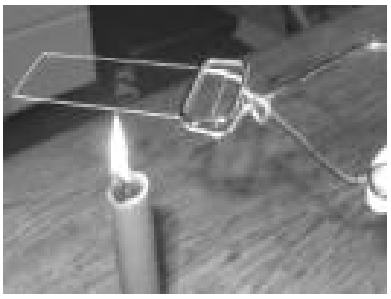
    a. Light a candle or have a match or lighter ready to ignite.

    b. Place a clean microscope slide on a flat surface and arrange it to hang over the side of the surface so you can later grasp it with the test tube clamp.

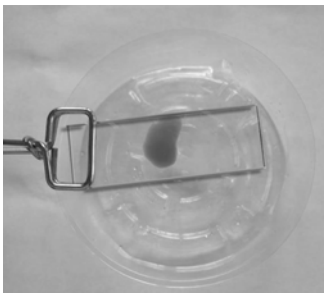
    c. Open the culture tube containing your selected culture. Carefully insert the LabPaq's metal spatula into the culture tube and scrape off a small portion of the targeted culture. Recap the culture tube.

    d. Grasp the clean microscope slide with the test tube clamp and smear the removed culture onto the center of the slide.

e. Sterilize the metal spatula tip by slowly passing it through the flame of a candle, match or lighter flame for five seconds. Lay the spatula on a clean paper towel that can be used to wipe off any soot or other material collected on it.



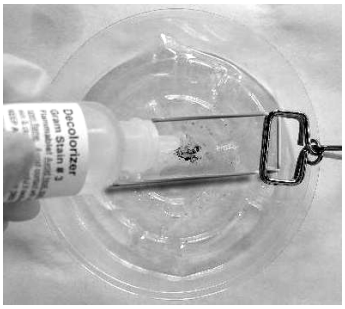
f. Pass <u>the underside</u> of the smeared microscope slide back and forth a few inches above the tip of a candle, match, or lighter flame. You want to heat the smear until it is dry, but not burn it. The heat fixes the culture on the slide so it will not wash off during the staining process



g. Hold the slide over the staining tray from the kit as shown in the photo on the right, place three drops of crystal violet solution (Gram stain #1) on the smear, and let it stand for one minute. Crystal violet is a very strong stain. Do not let it contact anything that can be damaged. If you must set the stained slide down, set it in the tray.

h. After one minute, lightly rinse the stained slide by briefly passing it through a thin stream of running tap water. Gently shake the excess tap water from the slide into the sink and rinse the excess down the drain. Note: these slides can also be rinsed by dunking them into a glass of clean water several times and then shaking the excess water into the staining tray or onto a paper towel.

i. While holding the slide over the staining tray, place three drops of PVP Iodine (Gram stain #2) on the smear and let it stand for one minute. Iodine is also a strong stain so do not let it contact anything that can be damaged.



j. After one minute, again lightly rinse the stained slide by and gently shake off excess tap water.

k. While holding the stained slide over the staining tray, slowly and repeated drop single drops of decolorizer (Gram stain #3) onto the smear, as shown, until the drops running off the slide no longer have any color. Briefly rinse the slide again r and gently shake off the excess tap water.

l. Place three drops of safranine (Gram stain #4) on the smear and let it stand for ***30 seconds***. Safranine is also a strong stain so do not let it contact anything that can be damaged.

m. After *30 seconds*, briefly rinse the smear again and gently shake off the excess tap water.

n. Gently blot the unsmeared areas of the slide with a paper towel or tissue, but NOT the actual smear area. Let the slide air-dry for a minute or two.

o. Note the color of the stained material on the slide. Examine the slide under your microscope. Describe and draw what you see. Do not wash the slide yet. Keep it for now so you can compare it to the other slides.

p. Repeat steps a. through o. above to successively stain and examine slides for each of the other cultures selected.

3. After you have finished examining all the slides and answered the following questions, wash the slides well with detergent, rinse them with distilled water, and allow them to air-dry before returning them to your LabPaq. Properly clean all other equipment. Place the sealed incubation tubes and used paper towels in the trash.

Questions:

A. Was the structure or arrangement of the colonies of microorganisms different among what you identified as bacteria versus yeast versus mold?

B. Four slides are, of course, a very small sample, but regardless of this limitation, what can you hypothesize about differences in the microbe growth patterns? Does your hypothesis match the descriptions at the beginning of the experiment?

C. Were you able to see any individual microorganisms? If so what would you guess they are (e.g., mold, yeast, etc.)?

D. Do any of the slides appear to have more than one type of microorganism? Did you determine this by physical appearance of the culture or by color of stain?

EXPERIMENT 13: Conditions to Grow Molds

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Part 1 takes 4 to 6 days to incubate. Part 2 takes a further 3 to 4 days. Plan accordingly.

Objectives: To hypothesize the conditions that a bread mold needs for growth,
To determine if bread mold can use a variety of food sources.

Materials:

Student Provides:
- Paper towels
- Distilled water
- Bread without preservatives
- Dehydrated potato flakes
- Raisins

From the LabPaq:
- Safety goggles
- Petri dishes, 4
- Thermometer
- Wax marker pencil
- Field microscope
- Hand magnifying lens

Discussion and Review: Molds are organisms that belong to the Kingdom Fungi. They are different from bacteria and protists in that they are usually multi-cellular, their cells have nuclei, and they are often large enough to be seen without a microscope.

Fungi have no chlorophyll and are therefore not able to make their own food. Organisms in this kingdom are either **saprophytes**, **mutualists**, or **parasites**. ***Saprophytic*** fungi feed on once-living material; ***mutualistic*** fungi obtain their food by living in close association with other living organisms such as algae; and ***parasitic*** fungi feed on living material.

PROCEDURES:

Part 1 – Growing Mold

It takes 4 to 6 days to grow bread mold. Plan accordingly

1. Form a hypothesis of how various factors such as temperature and light will affect the growth rate of mold.

2. Line the bottoms of three petri dishes with damp paper toweling, mark them #1, #2, and #3. Leave another petri dish dry and without paper toweling and mark it #4.

3. Obtain a thin slice of fresh bread from a bakery or other source that, unlike most commercial breads purchased in supermarkets, does NOT contain preservatives.

Homemade bread is best. Tear the bread into four approximately 5-cm diameter pieces that will fit inside the petri dishes. Use the soft part of the bread rather than the crust. Place one piece of bread in each of 4 petri dishes and replace the covers.

4. Place the containers in four different locations, each with a different temperature level.

5. Design a data table and record by its number each petri dish's location, and the location's temperature. One location should be cold, such as in the refrigerator. Another should be at room temperature or above but in the dark, such as in a drawer. The third should be at room temperature or above but in the light, such as in a window sill. The fourth dry petri dish should be placed somewhere warm, such as in the kitchen or over the water heater. Leave all the petri dishes undisturbed for at least four days. Check that the towel under the bread remains moist in the first three dishes. Add a few drops of water to remoisten the paper towel if needed.

6. Based on the conditions in which you have placed the four pieces of bread, record hypotheses as to which of the dishes will grow mold and relatively how fast.

7. On the fifth day observe the four specimens with a hand lens. Record observations for each dish. If there are no changes, replace the specimens in their respective petri dishes in their previous locations and leave them for three more days.

8. ***Save the two best cultures of mold for Parts 2 and 3.*** Wash the petri dishes and covers with dish detergent, rinse thoroughly, and allow them to dry.

Questions:

A. In which dishes under which conditions did mold grow?

B. Was your hypothesis proven correct? If not why do you think your hypothesis was wrong?

C. What combination of conditions do you conclude are the best for mold to grow?

Part 2 – A Closer Look

Bread mold has tiny black round structures called **sporangia** located on the tops of long stalks called **sporangiophores**. Reproductive spores form in these structures. As the mold grows, a mass of threadlike structures called **hyphae** spreads along the surface of the mold's food supply, enabling the mold to secure its food. The **hyphae** that penetrate the food supply are called **rhizoids**. Note: Though bread molds are generally considered harmless, it is best to wear a face mask when working with any molds.

1. Use a scalpel and cut a thin slice about 1 to 2 mm thick from the surface of the mold that has formed on the piece of bread. Place a piece of white paper on a table

surface, place the slice of mold on the paper, and place the field microscope over the piece of mold.

2. Examine the mold through the microscope and identify the sporangia, the sporangiophores, the hyphae, and the rhizoids. Draw what you see.

Part 3 – Other Food Sources

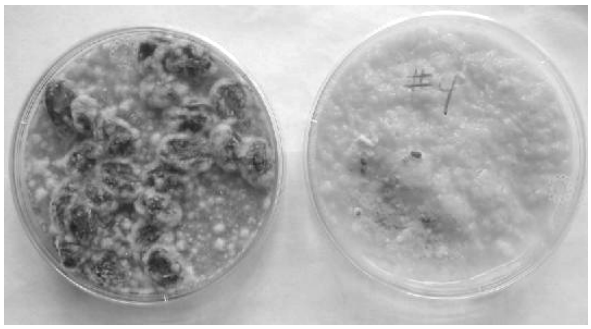
Molds will grow on a variety of food sources. This experiment will demonstrate the variety of food sources that can support molds and will also demonstrate whether or not molds require moisture to grow.

1. Place dehydrated potato flakes in the bottoms of two petri dishes. In one of the dishes add enough water to cover the bottom and make the flakes turn into a paste.

2. Place several dried raisins in the bottoms of two more petri dishes. In one of the dishes add enough water to halfway cover the raisins.

3. Dampen a cotton swab and rub it over the surface of the mold that has grown on a piece of bread saved from Part 1. Then rub the swab over the surface of the contents of the first petri dish.

4. Repeat step 3. above with a fresh damp cotton swab for each of the other three petri dishes.



5. Place covers on the petri dishes and leave them at room temperature for three days.

6. After three days examine each dish with the hand magnifying lens for the presence of bread mold. Record your observations.

7. Wash the petri dishes and covers with dish detergent, rinse thoroughly, and allow them to dry.

Questions:

A. In which dishes did mold grow?

B. What can you conclude about the variety of foods that will grow mold?

C. What is the effect of moisture on the growth of mold?

EXPERIMENT 14: Plant Structures

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow 30 - 45 minutes of observation time for Parts 1 and 2 plus 2 hours for Part 3

Objectives: To examine the structures of roots and identify their key parts
To examine the structures of stems and identify their key parts
To observe the effect of the transport of liquids in stems

Materials:

Student Provides:
- Fresh parsnip root, may substitute fresh carrot
- Fresh cut tree or shrub branch stem
- Fresh celery stalks
- Paper towels
- Drinking glasses, 3

From the LabPaq:
- Safety goggles
- Microscope
- Microscope cover slips, 4
- Red food coloring
- IKI Solution
- IKI Dropping pipet
- Scalpel and metric ruler from dissection kit

Discussion and Review: In spite of the almost endless diversity of plant life, from herbs and flowers to shrubs and trees, all **vascular plants** share the same basic structural elements. They are all composed of cells, tissues, and organs. Nutrients, minerals, and water are transported through them by two types of **vascular tissues**, **xylem** and **phloem**. Xylem's tubular cells transport water and minerals from the roots to the rest of the plant. There are three types of xylem cells in seed plants; they are: tracheids, vessel elements, and fibers.

Tracheids and **vessel elements** are both tubular cells that transport water throughout the plant. The cells of tracheids and vessel elements die as the plant matures. Yet, their cell wall structures remain and water and nutrients may continue to pass through and between the dead cells and into the living plant. The cell walls between tracheids have pits through which water and minerals flow. The vessel elements are wider and shorter than the tracheids and have openings in their end walls. In some plants, the mature vessel elements lose their end walls, and water and minerals flow freely and easily from one cell to another. While all plants have tracheids, not all have vessel elements. These are most commonly found in **angiosperms** or flowering plants**,** which may help to explain why these plants are the most successful plants on earth.

Phloem tissue transports sugars and other organic compounds from the leaves, where they are created, to all other parts of the plant down to and including its roots. Phloem is similar to xylem in that it is made up of cylindrical cells, but phloem cells do not die at maturity. Phloem cells are called **sieve tube members** and are unusual because, although they contain cytoplasm, they have no nucleus or ribosome.

