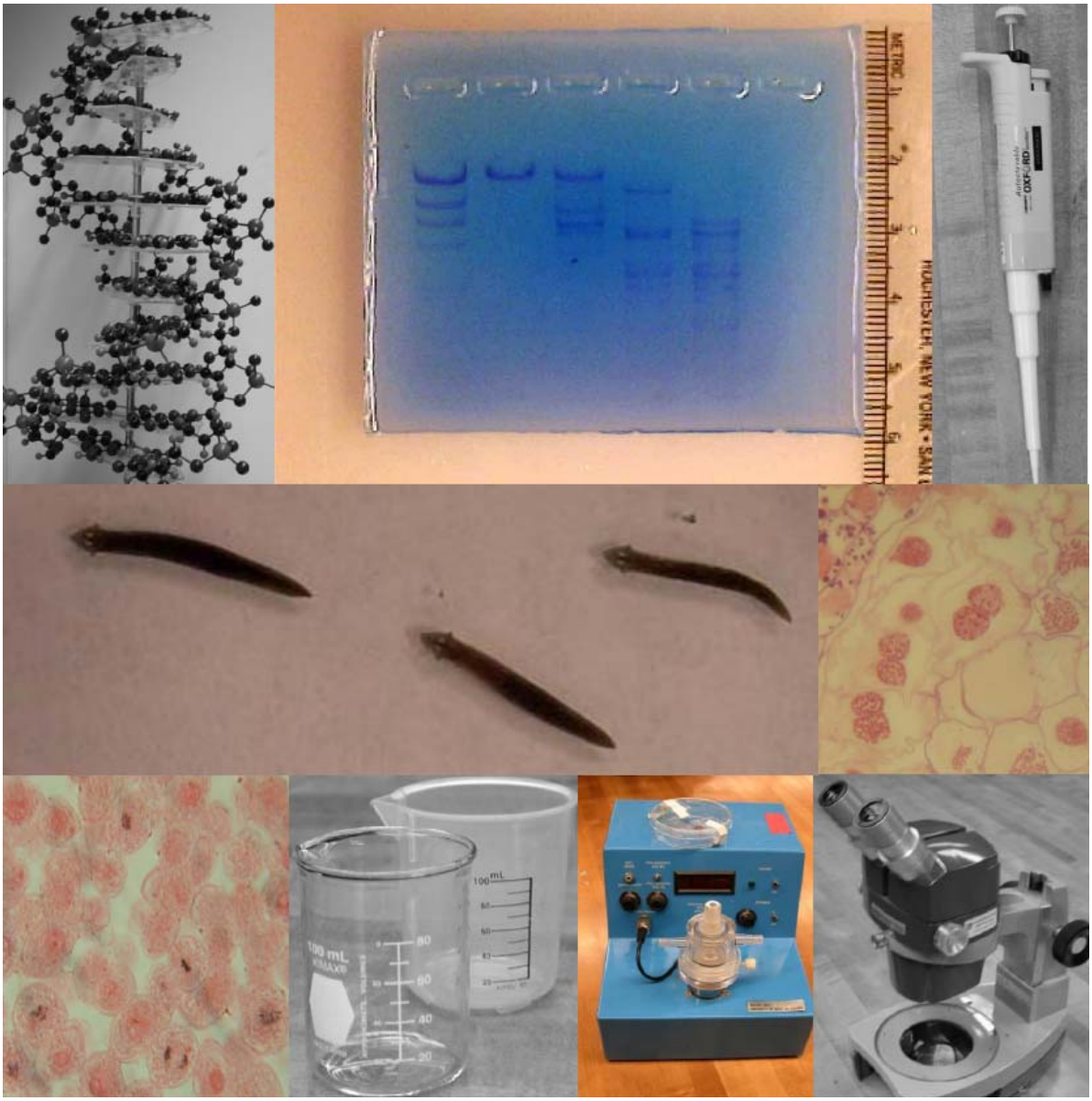
Biology 111 Lab Manual
Spring 2010

Collected writings of
Hawthorne, White, Stark, Hagar, Bennett, Vaillancourt



Name:_____
Lab Section:_____
TA:_____

Lab Syllabus & Table of Contents for Biology 111 Spring 2010 Laboratories

NO EATING OR DRINKING IS PERMITTED IN LAB.

IT IS EXPECTED THAT YOU WILL :

-PREPARE FOR LAB EACH WEEK BY READING THE LAB MANUAL AND TEXT BOOK PRIOR TO THE START OF LAB.

-USE CAUTION AND HANDLE EQUIPMENT PROPERLY.

-CLEAN UP AFTER YOURSELF. IF YOU HAVE QUESTIONS, REFER TO YOUR LAB MANUAL FIRST THEN ASK.

Lab Grade:

1. Multiple Choice Questions:
   Due at the end of lab every week (If absent, NO SCORE). 5 points each= total **60 points.**

2. Lab Report **50 points** on Photosynthesis. One rewrite (optional).
   Rewrite must be turned in with old lab report.

3. Lab Notebook Each week entry worth 15 points x 10 = **150 points**.
   If absent, you may submit TITLE through METHODS up to ONE week late.

There are no lab make ups.

Lab Notebook Entries:

All lab notebook entries should be neat, organized, legible, and follow the format EXACTLY as described below. Notebooks will be left IN THE LAB.

- Each entry should start at the top of the right hand page (5 points deducted otherwise).
- Leave ~1 inch margin on the RIGHT hand side below the name and date (2 points deducted per page without a FULL margin)
- The Words TITLE, OBJECTIVE, MATERIALS, METHODS, RESULTS, and CONCLUSIONS must be written in Capitals on the Left hand margin (deduct 1 point each word missing)
- The Title of the Lab must be EXACTLY the same as in Lab Notebook (1 point deducted if different)
- TITLE, OBJECTIVE, MATERIALS, and METHODS are graded during lab by TA
- TA enters grades on the right with initials at bottom of each page.
- TITLE, OBJECTIVE, MATERIALS, METHODS on Page 1
- RESULTS and CONCLUSIONS on Page 2 (If necessary page 3)
- Notes should be taken in the lab manual, then entered into the lab notebook in an organized fashion.
- No Lab notebook entry for Photosynthesis, Light, Pigments... or Flower Lab I
- As a general rule of thumb: Be succinct (keep it simple but include all necessary information). For example: "We applied light to the planaria" would receive fewer points than "We applied white light to the tails of the planaria for 60 seconds".

Page 1 Lab Notebook Entry Format Example:

Name
Date

TITLE: (**1 pt**)

OBJECTIVE: 2-3 sentences describing the goal and hypotheses of the lab exercise (**2 pts**)

MATERIALS: Bullet point list of BioTools used in the lab. If many items are used they can be in columns (**1 pts**)

METHODS: outline the methods in an organized presentation (either numbered or by flow chart) (**3 points**)

Page(s) 2-3 Lab Notebook Entry Format Example:

RESULTS: 3-4 sentences describing the results of the lab experiment. Figures such as Hand drawings, charts, tables, or