Next to each sieve tube member is another type of phloem cell, a companion cell that is nucleated and helps to manage the transport of sugars and other organic compounds. In flowering plants**,** the end walls between two sieve tube members are called **sieve plates**. They have large pores. Sugar and other compounds move from cell to cell through these plates. The vascular tissues of many plants also contain **fibers,** but fibers do not transport materials but provide essential support for the plant.

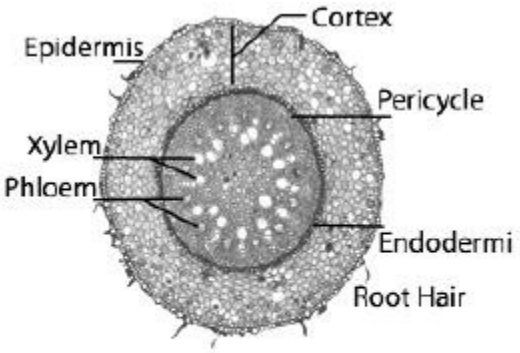
PROCEDURES: Part 1: Structure of Roots:

Roots are the underground parts of plants. They anchor the plant, absorb water and minerals from the soils, and transport these materials to the stem and the rest of the plant. Some plants like carrots, potatoes, and parsnips also accumulate and store food in their fleshy roots. The total surface area of a plant's roots can be as much as 50 times larger than that of its leaves. Roots systems vary widely according to the needs of the plant.

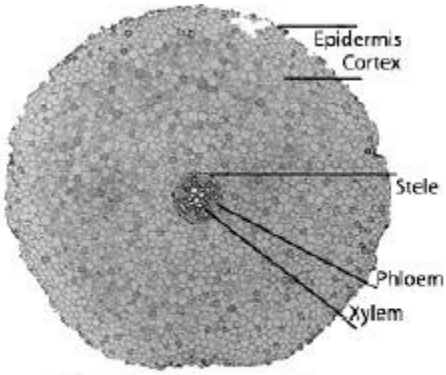
In the cross section of a typical root you will see that the **epidermis** forms the outermost layer of cells. **Root hairs** like those of the carrot in the photo at the right are extensions of a single epidermal cell that increases the contact area of the root with the soil and improves its ability to absorb water and dissolved minerals. The next layer is a part of the ground tissue called the **cortex**; it includes all tissues other than the dermal tissues and the vascular tissues. The cortex is involved in the transport of water and ions into the vascular core at the center of the root. It is made up of **parenchyma cells** that can store food and water.

At the inner edge of the cortex is the **endodermis**, a single layer of cells that forms a waterproof seal surrounding the vascular tissue. This waterproof seal between each cell of the endodermis forces all water and minerals to pass through the endodermis, controlling the flow into the root and thus into the rest of the plant.

Xylem and phloem are located in the center of the root. The arrangement of these tissues is one of the major differences between monocots and dicots. In **dicot roots** the xylem forms a small central star shaped mass with phloem cells between the rays of the star. **Monocot roots** have strands of xylem alternating with strands of phloem in a band around a central core of parenchyma cells that is called pith.



Monocot Root Cross section
Vascular bundles exist as a wide ring around the central core.



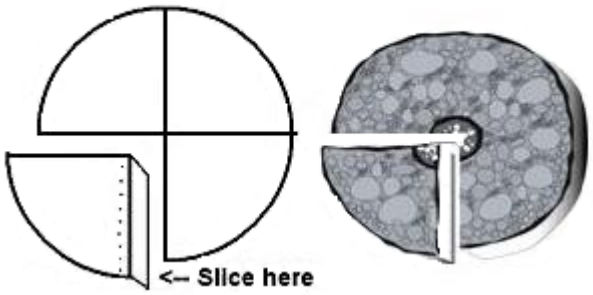
Dicot Root Cross section
Vascular bundles are centralized within a stele.

1. Select an area of a fresh parsnip root that is about 20 mm in diameter. Note a parsnip is shaped like a carrot but paler in color like a turnip. If your grocery does not carry parsnips, you may substitute a carrot root.

2. Make a clean straight cut across the root, exposing a cross section. Examine the cross section with your hand lens. Draw and label the parts that you can see.

3. Make a second cut across the root to obtain a cross section of the root about 0.5 mm thick. You will then have a small disk of root, a bit smaller and thinner than a dime. Cut this disk into quarters. Slice a thin section from the edge of one of the quarters. You should then have a very thin rectangle of root, about 5 mm x 10 mm. This is called a longitudinal section.

How to cut a longitudinal section of a root



4. Place the cut longitudinal section on a microscope slide. Add a several drops of IKI solution. Allow it to soak for 15 seconds and then blot the excess dye from the edges of the slide with a paper towel. Add a cover slip to the slide.

5. Examine the slide under the microscope. Slowly move the slide across the field of view so you can observe all the different types of the root section's tissues. You should see two different types of cells. Those toward the center of the root look like parallel tracks and are transporting cells. Those toward the outer circle of the root are rounded or squared and packed tightly together; they are storage cells. Storage cells are much smaller than the transporting cells. Since they contain starch, they will appear blue from the IKI stain. Draw what you see and label all the different tissues and cells.

6. Carefully slice another cross section of the root. This time slice it as thin as possible while still keeping the section intact. It should be thin enough that when you hold it up to a light, you can see light shining through the section. This is what is formally called a cross section or a thin section.

7. Place the thin section on a microscope slide. Add several drops of IKI solution. Allow it to soak for 15 seconds and then blot the excess dye solution from the edges of the slide with a paper towel. Add a cover slip to the slide.

8. Examine the slide under the microscope. Slowly move the slide across the field of view so you can observe all the different types of the cross section's tissues. Draw what you see and label all the different tissues and cells.

Questions:

A. Identify in your drawings and describe the function of each of the following:

- xylem
- phloem
- epidermis
- root hairs
- endodermis
- cortex

B. What are the overall functions of the two major regions of the root?

Part 2 - Structure of Stems

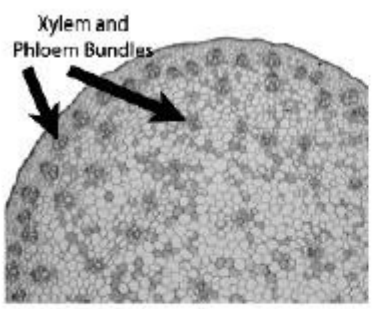
Stems are the above ground parts of plants that support the leaves and flowers and transport water and nutrients through the plant. Stems range from the **herbaceous** stems of annuals that die back every year, to the massive trunks of trees that live for centuries. Green herbaceous stems are soft and flexible and usually also carry out some photosynthesis. Woody stems from most trees, shrubs, and perennials, are hard and rigid and contain strands of xylem and **schlerenchyma** fibers.

Stems provide support for all the above ground parts of the plant. The vascular tissues that run the length of the stem transport mineral ions and sugars to and from the leaves and roots. Like roots, certain adapted forms of stems can also store food.

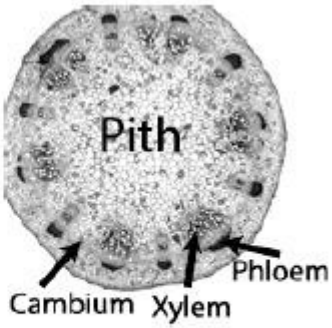
Also like roots, stems have vascular tissues, though they are arranged differently than in roots. Stems have a bundled or circular arrangement of vascular tissues within a surrounding mass of **parenchyma** tissue. Dicots and monocots have different arrangements of these vascular tissues in their stems. In dicots, the xylem and the

phloem are bundles that form a ring in the cortex. In monocots, the bundles of xylem and phloem are scattered throughout the stem.

Conifers and perennial dicots produce thick woody stems that may live years or decades. As the stems of these plants grow in length they also grow in thickness. This growth in thickness, called secondary growth, is a product of cell division in the **vascular cambium**. Mature xylem cells are dead, and the xylem tissue produced by secondary growth is called wood. In temperate regions, the layers of vascular tissue produced each year by secondary growth make up the tree's annual growth rings. This vascular tissue also often contains sclerenchyma fibers that support the plant.



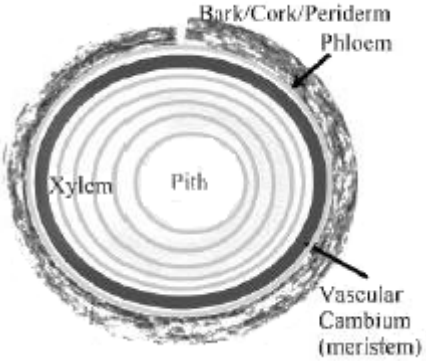
Monocot Stem Cross section with Scattered Vascular Bundles



Dicot Stem Cross section with Vascular Bundles arranged around Vascular Cambium

As secondary growth continues, a new band of xylem is produced each year and the previous layers of xylem are compacted into the core of the stem. The normal abundance of water each spring produces large xylem cells, and the shortage of water later in the summer produces smaller cells. The difference between the sizes of spring and summer xylem cells is what forms tree rings, the visible line that separates one year of growth from another.

The outer portion of woody stem develops a layer of bark, composed of phloem cells and **cork cambrium**. Bark is a tough, cork-like tissue that protects the stem from damage by browsing herbivores and burrowing insects. Under the bark is a layer of phloem and under that is the vascular cambium that produces the cells that make up the secondary growth. Next is the most recent layer of xylem. Water, required by the plant leaves, moves up from the roots through the fresh xylem, with water molecules forming an unbroken column within the xylem. Sugars and minerals are transported along the stem through the phloem.



1. Select a stem of a young bush or tree that is 15 to 20 mm in diameter. Make a clean straight cut across the stem, exposing a cross section. Examine the cross section with the hand magnifying lens. Draw and label the parts that you can see.

2. Make a second cut across the stem to get a cross section of the stem about 0.5 mm thick. You will then have a small disk of stem, a bit smaller and thinner than a dime. Cut this disk into quarters, and then, from one of the quarters, slice a thin

longitudinal section from one cut edge to yield a very thin rectangle of stem about 5 x 10 mm.

3. Place the thin section on a microscope slide. Add several drops of IKI solution. Allow it to soak for 15 seconds and then blot the excess dye from the edges of the slide with a paper towel. Add a cover slip to the slide.

4. Examine the slide under the microscope. Slowly move the slide across the field of view so that you observe all the different types of tissues of the stem thin section.

5. Draw what you see and label all the different tissues and cells you can see.

6. Carefully make another cut across the original cross section of the stem slicing off the thinnest cross section that you can while keeping the section intact.

7. Place the thin section on a microscope slide. Add several drops of IKI solution. Allow it to soak for 15 seconds and then blot the excess dye from the edges of the slide with a paper towel. Add a cover slip to the slide.

8. Examine the slide under the microscope. Slowly move the slide across the field of view so that you observe all the different types of tissues of the stem section.

9. Draw what you see and label all of the different tissues and cells.

Questions:

A. Identify in your drawings and describe the function of each of the following:

Cork	Cork cambrium
Cortex	Pith
Spring xylem	Summer xylem
Vascular cambium	Phloem
Bast fibers	

B. Each new band of xylem forces the older bands of xylem toward the center of the stem. Next to which stem tissue is the oldest band of xylem found?

C. Did the IKI solution cause any of the tissues in the stem to turn blue? If so, what would you guess is the function of that tissue?

Part 3 – Transport of Water:

Water travels through the xylem of plant tissues toward the leaves. This exercise will demonstrate the rate of that movement and how it is, in part, related to the transpiration pull of the leaves.

1. Dispense equal amounts the red food coloring supplied into the three clear glasses. Then measure and add 2.5 cm of tepid tap water into each of glasses.

2. Select three stalks of fresh celery, one with no leaves, one with few leaves, and one with many leaves. Trim 1 - 3 mm off the bottom of the stalks with a sharp knife. Place one stalk into each of the three separate glasses of colored water.

3. Measure how far the colored water has traveled up each celery stalk after 5, 10, 20, 40, 60, and 120 minutes. Create a data table and record your measurements.

Questions:

A. How did the data differ among the three celery stalks? Explain your answer.

B. What do you predict will happen after 24 hours?

C. How did the number of leaves affect the water uptake of the celery stalks?

D. Explain how transpiration pull is involved in your results.

EXPERIMENT 15: Homeostasis

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow at least 90 minutes to perform this experiment

Objectives: To observe how the human body responds to changes detected in the external environment to maintain homeostasis
To identify the location of some of the body's receptors that detect changes in the external environment.

Materials:

Student Provides:
- An assistant
- Wall mirror
- Paper
- Hot and cold water

From the LabPaq:
- Metric ruler
- Graduated cylinder
- Beaker
- Thermometer
- Wax marker pencil
- Cork
- Pins
- Plastic glasses, 4

Discussion and Review: Homeostasis is the tendency of a body to maintain internal constancy and independence from its surrounding environment. It is a characteristic of life and a process that occurs in all living things. The ability to respond to stimuli in the environment is vitally important to maintaining life. This ability is an obvious characteristic since many of the structures and behaviors of living organisms are specifically adapted to their environment and to maintaining their species' survival.

In addition to responding to external stimuli, living things also respond to internal changes. Organisms must make constant internal adjustments to maintain a proper body temperature and to keep the correct amount of water and minerals in their cells. Such responses require the controlled use of energy within the organism's cells. Changes in the internal environment must be stabilized by the physiological mechanisms of homeostasis. Internal changes arise from two basic sources:

1. **The External Environment:** An organism's internal environment responds to changes in its external environment, such as temperature, pressure, or chemical assault.

2. **Metabolic Activities:** All metabolic activities require a constant supply of oxygen, nutrients, salt, and other minerals that cells withdraw from their environment and that must be replaced.

Homeostasis is maintained by the coordinated activities of the nervous, circulatory, and endocrine systems, and especially by organs such as the lungs, skin, eyes, ears, and digestive tract. These are all specific sites where materials and/or stimuli exchange with the external environment.

PROCEDURES: Part 1 – Vision:

The eyes are a primary organ for warning the body of any assault from the external environment. Our body reacts reflexively to danger perceived by the eyes rather than waiting to make a conscious thought or decision. The eyes themselves react to the amount of light striking them by changing the diameter of the pupil through which light reaches the eyes' sensors. This protects the eyes from too much light and makes them more sensitive in the dark.

1. Make several small balls of any kind of crumpled paper, notebook, newspaper, etc.

2. Put on your safety goggles and stand facing a wall mirror in a well lit room. If you do not have a wall mirror, stand in front of a glass window at night when it is dark outside; when you turn on bright light in the room, the window will act as a mirror and reflect your image. Stand about three feet from the mirror or window.

3. Have an assistant stand behind and a little to one side of you, and throw the paper balls, one at a time, at the image of your face in the mirror. Describe how you feel as your eyes respond. Ask your partner to describe how your eyes responded. Record these observations.

4. Still facing the mirror or window, have your partner pinch the skin at the back of your neck. Describe how you feel as your eyes respond. Ask your partner to describe how your eyes responded. Record these observations.

5. Stand one foot from the mirror. Observe the diameter of the pupils of your eyes.

6. Cover one eye with your hand for one minute, leaving the other eye open. Remove your hand and quickly observe the diameter of the pupils in both eyes. Repeat the same procedure with the other eye. Record these observations.

7. Close both eyes for one minute and have your assistant observe the size of the pupils of your eyes when you open them. Have the assistant continue to observe for one minute and describe any changes in the diameter of the pupils of your eyes. Record these observations.

8. Have your assistant slice open an onion a few feet in front of you while watching your eyes. Describe how you feel as your eyes respond. Ask your partner to describe how your eyes responded. Record these observations.

Questions:

A. Did any of the responses of your eyes require conscious thought?

B. Do you think that you could have controlled or prevented any of the reactions?

C. In seeing the onion cut what is your body anticipating?

D. What are the four basic reactions that you have observed in the experiment? For each of the four describe how this reaction or reflex is a defensive mechanism for your body and how it maintains homeostasis.

Part 2 – Touch:

There are sense receptors in your skin that warn your body if it is being touched by something in the external environment. The concentration of the skin's receptors varies in different parts of the body. Some areas like fingertips have a very high concentration of receptors and are used to feeling and determining the shapes of objects, but other skin surfaces may have only enough sensors to detect larger objects. When two separate points are simultaneously touched to the skin, the minimum distance apart at which they are sensed as two separate and distinct points is the same distance apart that that the receptors are located in that area of skin.

1. Insert two dissection pins into a cork so that the heads are 0.5 cm apart. Gently touch the heads of the pins to your forearm, to the back of your hand, to your palm, to a fingertip, to your cheek, and to your lip. For each touch note if it feels like two points or one. Set up a data table of touch points by distance between points like the one below to record your observations. Feel free to add other skin location columns such as stomach, thigh, etc.

Pin Point	Nerve Receptor Concentration Test					
Distance	Forearm	Hand Back	Palm	Fingertip	Cheek	Lip
0.5 cm						
1.0 cm						
1.5 cm						
2.0 cm						
.... etc.						

2. Rearrange the pins so that the heads are 1 cm apart. Repeat the process of touching the same areas and observe for each if it feels like one point or two. Record your observations.

3. Repeat the process increasing the distance between the points by 0.5 cm each time until all skin location contacts distinctly feel the two points.

Questions:

A. Which areas touched recognized the two points as two points from the least distance apart?

B. Would these areas coincide with areas of skin that you consider generally more sensitive?

C. What role does the greater sensitivity of these areas play in defending the body against external contact?

D. How does this defense sustain homeostasis?

Part 3 – Temperature:

Detection of temperature is another critical defense of the body. Homeostasis demands that the human body reflexively avoid objects or environments that are significantly warmer or cooler than the core body temperature of about 37 degrees C (98.6 degrees F). Receptors in our skin are constantly telling the body that the environment is too hot or too cool, and that the body is therefore gaining or losing thermal energy. Homeostasis continually causes our bodies to try to adjust this imbalance through changes in our behavior and/or in our metabolic rate.

Temperature senses can be unreliable, sometimes sensing the wrong imbalance because of contrast to another sensation or because the sensors have been desensitized or super sensitized. However, this is complicated by the fact that in sensing contrasts, the skin sensors are also defending the body not only against imbalance of temperature but also against abrupt changes of temperature. The following will demonstrate such deceptions.

1. Prepare four glasses of tap water at different temperatures. Use combinations of hot and cold water from the faucet and some crushed ice achieve temperatures of approximately 10°C, 20°C, 30°C, and 45°C respectively in the four glasses. Use the wax pencil to write the temperature on each glass.

2. Place the index finger from one hand into the 45°C water and the index finger from the other hand into the 20°C water; hold them there for 30 seconds.

3. After the 30 seconds place both fingers in the glass of 30°C water. Note the sensation in each finger. Dry your hands and record your observations.

4. Place the index finger from one hand into the 45°C water and the index finger from the other hand into the 30°C water; hold them there for 30 seconds.

5. After the 30 seconds place both fingers in the glass of 10°C water. Note the sensation in each finger. Dry your hands and record your observations.

Questions:

A. In what conditions were the sensations of temperature false? That is, where did you sense heat or cold when the water contacted was not actually hotter or colder respectively, than core body temperature? Why do you think this happens?

B. How is the sense of temperature important to homeostasis?

C. How can the false senses of temperature be also important in homeostasis?

EXPERIMENT 16: Muscle Fatigue

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow at least one hour to perform this experiment

Objectives: To observe how fatigue affects the number of repetitions of an exercise one can accomplish and
To hypothesize how this relates to muscle function.

Materials: Student Provides: Timer or clock
Exercise equipment (optional)
Partner (optional but preferable)

Discussion and Review: Nearly half of normal body mass is muscle; the contraction and relaxation of those muscles produce the movement of body parts. In this process, muscles use up energy in the form of **ATP, adenosine triphosphate**. ATP is produced from aerobic and anaerobic cellular respiration in the muscles. The aerobic respiration dominates when adequate oxygen is delivered to the muscle cells if a muscle is at rest or during moderate activity. But when an adequate supply of oxygen cannot be maintained during vigorous activity, anaerobic respiration, specifically the process of lactic acid fermentation, becomes the primary source of ATP production.

As you continue to exercise, at some point your muscles are unable to get oxygen fast enough to sustain the anaerobic respiration. ATP becomes in short supply. As the anaerobic process becomes dominant, lactic acid builds up in your muscles. As excess lactic acid passes into the blood stream, the blood becomes more acidic. Rapid breathing occurs as your system tries to capture more oxygen for aerobic respiration. Cramping can occur in the muscles from the excess lactic acid, and you experience fatigue. As you catch your breath following exercise, more oxygen is supplied to your muscles and the lactic acid is broken down. Regular exercise can result in improved efficiency of your lungs and in improved maintenance of aerobic cellular respiration in your muscles.

PROCEDURES:

1. Select a form of repetitive exercise that will work one set of muscles. Make sure you can count single repetitions of the exercise over time such as one jumping jack, push up, sit up, or free weight lift. Describe the exercise.

2. The purpose of the exercise is to count how many repetitions you can accomplish within a set amount of time. You will repeat this process 5 times for 5 trials. Create a data table to record the number of repetitions completed for each trial.

3. This exercise can be done alone but it is much easier and perhaps more accurate to do it with a partner. Agree on a fixed amount of time for each trial such as 30 seconds or 1 minute. While your partner records your time, begin your exercise. Count and record the number of repetitions within each trial period.

4. Take a one minute rest and repeat the exercise. Repeat at the same rest intervals until you have completed five trials.

5. Record your results in a graph; plot the number of repetitions on the y-axis and the trial number on the x-axis. Connect the points on your graph with a line.

6. Calculate the slope of the line in the graph for the overall sum of the set of trials and between each set of points (i.e., between each pair of trials). To calculate the slope of a line, divide the distance between the points along the *y*-axis by the distance between the same points along the *x*-axis. The formula for this is $(y_2-y_1)/(x_2-x_1)$ where y_1 x_1 are the coordinates of one trial data point and y_2 x_2 are the coordinates for another.

Questions:

A. What effect did repeating the exercise over time have on the muscle group?

B. As you repeated the exercise over time, how did your muscles feel?

C. Reflect on how your muscles work. What physiological factors are responsible for fatigue?

D. How well do you think your fatigued muscles would work after 30 minutes of rest?

E. What can you learn form the slope of the line in your graph? How does the slope change as you become more tired? What does a steeper or flatter slope mean?

EXPERIMENT 17: Respiration

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow at least 45 minutes to perform this experiment

Objectives: To measure the resting breathing rate,
To estimate the amount of air inhaled per minute.

Materials:

Student Provides:
- Timer or clock
- An assistant

From the LabPaq:
- Balloon
- String
- Metric ruler from dissection kit

Discussion and Review: In **external respiration**, air is drawn into the lungs where the oxygen in the air is exchanged for carbon dioxide that is then exhaled. External respiration should not be confused with cellular respiration in which chemical reactions take place within the cell to provide energy. In the doctor's office, the volume of air that moves in and out of the lungs is measured by use of an instrument called a **spirometer**. This volume of air can also be measured, though less accurately, using a balloon.

PROCEDURES:

1. Sit at rest. Watch the second hand on a clock and count the number of times you breathe in 30 seconds. Count one complete cycle of inhaling and exhaling as one breath. Record the number of breaths.

2. Repeat Step 1 two more times, recording the number of breaths each time.

3. Calculate the average number of breaths you take in 30 seconds. Multiply this average by 2 to get your average resting breathing rate per minute.

4. Stretch the balloon you'll be using and blow it up once or twice to make it flexible before beginning the next part of the experiment.

5. Take a regular breath and exhale normally into the balloon. With one hand, squeeze the balloon neck and hold it tightly closed so that air does not escape. With the other hand, wrap a string around the balloon at its widest point and grasp the string where it marks the balloon's circumference. Release the neck of the balloon. Measure the length of the string in cm from its tip to where you grasped it. Record this length as the circumference of the balloon.

6. Repeat the above step four more times. Calculate the average of the five measures of the balloon's circumference.

7. Calculate the average radius of the balloon by dividing the average circumference by 6.28 which is approximately equal to 2 π.

8. Calculate the average volume of the balloon as $\frac{4 \pi r^3}{3}$. This is the tidal volume of one average breath in cm^3 or cc, also in mL.

9. Multiply the average tidal volume in mL by the average number of breaths you take per minute. Divide the product by 1000 to get the tidal volume per minute in liters. Record your calculations and results.

10. Repeat the procedures above with an assistant. Compare and contrast the results.

Questions:

A. How do your average number of breaths and your tidal volume per minute compare with the measurements for your assistant? Can you account for differences by the size of the person or their physical condition?

B. An average adult inhales 6 liters of air per minute. How does this compare to your tidal volume and that of your partner?

C. What do you think would happen to your number of breaths and your tidal volume just after exercising? Why?

EXPERIMENT 18: Spread of Contagion

Read the entire experiment and organize time, materials, and work space before beginning.
Remember to review the safety sections and wear goggles when working with chemicals.
Allow 60 minutes to perform this experiment

Objective: To demonstrate the ease in which contagion is spread

Materials:

Student provides:
- Assistant(s), 1 minimum and up to 7 if possible
- Distilled water (DO NOT use tap water)

From the LabPaq:
- 50-mL Graduated cylinder
- Clear plastic cups, 8
- Scissors from dissection kit

Experiment Bag:
- 30 mL 0.1M Sodium hydroxide
- Pipet with 2 mL of phenolphthalein

Discussion and Review: Contagious diseases can spread rapidly through a group of people from very little contact. Many germs thrive in water or moisture and can spread rapidly from contact such as drinking from the same glass or eating from the same plate. This experiment demonstrates the speed with which such spread of contagion can occur.

The experiment requires at least one assistant and preferably 7 assistants. The more the better! Try to do it when you can arrange to have a group of people who are willing to help you and who can all get together at the same time. Or you can simply call friends and/or neighbors and ask them to come over at a specific time to assist you with a science experiment. If you have everything set up ahead of time, the experiment should not take more than 10 minutes of their time. This is a great experiment that will impress your friends, so don't be embarrassed to ask for their help.

Your assistants can be any age. The only criterion is that they be able to understand and exactly follow the directions you will give them. The most important direction will be that **they must NOT drink the solutions used in this experiment!** If it is impossible for you to gather a group of people to help you, the experiment can be done with just one assistant by following the alternate instructions in step 10 of the procedures.

PROCEDURES:

1. Read through all the procedures below and form your hypothesis as to how many of the glasses will indicate contagion at the end of the experiment.

2. Set up the experiment a few minutes before you plan to perform it. Your assistants should not be in the same room, or should have their backs turned so they cannot see what you are doing during this set up.

3. Line up the 8 clear plastic cups from the LabPaq along a table or counter. There should be 1 clear plastic cup for every participant including you. Use 8 cups regardless of how many assistants you have.

4. Empty the contents of the bottle of sodium hydroxide (30 mL) into one cup.

5. Use the graduated cylinder to measure and pour exactly 30 mL of distilled - NOT tap - water into each of the remaining cups.

6. Place scissors, pencil, and paper in a convenient place for when you need them.

7. Ask your principal assistant to rearrange the cups while you turn your back or leave the room.

8. Then have the principal assistant turn away while you rearrange the cups again. The sodium hydroxide is clear and odorless. The objective is to make sure that no one, not even you, knows which cup it is actually in.

9. Call all of your assistants together and read them the following instructions:

   We are going to pretend that we are at a party and enjoying these "beverages."

   Each of us will be responsible for one glass after I have finished explaining the procedures.

   *Do **NOT**, I repeat, **DO NOT DRINK** any of the "beverage" solutions or you could get very, very sick and have to go to the hospital! Remember, this is pretend!*

   You are going to pretend to mingle at a party and consecutively exchange "beverages" with 3 different people.

   Pour the "beverage" solution from your glass into another person's glass while being very careful to not spill any. Then that person will very carefully pour half of the combined "beverage" solution back into your glass.

   *Do this same exchange with two other people - 3 in total. After you and your glass have exchanged "beverages" with 3 different people's glasses, place your glass on the t*able or counter and stand next to it.

10. If you do not have many assistants, you and the assistant(s) you do have can act the parts of other imaginary assistants by taking each of the glasses and going through the exchange process with three other glasses. Be sure that each glass has

exchanged with three other randomly selected glasses before the next glass begins its exchange.

11. Ask these questions of the assistant(s) and record the responses:

    **** One person at our party has a cold; do you think you caught this person's cold? If you feel 100% sure you caught this person's cold, raise your hand.***

    **** How many people at our party do you think caught this person's cold?***

12. Snip off the tip of the pipette of phenolphthalein and carefully dispense 3 drops of phenolphthalein into each glass as shown at right and observe what happens.

13. Now tell your assistants: "At the beginning of this experiment before we began to mingle and exchange 'beverages,' one glass contained sodium hydroxide, a clear chemical that reacts with phenolphthalein. That glass represents a cold or any other contagious disease. If your ending 'beverage' reacted to the phenolphthalein that means you caught the cold or other disease."

14. Ask your assistants if they were surprised by the results of this experiment. Encourage them to express their thoughts about the experiment, its results, and what those results mean.

15. Pour all the "beverages" down a sink and flush with running water. Either throw the plastic cups away or wash them with soap and water and keep them for future use.

Questions:

A. What happened when the phenolphthalein was dropped into the cups?

B. Were your assistants surprised by the results of this experiment? Why or why not?

C. What where some of the comments they made?

D. Did this experiment confirm your initial hypothesis? Why or why not?

E. What do the results of this experiment suggest to you? How is the experiment a model of the way in which real contagion can spread?

APPENDIX A: USING STATISTICS

This is a short introduction to statistics. It is designed to allow you to perform a few simple statistical analyses on some of your labs. If you want to learn more about statistics, you may want to review a statistics textbook, download one of the many statistics tutorials on the Internet (search: statistics tutorials), or take a statistics class. This brief introduction to statistics only scratches the surface. Don't be intimidated by the scary-looking equations shown in the statistics review below. The final section will show you how to let your Excel® software calculate most of the statistics automatically.

Statistics is a branch of applied mathematics. It specifically deals with the collection and interpretation of quantitative data and the use of probability theory to estimate population parameters.

Statistics can be categorized into two sub-groups: **Descriptive Statistics** and **Inferential Statistics**. Descriptive Statistics describe large amounts of data in an abbreviated form. They describe important characteristics of your data, like the mean, median, range, variance, standard deviation, etc. Inferential Statistics is the process of using data obtained from a small group of elements called the "sample" to make estimates and test hypotheses about the characteristics of a larger group of elements called the "population)"

Descriptive Statistics: There are a number of measures of **Central Tendency** used describe the center of a distribution and the scatter of observations around the center:

Mean: The arithmetic average of all observations in a distribution. The mean is equal to the sum of all observations divided by the sample size.

$$\overline{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Mode: The mode is the most common value or class in the distribution.

Median: If all of the observations are arranged in rank order from smallest to largest, the median is that value which is bound by 50% of the observations on each side. If the number of observations in the distribution is odd, then the median is simply the middle value in the ranked observations. If the number of observations is even, then the median is mean of the two most central observations.

The measurement of the scatter of observations around the center of a distribution is extremely important. What if you had two means from two populations and the means of both were 50, but the values going into sample #1 were 1, 50, 100 and the values going into sample #2 were 49, 50, 51. It would appear that these are two very different distributions, but with the same mean.

The **scatter of observations around the center of a distribution** can be depicted in the following ways:

Range: The range of a distribution is the difference between the largest and the smallest value, and is typically expressed as "range = 1 – 22", which means that the lowest value was 1 and the highest was 22.

Variance and standard deviation: The variance is a measure of the average squared deviation from the mean. Variance differs from the range in that the variance takes into account the distribution of all data points, whereas the range simply describes the single lowest and the highest extremes. To calculate variance you take the deviation (or differences) of each value x_i (i.e., the *i*th value of x) from the mean, $\overline{x}$ (i.e., *Xi* - $\overline{x}$). Then square these differences and divide by the number of values minus one (i.e., *n*-1).

$$s^2 = \frac{\sum_{i=1}^{n} (x_i - \overline{x})^2}{n-1} = \frac{\sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{n} x_i\right)^2}{n}}{n-1}$$

The standard deviation (*s*) is the square root of the variance. The advantage of the standard deviation is that if your data conform to a normal distribution, 95% of the values will fall within ~two standard deviations (actually 1.96*s*) on either side of the mean.

$$s = \sqrt{s^2} = \sqrt{\frac{\sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{n} x_i\right)^2}{n}}{n-1}}$$

If you were contrasting the weight of two populations of acorns, it might be nice to see a statement something like" "Acorns from Plot A (mean = 4.58 g, S.D. = .59, range = 3.99 – 4.91) were heavier than the acorns from Plot B, (mean = 3.64, S.D. = .71, range = 2.99 – 4.29). (*At this point you could also give the results of a statistical test comparing these two samples: see the next section on Hypothesis Testing*).

Inferential Statistics: There are two types of statistical inferences: estimation of population parameters and hypothesis testing. Hypothesis testing refers to a general class of procedures for weighing the strength of statistical evidence, or for determining whether the evidence supporting one hypothesis over another is sufficiently strong. Hypothesis testing is one of the most important tools statistical applications bring to real-life problems. Most often, decisions are required concerning populations on the basis of sample information. Statistical tests are used in arriving at these decisions.

There are five ingredients to any statistical test:

1. Null Hypothesis
2. Alternate Hypothesis
3. Test Statistic
4. Rejection/Critical Region
5. Conclusion

Here is a simple **example of hypothesis testing,** and the application of a null hypothesis and an alternative hypothesis. One may wish to test whether or not a coin is fair (that is, whether there is an equal chance of it coming up heads or tails when tossed). The null hypothesis is that the coin is fair, and the alternative hypothesis is that it is biased. If a series of coin tosses produces a result that is only 4% likely given a fair coin, one would reject the null hypothesis, assuming 95% confidence is required. If, by contrast, the experiment produces a result that is 30% likely given a fair coin, one would fail to reject the null hypothesis that the coin is fair. It is not permissible to accept the alternative hypothesis. Only acceptance or failure to reject the null hypothesis is allowed in hypothesis testing. If a test fails to reject the null hypothesis, it is said to lack sufficient power to accept the alternative hypothesis.

The **Null Hypothesis** is a hypothesis which states that there is no effect or difference between procedures and is denoted by H_0. The objective of hypothesis testing is to either accept or reject the null hypothesis

Alternative Hypotheses state that there is a statistically significant difference in the outcome of an experimental procedure. Typically, we state the null hypothesis first followed by the alternative hypothesis (Ha). This alternative can be stated simply as "there is a true difference". Only one of the two statistical hypotheses can be true. Consider a simple example in which we wish to compare the size of male and female fish. Our null hypothesis might be that the males and females are the same size (i.e., that samples we obtain were drawn from the same underlying population). Our alternative hypothesis is that males and females are different sizes. We test the null hypothesis with an appropriate statistic. If we reject the null hypothesis, we are left with the alternative that there is a difference (i.e., males and females are of different sizes). This sounds pretty simple, but statistical tests provide a formal means to tell us if the evidence we have is sufficiently compelling to reject the null and decide that "something is going on" and might therefore be worthy of further investigation.

Hypotheses can be directional (e.g., "males are smaller than females") or non-directional (e.g., "males and females are of different sizes") and this determines whether you use what is called a one-tailed or a two-tailed test.

Example - Two-Tailed Hypothesis:
Ho = There is no difference in size between male and female fish.
Ha = There is a difference in size between male and female fish.

Example - One-Tailed Hypothesis:
Ho = Male fish are not smaller than female fish.
Ha = Male fish are smaller than female fish.

In the example for a one-tailed test, failure to reject the null hypothesis might mean that there was no difference in size of male and female fish or that female fish were bigger than male fish.

Decision Making and the Level of Significance: After stating our hypothesis, we must select and carry out an appropriate statistical test. Each test is based upon a different "test statistic" [e.g., given the symbols, *t* (for a *t*-test), *F* (in an analysis of variance), *r* (for a correlation analysis), χ2 (for a chi-square test), etc.]. By plugging the values from our sample into a formula for the statistical test, we end up with an observed value for the test statistic. We must then compare this observed value of the test statistic with a theoretical distribution of values that we would obtain if the null hypothesis was true. This distribution of expected values is generated from the assumptions that underlay the test and, in the case of parametric tests, from some of the data that we've collected, such as the variance among multiple observations within a group. These distributions are typically summarized in tables that are published in statistics books or are readily available on the Internet. (search: Statistical Tables)

With these tables, we can then ask "how likely is it that we would have obtained the observed results. The table provides the entire distribution and determines how much of the distribution lies beyond the observed value of the test statistic. This yields the "*P*-value", or the probability of obtaining our observed results or something more extreme under the assumption that the null hypothesis is correct. For example, a *P*-value of 0.13 means that if the null hypothesis were true, 13% of all possible samples would lead to results as extreme as ours (i.e., with the same or more extreme differences between our two groups).

The smaller the *P*-value, the less likely it is that the null hypothesis is true. But how small should the *P*-value be before we reject the null hypothesis? We give this cut-off the symbol α (alpha), which by convention we typically set at 0.05. In other words, 5% of the times when the null hypothesis is correct, we will conclude that the null hypothesis is wrong. This is called a Type I error. The probability of a Type I error is equal to α.

To interpret our results, we compare the *P*-value to α. If P<α (i.e., if *P*<0.05) then we reject the null hypothesis. If P> α or P= α, we tentatively accept the null, recognizing that it might be wrong, but that we have insufficient evidence to reject it. If we are able to calculate a *P*-value exactly from a distribution or from a statistics software program, it's useful to report the exact value as in P=0.05, rather than *P*<0.05 or *P*>0.05. In the second and more common case, the entire distribution is not published so we cannot exactly determine the *P*-value. Instead, the tables provide particular values of the test statistic associated with different *P*-values or "levels of significance" or α. If our observed test statistic is greater than this critical value of the test statistic, then we can reject the null hypothesis because P < α.

The T-Test

One of the most common comparative statistical tests is the t-test, also called student's t-test, which is used when there are just two sets of normally-distributed data to compare. Normally-distributed data means that the data distribution looks like a bell-

shaped curve. There are several types of t-tests, each designed mathematically for a specific application. Here we will look at the t-test which is used to compare two independent samples. This is the test one would use in this experiment where we compare the average height of plants in the two squares sampled.

In this laboratory exercise you will ask if the difference between the mean heights of the plants in the two plots is statistically significant. Our null hypothesis is that the species of plants present and the conditions in which they have grown have made no difference in the height of the plants and that the mean heights of the two plots are essentially the same, allowing for some minor variance:

$$H_o: \mu_1 = \mu_2$$

The alternative hypothesis is that differences in the plant species and the growing conditions have made a difference and that the mean heights are not the same:

$$Ha: \mu_1 \neq \mu_2$$

If they are not the same then the question is if the mean height of one sample is significantly larger or smaller than the other. As we have two means we will use a two-tailed test. Don't worry if this sounds confusing, below are a step-by- step examples of how this analysis is performed.

Sample Problem 1: Weights of acorns collected from two different plots

Plot A	Plot B
2.33	2.02
2.52	1.90
2.23	2.13
2.70	2.50
2.00	2.30
2.42	2.21
2.54	2.21
2.60	1.80
2.44	2.64
2.53	2.14

1. Calculate the mean (average) of the weights in grams. Add all data point values for each plot and divide by the number of data points.

   Plot A: (2.33 + 2.51 +2.12 +2.7 +2 +2.42 +2.54 +2.6 +2.44 +2.53)/10 = 2.419

   Plot B: (2.02 +1.9 +2.13 +2.5 +2.3 +2.5 +2.3 +2.21 +2.21 +1.8 +2.64+2.14)/10 = 2.185

2. Calculate the variance (s^2) of each plot: $$s^2 = \frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}$$

   a. Square each data value and enter it in a data table, Ex: $2.33^2 = 5.4289$ etc.

   b. Add all the data values in the last row: 2.33+2.51+2.12 +……= 24.19

   c. Add all the squared data values: 5.4289 + 6.3001 + ------- = 58.94 (rounded)

3. Enter the values calculated in Step 2 into the equation:

Plot A: s^2 = {58.94 – [(24.19*24.19)/10]/9}
= 0.047

Plot B: s^2 = {48.32 – [(21.85*21.85)/10]/9}
= 0.065

Plot A		Plot B	
X	**x^2**	**x**	**x^2**
2.33	5.4289	2.02	4.0804
2.51	6.3001	1.9	3.61
2.12	4.4944	2.13	4.5369
2.7	7.29	2.5	6.25
2.0	4.0	2.3	5.29
2.42	5.8564	2.21	4.8841
2.54	6.4516	2.21	4.8841
2.6	6.76	1.8	3.24
2.44	5.9536	2.64	6.9696
2.53	6.4009	2.14	4.5796
24.19	**58.9359**	**21.85**	**48.3247**

4. Calculate the t-value $t = \frac{(\bar{x}_A - \bar{x}_B)\sqrt{n}}{\sqrt{s_A^2 + s_B^2}}$

a. Calculate the numerator of the equations above: $(\bar{x}_A - \bar{x}_B)$. Subtract the mean of plot B from the mean of plot A: 2.419 - 2.185 = 0.234

b. Multiply the difference of the means (0.234) by the √ n (for the sample in the example n=10, and the √10 is 3.162) which = 0.74

c. Calculate the denominator: Take the square root of the sum of the two variances calculated earlier: √(0.047 + 0.065) = 0.334

d. Divide the numerator by the denominator: 0.74/0.334 = 2.24 = t

The calculated t-value is 2.24.

5. Now we need to look at the table of critical values for *t* and compare the values in the table to our calculated *t*.

In order to use the critical values table we need alpha (α) and degrees of freedom (df). The total number of data points is n, in our case 20 acorns. For a t-test involving two independent means, df = n – 2, and in our case n = 20 so df = 20 – 2 = 18.

Alpha refers to the degree of confidence, and the degree needed to accept the null hypothesis is normally 5% or 0.05. Since we are using a two-tailed test our alpha has to be split between the two tails, giving an alpha of 0.025 for each tail. Now go to the table and look under 0.025 and 18 df. You can find such tables also on the Internet with a search under *t-test critical values.*

df\α	0.40	0.25	0.10	0.05	0.025	0.01	0.005	0.0005
1	0.324920	1.000000	3.077684	6.313752	12.70620	31.82052	63.65674	636.6192
2	0.288675	0.816497	1.885618	2.919986	4.30265	6.96456	9.92484	31.5991
3	0.276671	0.764892	1.637744	2.353363	3.18245	4.54070	5.84091	12.9240
4	0.270722	0.740697	1.533206	2.131847	2.77645	3.74695	4.60409	8.6103
5	0.267181	0.726687	1.475884	2.015048	2.57058	3.36493	4.03214	6.8688
6	0.264835	0.717558	1.439756	1.943180	2.44691	3.14267	3.70743	5.9588
7	0.263167	0.711142	1.414924	1.894579	2.36462	2.99795	3.49948	5.4079
8	0.261921	0.706387	1.396815	1.859548	2.30600	2.89646	3.35539	5.0413
9	0.260955	0.702722	1.383029	1.833113	2.26216	2.82144	3.24984	4.7809
10	0.260185	0.699812	1.372184	1.812461	2.22814	2.76377	3.16927	4.5869
11	0.259556	0.697445	1.363430	1.795885	2.20099	2.71808	3.10581	4.4370
12	0.259033	0.695483	1.356217	1.782288	2.17881	2.68100	3.05454	4.3178
13	0.258591	0.693829	1.350171	1.770933	2.16037	2.65031	3.01228	4.2208
14	0.258213	0.692417	1.345030	1.761310	2.14479	2.62449	2.97684	4.1405
15	0.257885	0.691197	1.340606	1.753050	2.13145	2.60248	2.94671	4.0728
16	0.257599	0.690132	1.336757	1.745884	2.11991	2.58349	2.92078	4.0150
17	0.257347	0.689195	1.333379	1.739607	2.10982	2.56693	2.89823	3.9651
18	0.257123	0.688364	1.330391	1.734064	2.10092	2.55238	2.87844	3.9216
19	0.256923	0.687621	1.327728	1.729133	2.09302	2.53948	2.86093	3.8834
20	0.256743	0.686954	1.325341	1.724718	2.08596	2.52798	2.84534	3.8495
21	0.256580	0.686352	1.323188	1.720743	2.07961	2.51765	2.83136	3.8193
22	0.256432	0.685805	1.321237	1.717144	2.07387	2.50832	2.81876	3.7921
23	0.256297	0.685306	1.319460	1.713872	2.06866	2.49987	2.80734	3.7676
24	0.256173	0.684850	1.317836	1.710882	2.06390	2.49216	2.79694	3.7454
25	0.256060	0.684430	1.316345	1.708141	2.05954	2.48511	2.78744	3.7251
26	0.255955	0.684043	1.314972	1.705618	2.05553	2.47863	2.77871	3.7066
27	0.255858	0.683685	1.313703	1.703288	2.05183	2.47266	2.77068	3.6896
28	0.255768	0.683353	1.312527	1.701131	2.04841	2.46714	2.76326	3.6739
29	0.255684	0.683044	1.311434	1.699127	2.04523	2.46202	2.75639	3.6594
30	0.255605	0.682756	1.310415	1.697261	2.04227	2.45726	2.75000	3.6460
inf	0.253347	0.674490	1.281552	1.644854	1.95996	2.32635	2.57583	3.2905

From the previous table we see that the value under α = 0.025 and df = 18 is approximately 2.1

Interpretation of the results: Since the calculated t-value of 2.24 is greater than the critical value of 2.1 we accept the null hypothesis that the two means are statistically equal. It indicates that the difference between the means is insignificant at the 95% level (100% minus alpha). In other words, the means of the two samples differ by less than can be accounted for by minor variations and the size of the sample taken.

Sample Problem 2: Weights of acorns collected from two different plots with different number of data points in each plot.

Sample 1	Sample 2
2.33	2.02
2.51	1.9
2.12	2.13
2.7	2.5
2.0	2.3
2.42	2.21
2.54	2.21
2.6	1.8
2.44	2.64
2.53	2.14
2.5	
2.55	

1. Calculate the mean (average) of the weights in grams. Add all data point values for each plot and divide by the number of data points.

   Plot A: (2.33 + 2.51 +2.12 +2.7 +2 +2.42 +2.54 +2.6 +2.44 +2.53+2.5+2.55)/12 = 2.437

   Plot B: (2.02 +1.9 +2.13 +2.5 +2.3 +2.5 +2.3 +2.21 +2.21 +1.8 +2.64+2.14)/10 = 2.185

2. Calculate the variance (s²) of each plot:

$$s^2 = \frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}$$

   in

   a. Square each data value and enter it a data table, Ex: 2.33² = 5.4289, etc.

   b. Add all the data values: 2.33+2.51+2.12 +……= 29.24

   c. Add all the squared data values: 5.4289 + 6.3001 + ------- = 71.69 (rounded).

Plot A		Plot B	
X	x^2	x	x^2
2.33	5.4289	2.02	4.0804
2.51	6.3001	1.9	3.61
2.12	4.4944	2.13	4.5369
2.7	7.29	2.5	6.25
2.0	4.0	2.3	5.29
2.42	5.8564	2.21	4.8841
2.54	6.4516	2.21	4.8841
2.6	6.76	1.8	3.24
2.44	5.9536	2.64	6.9696
2.53	6.4009	2.14	4.5796
2.5	6.25		
2.55	6.5025		
29.24	**71.6884**	**21.85**	**48.3247**

3. Enter the values calculated in Step 2 into the equation:

$$s^2 = \frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}$$

   Plot A: s^2 = {71.69 – [(29.24*29.24)/12]/10} = 0.04

   Plot B: s^2 = {48.32 – [(21.85*21.85)/10]/9} = 0.065

4. An additional step is now needed to calculate **pooled variance** since we have an unequal number of data points in each plot.

$$s_p^2 = \frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1 + n_2 - 2}$$

So, what does this mean?

n_1-1 = number of data points in plot A minus 1
s_1^2 = variance for plot A
n_2-1 = number of data points in plot B minus 1
s_2^2 = variance for plot B
$n_1 + n_2 - 2$ = number of data points in plot A + plot B minus 2 (which also = df)

$$s_p^2 = \frac{(11)0.04 + (9)0.065}{12 + 10 - 2} = \frac{1.025}{20} = 0.051$$

5. Now, having adjusted the variance for different sample sizes we can calculate the t-value using a slightly different equation.

$$t_{(pooled)} = \frac{\bar{x}_A - \bar{x}_B}{\sqrt{\frac{s_p^2}{n_1} + \frac{s_p^2}{n_2}}}$$

$$\frac{\bar{x}_A - \bar{x}_B}{\sqrt{\frac{s_p^2}{n_1} + \frac{s_p^2}{n_2}}} = 2.437 - 2.185 = 0.2545$$

$$= 0.051/12 + 0.051/10 = .00935$$

$\sqrt{}$.00935 = 0.0967

Final step: 0.2545/0.0967 = 2.63 calculated t = 2.63

6. Now we look up the critical t-value from the table above, using alpha = 0.05/2 = 0.025 and df = 22 – 2 = 20. We find that $t_{critical}$ = 2.086.

7. Interpretation of results: Since the calculated t-value of 2.63 is greater than the critical value of 2.086 we reject the null hypothesis that the two means are equal. It indicates that the difference between the means is significant at the 95% level (100% minus alpha).

The Chi-Square Test

The chi-square (X^2) test is one of the most useful non-parametric statistical tests for the biologist. It is used with count data or frequencies organized in a matrix defined by two or more variables.

The chi-square test is based on the differences between the observed results and the expected values (those results that would be obtained if the null hypothesis were true). The formula for X^2 is as follows:

$$\chi^2 = \sum (o-e)^2 / e$$

where *o* is the observed frequency and *e* is the frequency expected under the null hypothesis of no difference between groups.

Example: Suppose a fisheries biologist samples adult fish from two lake populations (100 from Lake 1 and 150 from Lake 2). S/he records whether or not they are infested with a nematode parasite that encysts in their muscles. S/he wants to know whether the presence of the parasite is independent of the lake from which they were taken.

1. Arrange the data in a tabular form.

2. Then calculate the sums for each row and column of the table.

Table of observed values:

Site	# fish w/parasites	#fish w/out parasites	Total
Lake A	15	85	100
Lake B	50	100	150
Totals	65	185	250

3. Compute the table of expected values. For example, the expected value for the number of fish with parasites in Lake 1= (100x65)/250=26 [i.e., (the row total x the column total)/total].

Table of expected values:

Site	# fish w/parasites	#fish w/out parasites	Total
Lake A	26	74	100
Lake B	39	111	150
Totals	65	185	250

Notice that the row and column totals are the same in the tables of expected and observed values.

4. Compare the observed and expected frequencies using the χ^2 statistic. $\chi^2 = (15\text{-}26)^2/26 + (85\text{-}74)^2/74 + (50\text{-}39)^2/39 + (100\text{-}111)^2/111 = 10.5$

5. Determine the degrees of freedom for the test = (2 rows-1) x (2 columns-1) = 1 df.

6. Compare the calculated χ^2 value (10.5) with the value for 1 degree of freedom from a stats table. Since our calculated value is greater than 3.84 (from the table) we can reject our null hypothesis that the presence of parasites in fish is independent of the lake.

Chi-square table:

	Probability of exceeding the critical value				
ν	0.10	0.05	0.025	0.01	0.001
1	2.706	3.841	5.024	6.635	10.828
2	4.605	5.991	7.378	9.210	13.816
3	6.251	7.815	9.348	11.345	16.266
4	7.779	9.488	11.143	13.277	18.467
5	9.236	11.070	12.833	15.086	20.515
6	10.645	12.592	14.449	16.812	22.458
7	12.017	14.067	16.013	18.475	24.322
8	13.362	15.507	17.535	20.090	26.125
9	14.684	16.919	19.023	21.666	27.877
10	15.987	18.307	20.483	23.209	29.588
11	17.275	19.675	21.920	24.725	31.264
12	18.549	21.026	23.337	26.217	32.910
13	19.812	22.362	24.736	27.688	34.528
14	21.064	23.685	26.119	29.141	36.123
15	22.307	24.996	27.488	30.578	37.697
16	23.542	26.296	28.845	32.000	39.252
17	24.769	27.587	30.191	33.409	40.790
18	25.989	28.869	31.526	34.805	42.312
19	27.204	30.144	32.852	36.191	43.820
20	28.412	31.410	34.170	37.566	45.315

APPENDIX B: MS EXCEL© AND STATISTICS

The Microsoft Excel© spreadsheet can be used to perform most of your statistical calculations.

Analysis ToolPak add-in: MS Excel® provides a set of data analysis tools, called the Analysis ToolPak, which you can use to save steps when you develop complex statistical or engineering analyses. You provide the data and parameters for each analysis; the tool uses the appropriate statistical or engineering macro functions and then displays the results in an output table. Some tools generate charts in addition to output tables.

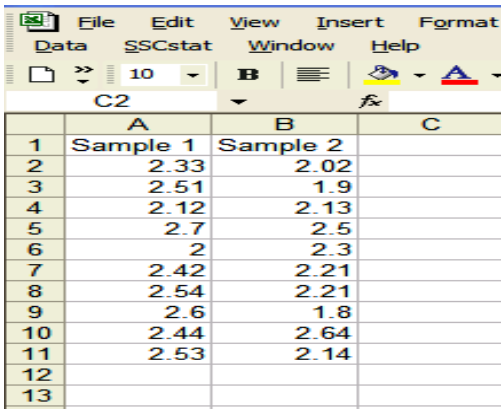
Available tools: To view a list of available analysis tools, go to the **Tools** menu and click **Data Analysis**. If the **Data Analysis** command is not on the **Tools** menu, you need to install the Analysis ToolPak.

How to install and use the Analysis ToolPak:

A. On the **Tools** in Excel© menu, click **Add-Ins**. If **Analysis ToolPak** is not listed in the **Add-Ins** dialog box, click **Browse** and locate the drive, folder name, and file name for the Analysis ToolPak add-in, Analys32.xll — usually located in the Microsoft® Office\Office\Library\Analysis folder — or run the Setup program if it isn't installed.

B. Select the **Analysis ToolPak** check box.

Related worksheet functions: Excel provides many other statistical, financial, and engineering worksheet functions. Some of the statistical functions are built-in and others become available when you install the Analysis ToolPak.

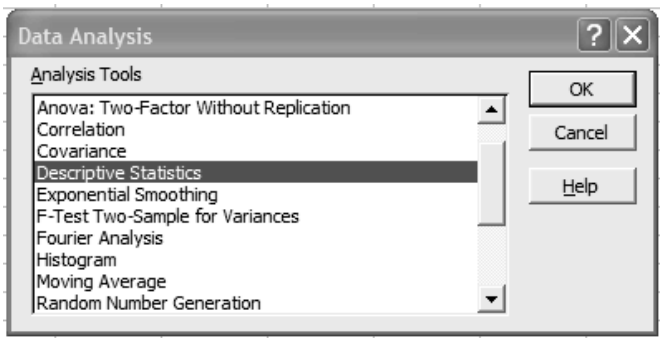


	A	B	C
1	Sample 1	Sample 2	
2	2.33	2.02	
3	2.51	1.9	
4	2.12	2.13	
5	2.7	2.5	
6	2	2.3	
7	2.42	2.21	
8	2.54	2.21	
9	2.6	1.8	
10	2.44	2.64	
11	2.53	2.14	
12			
13			

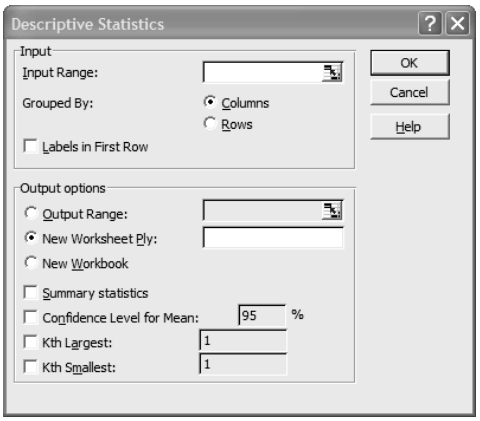
It is easy to analyze data using descriptive statistics in Excel® because Excel® includes all common statistics such as mean, median, mode, standard deviation, etc. In this example we will create a data file. Open Excel®, and then enter the data at left:

1. Click once on the cell C1 (the cell to the right of the sample 2 column).

2. Click on Tools, then on Data Analysis. When you do this, you will see the screen at right.



Click once on Descriptive Statistics, and then click on OK. You will see the following screen.

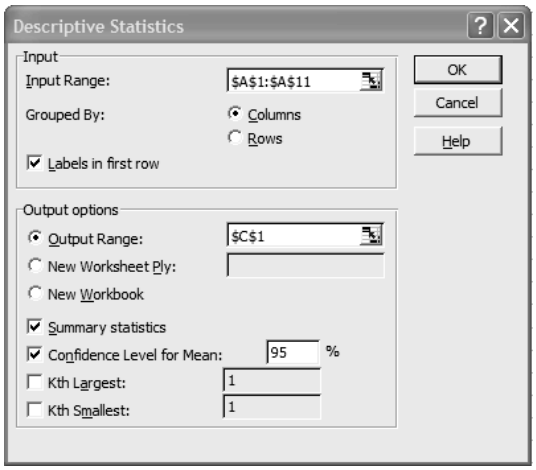


3. Enter the cell reference for the range of data you want to analyze. The reference must consist of two or more adjacent ranges of data arranged in columns or rows. For our example we are going to choose the values for Sample 1. You should type the range, $A$1:$A$11, into the Input Range box. Or, you can select the range from your spreadsheet by dragging you mouse.

4. Next, you need to indicate whether the data in the input range is arranged in rows or in columns. In this case it is Columns. If the first row of your input range contains labels, select the **Labels in First Row** check box. If the labels are in the first column of your input range, select the **Labels in First Column** check box. This check box is clear if your input range has no labels; MS Excel® generates appropriate data labels for the output table.

5. For our example, we will check the box Grouped by **Columns**.

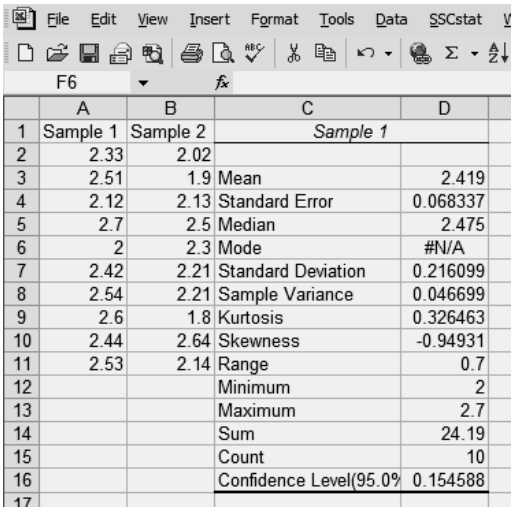
6. Select if you want to include a row in the output table for the confidence level of the mean. In the box, enter the confidence level you want to use. For example, a value of 95 percent calculates the confidence level of the mean at a significance of 5 percent. For this example we are going to use a confidence level of 95. With the choices we have made thus far, you should see the screen at right.



7. Kth Largest: Select if you want to include a row in the output table for the kth largest value for each range of data. In the box, enter the number to use for k. If you enter 1, this row contains the maximum of the data set. We will leave this blank.

8. Kth Smallest: Select if you want to include a row in the output table for the kth smallest value for each range of data. In the box, enter the number to use for k. If you enter 1, this row contains the minimum of the data set. We will leave this blank.

9. Output Range: Enter the reference for the upper-left cell of the output table. This tool produces two columns of information for each data set. The left column



	A	B	C	D
1	Sample 1	Sample 2	*Sample 1*	
2	2.33	2.02		
3	2.51	1.9	Mean	2.419
4	2.12	2.13	Standard Error	0.068337
5	2.7	2.5	Median	2.475
6	2	2.3	Mode	#N/A
7	2.42	2.21	Standard Deviation	0.216099
8	2.54	2.21	Sample Variance	0.046699
9	2.6	1.8	Kurtosis	0.326463
10	2.44	2.64	Skewness	-0.94931
11	2.53	2.14	Range	0.7
12			Minimum	2
13			Maximum	2.7
14			Sum	24.19
15			Count	10
16			Confidence Level(95.0%	0.154588

contains statistics labels, and the right column contains the statistics. MS Excel® writes a two-column table of statistics for each column or row in the input range, depending on the **Grouped By** option selected. If you don't enter an output range, Excel® might overwrite your data table. For this example, we will enter $C$1 into the **Output Range** box.

10. Now, for the last step, click on OK. After some calculation time, the table at left will appear in your spreadsheet with all your descriptive statistics.

Perform a t-Test Analysis

This tool is a part of the Analysis ToolPak. When two populations are both normally or approximately normally distributed and when at least one sample size is small (less than 30), the t-test is used to make decisions about differences between the population means.

The Analysis ToolPak provides three tools that you can use to test the means of different types of populations. There are four different ways of doing a t-test in Excel®.

1. t-test: One Sample t-test
2. t-test: Two-Sample Assuming Equal Variances Analysis
3. t-test: Two-Sample Assuming Unequal Variances Analysis
4. t-test: Paired Two Sample for Means Analysis

Single Sample t-Test

Definition: Used to compare the mean of a sample to a known number (often 0).

Assumptions: Subjects are randomly drawn from a population and the distribution of the mean being tested is normal.

Test: The hypotheses for a single sample t-test are:

H_o: $u = u_0$

H_a: $u < > u_0$

(where u_0 denotes the hypothesized value to which you are comparing a population mean).

Test statistic: The test statistic, t, has N-1 degrees of freedom, where N is the number of observations.

Results of the t-test: If the p-value associated with the t-test is small (usually set at $p < 0.05$), there is evidence to reject the null hypothesis in favor of the alternative. In other

words, there is evidence that the mean is significantly different than the hypothesized value. If the p-value associated with the t-test is not small ($p > 0.05$), there is not enough evidence to reject the null hypothesis, and you conclude that there is evidence that the mean is not different from the hypothesized value.

To use this function in Excel®, you use the tdist function.

Example:
TDIST (1.96,60,2) equals 0.054645, or 5.46 percent

Exercise - Single Sample t-test
from http://www.mastep.sjsu.edu/learn/t_test.htm
You have been told that the average employee for your industry has an average dexterity score of 100 on a standardized test. You think your employees will score differently, so you give a random sample of 12 the test. The results are:

Subj.	Test Score
1	98
2	102
3	120
4	140
5	123
6	101
7	89
8	99
9	119
10	103
11	132
12	107

First, we need to construct hypotheses.
Ho: The average dexterity score for our employees is 100.
Ha: The average dexterity score for employees is not 100.

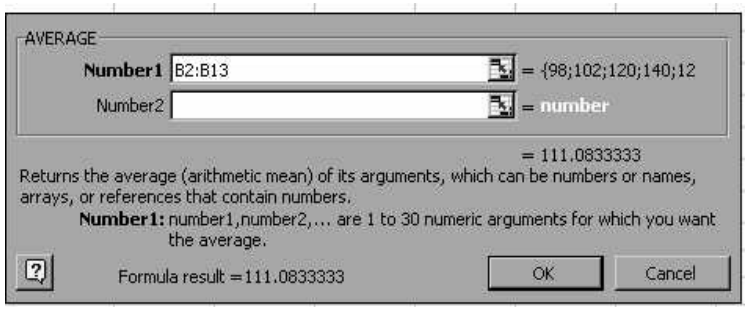
1. Open the Excel® spreadsheet and enter the data from the table.

2. Move your cursor to cell E4.

3. First, we need to calculate the sample mean and the standard deviation of our sample.

4. Click on **Insert,** then **Function**. In the left column, click on **Statistical**. You will see a list of statistical functions appear on the right.

5. Scroll down until you see AVERAGE. Click on AVERAGE and you will see the screen at right.

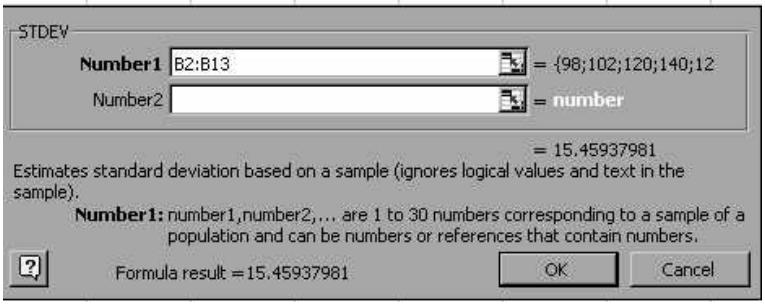


6. Either type the range B2:B13 into the **Number1** box or use your mouse to select the range from the worksheet. After you have selected the range, click on OK. For this example, you should get a mean of 111.0833.

7. Next, click on cell E3.

8. Click on **Insert,** then **Function**. In the left column, click on **Statistical**. You will see a list of statistical functions appear on the right.

9. Scroll down until you see STDEV. Click on STDEV and you will see the screen at right.



10. Either type the range B2:B13 into the **Number1** box or use your mouse to select the range from the worksheet. After you have selected the range, click on OK. For this example you should get a standard deviation of 15.45938. We are using the standard deviation of a sample [with n-1] because we know it is a sample, not the entire population.

11. Next, we calculate the t ratio using the following formula.

$$t = \frac{\text{Sample Mean} - \text{Population Mean}}{\text{Sample SD} / (\text{SQRT (sample size)})}$$

For our example, this calculation would be

$$t = \frac{111.0833 - 100}{15.45938/(\text{SQRT}(12))}$$

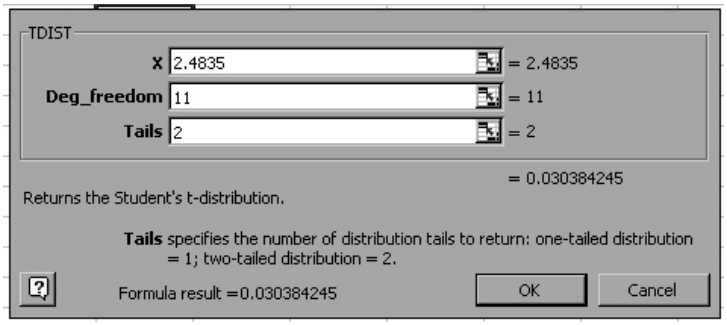
$$t = 2.4835$$

12. To test the hypothesis that our sample is different from the population, we need to find the two-tailed probability of a t ratio of 2.4835 with 11 degrees of freedom Remember degrees of freedom is Sample Size - 1.

13. Next, click on cell E4.

14. Click on **Insert,** then **Function**. In the left column, click on **Statistical**. You will see a list of statistical functions appear on the right.

15. Scroll down until you see TDIST. Click on TDIST and the window at right will appear.



16. Type the above values into your table, and then click OK. You should get a value of 0.030384. This means that there is a 3% chance that your sample is representative of the population. Related to significance levels, you would reject the null hypothesis at the 5% significance level, **but** you would not reject the null hypothesis at the 1% level.

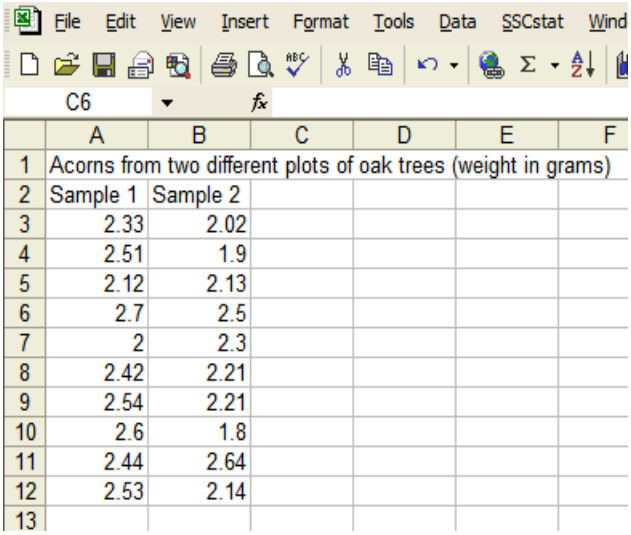
About the t-Test: Two-Sample Assuming Equal Variances

Two samples are referred to as independent if the observations in one sample are not in any way related to the observations in the other. This is also used in cases where you randomly assign subjects to two groups, give the first group treatment 1, give the second group treatment 2, and compare the two groups.

This analysis tool performs a two-sample student's t-test. This t-test form assumes that the means of both data sets are equal; it is referred to as a homoscedastic t-test. You can use t-tests to determine whether two sample means are equal.

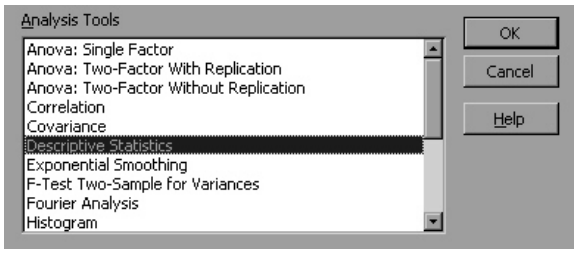
For this example, we are going to use the data from the file space.xls. In a NASA-funded study, 7 men and 8 women spent 24 days in seclusion to study the effects of gravity on circulation. Without gravity, there is a loss of blood from the legs to the upper parts of the body. The study started with a 9-day control period in which the subjects were allowed to walk around. This was followed by a 10-day bed rest period in which the subjects' feet were somewhat elevated to simulate weightlessness. The study ended with a 5-day recovery period in which the subjects were allowed to walk around. Every few days, researchers measured the electrical resistance at the calf, which increases when there is blood loss. The electrical resistance gives an indirect measure of the blood loss. (Example from **Data Analysis with MS Excel®**, p. 137).

1. Open Excel® and enter the data set at right.



2. We are going to use Excel's® Analysis ToolPak to perform a 2-sample t-test.

3. Click on Tools, then on Data Analysis. When you do this, you will see the following screen.

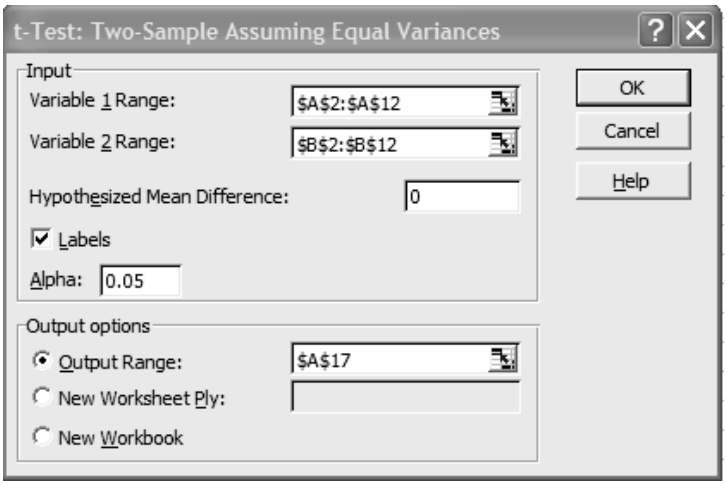


4. Scroll down until you see t-test: Two-Sample Assuming Equal Variances. Click once on this t-test and the following window will appear.

5. First, we must give the **Variable 1 Range.** This is the cell reference for the first range of data you want to analyze. The range must consist of a single column or row of data. In this case, we are going to select the range $A$2:$A$12. You can either type this range or use your mouse to select the range from the worksheet.

6. Next, we need to select the Variable 2 Range. We are going to select the range $B2:$B$12.

7. Hypothesized Mean Difference: Enter the number that you want for the shift in sample means. A value of 0 (zero) indicates that the sample means are hypothesized to be equal. Enter 0.

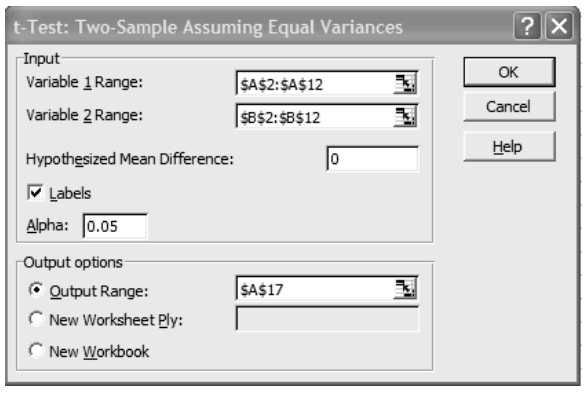


8. Labels: Select if the first row or column of your input ranges contains labels. Clear this check box if your input ranges have no labels; MS Excel® generates appropriate data labels for the output table. Since our first row contains labels (Sample 1 and Sample 2) you should check the **Labels** box.

9. Alpha: Enter the confidence level for the test. This value must be in the range 0 to 1. The alpha level is a significance level related to the probability of having a type I error (rejecting a true hypothesis). We will enter 0.05 for our Alpha.

10. Output Range: You have three options for the output range. Generally, it is safer to choose either **New Worksheet Ply** or **New Workbook**.

    Output Range: If you place a range here, your t-test values will appear on the same worksheet. Enter the reference for the upper-left cell of the output table. MS Excel® automatically determines the size of the output area and displays a message if the output table will replace existing data.



    New Worksheet Ply: Click to insert a new worksheet in the current workbook and paste the results starting at cell A1 of the new worksheet. To name the new worksheet, type a name in the box.

    New Workbook: Click to create a new workbook and paste the results on a new worksheet in the new workbook.

11. For this example, we have selected “Output Range” A17. That means the results will be displayed below the data table. After you have entered all of these values, the screen should look like the previous screen.

12. When all of your choices are correct, click OK. You should see the following information in a new worksheet.

16			
17	t-Test: Two-Sample Assuming Equal Variances		
18			
19		*Sample 1*	*Sample 2*
20	Mean	2.419	2.185
21	Variance	0.046698889	0.06471667
22	Observations	10	10
23	Pooled Variance	0.055707778	
24	Hypothesized Mean Difference	0	
25	df	18	
26	t Stat	2.216883864	
27	P(T<=t) one-tail	0.019873325	
28	t Critical one-tail	1.734063062	
29	P(T<=t) two-tail	0.03974665	
30	t Critical two-tail	2.100923666	

Let's translate this data into "regular" English. Based on the table, we can see that, on the average, the sample 1 acorns have higher weight (2.419g as compared to 2185 g for sample 2 acorns). The variances in the two samples are not exactly the same, so we may want to recheck our calculations using the t-test with unequal variances option in Excel®. Generally, if the variance is close, the difference in t value will not be great.

The value of the t statistic is 2.217. The two-tailed p-value is given as 0.0397, which is less than 0.05, so the difference is significant at the 5% level. Because the probability level is so small, the null hypothesis of no difference between sample 1 and sample 2 seems incompatible with the data. Therefore, you would reject the null hypothesis and state that the weights are different for sample 1 and sample 2 acorns.

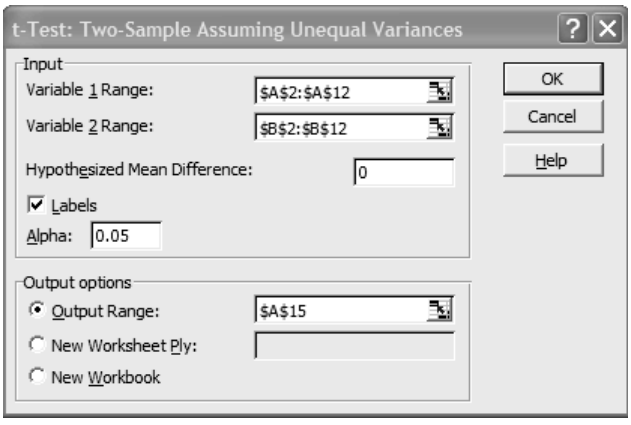
About the t-Test: Two-Sample Assuming Unequal Variances

One would use this test if the variances in the two groups are extremely different. The worst situation would be if the two samples are of very different sizes, and the small sample has a much larger standard deviation. We will use the same data file as above, Space.xls to do this calculation.

1. Open Excel®, and use the same acorn data as before.

2. Again click on Tools, then on Data Analysis.

3. Scroll down until you see t-test: Two-Sample Assuming Unequal Variances. Click once on this t-test and the window at right will appear.



   Enter the same data as you did in the **t-test: Two Sample Assuming Equal Variances** example above. Then, click OK. After you have, you will see the following information.

14			
15	t-Test: Two-Sample Assuming Unequal Variances		
16			
17		*Sample 1*	*Sample 2*
18	Mean	2.419	2.185
19	Variance	0.046698889	0.06471667
20	Observations	10	10
21	Hypothesized Mean Difference	0	
22	df	18	
23	t Stat	2.216883864	
24	P(T<=t) one-tail	0.019873325	
25	t Critical one-tail	1.734063062	
26	P(T<=t) two-tail	0.03974665	
27	t Critical two-tail	2.100923666	

4. As you can see, the values are identical to those for equal variances, and our assumption about equal variances was correct. If the variances had been unequal, we might have gotten slightly different results. But as long as the standard deviations and the sample sizes are close, the results will often be very close to those of the equal variances t-test.

t-Test: Paired Two Sample For Means Analysis

This analysis tool and its formula perform a paired two-sample student's t-test to determine whether a sample's means are distinct. This t-test form does not assume that the variances of both populations are equal. You can use a paired test when there is a natural pairing of observations in the samples, such as when a sample group is tested twice — before and after an experiment.

Another reason data is dependent is when results on one measure are presumed to be related to another measure. For example, if a student does well in one subject, English, it is likely that he will do well in another subject, for example, history. In fact, this is the situation we are going to use to demonstrate this t-test.

Paired t-test:
Definition: Used to compare means on the same or related subject over time or in differing circumstances.

Assumptions: The observed data are from the same subject or from a matched subject and are drawn from a population with a normal distribution.

Characteristics: Subjects are often tested in a before-after situation (across time, with some intervention occurring such as a diet), or subjects are paired such as with twins, or with subject as alike as possible. An extension of this test is the repeated measure ANOVA.

Test: The paired t-test is actually a test that the differences between the two observations is 0. So, if *D* represents the difference between observations, the hypotheses are:

H_o: *D* = 0 (the difference between the two observations is 0)
H_a: *D* 0 (the difference is not 0)

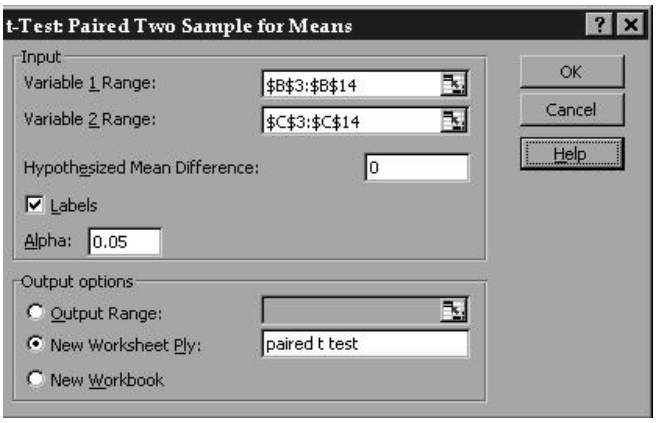
The test statistic is *t* with n-1 degrees of freedom. If the p-value associated with t is low (< 0.05), there is evidence to reject the null hypothesis. Thus, you would have evidence that there is a difference in means across the paired observations.

Sample Calculation: We want to find out whether a student's performance in English is, on the average, different from his/her performance in history. Suppose that a sample of 11 students is selected and their grades for these two subjects are obtained.

1. Open Excel® and enter the data at right.

	A	B	C	D
1	Performance in English and History			
2				
3	Student #	English	History	
4	1	88	85	
5	2	93	96	
6	3	75	65	
7	4	68	73	
8	5	98	88	
9	6	58	65	
10	7	79	80	
11	8	83	75	
12	9	88	93	
13	10	52	48	
14	11	67	70	

2. Click on Tools, then on Data Analysis. Scroll down until you see **t-Test: Paired Two-Sample for Means**. Click once on this t-test and the following window will appear.



3. In this case, we are going to select the range $B$3:$B$14. You can either type this range or use your mouse to select the range from the worksheet.

4. Next, we need to select the Variable 2 Range. We are going to select the range $C$3:$C$10.

5. Hypothesized Mean Difference: Enter the number that you want for the shift in sample means. A value of 0 (zero) indicates that the sample means are hypothesized to be equal. Enter 0.

6. Labels: Select if the first row or column of your input ranges contains labels. Clear this check box if your input ranges have no labels; MS Excel® generates appropriate data labels for the output table. Since our first row contains labels (English and History), you should check the **Labels** box.

7. Alpha: Enter the confidence level for the test. This value must be in the range 0...1. The alpha level is a significance level related to the probability of having a type I error (rejecting a true hypothesis). We will enter 0.05 for our Alpha.

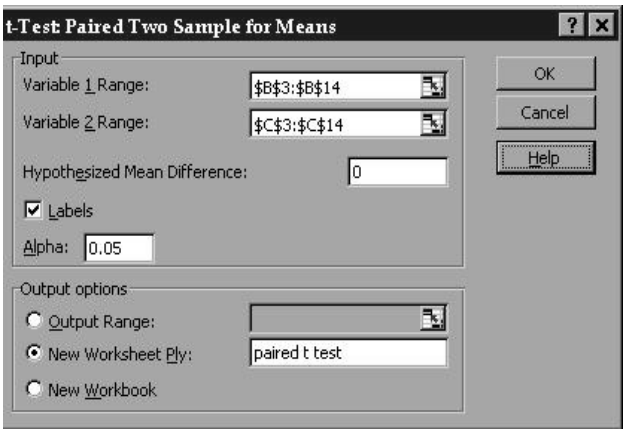
8. Output Range: As before, you have three options for the output range. Generally, it is safer to choose either **New Worksheet Ply** or **New Workbook**.

   Output Range: If you place a range here, your t-test values will appear on the same worksheet. Enter the reference for the upper-left cell of the output table. MS Excel® automatically determines the size of the output area and displays a message if the output table will replace existing data.

New Worksheet Ply: Click to insert a new worksheet in the current workbook and paste the results starting at cell A1 of the new worksheet. To name the new worksheet, type a name in the box.

New Workbook: Click to create a new workbook and paste the results on a new worksheet.

9. Choose **New Worksheet Ply** and type the name, paired t-test, to the right. After you have entered all of these values, the screen should look like the screen at right.



10. Click OK. You should see the following table.

	A	B	C
1	t-Test: Paired Two Sample for Means		
2			
3		*English*	*History*
4	Mean	77.18182	76.18182
5	Variance	214.9636	200.1636
6	Observations	11	11
7	Pearson Correlation	0.905183	
8	Hypothesized Mean Difference	0	
9	df	10	
10	t Stat	0.527046	
11	P(T<=t) one-tail	0.304827	
12	t Critical one-tail	1.812462	
13	P(T<=t) two-tail	0.609654	
14	t Critical two-tail	2.228139	

What does this mean in everyday language? Based on the table, we can see that the means for English and History are almost the same. The variances in the two samples are not exactly the same. The value of the t (two-tailed) statistic is 0.52. The two-tailed p-value is given as 0.61, which is greater than 0.05, so the difference is not significant at the 5% level. In other words, we do not reject the null hypothesis.

Additional Information:

The Statistical Services Centre of the University of Reading, Reading, RG6 6FN, UK has developed a statistical package as an add-on for Excel spreadsheets. It can be downloaded from http://www.rdg.ac.uk/ssc/software/sscstat/sscstat.html. At the time of this manual, an excellent tutorial is available from the same site with a free single user license.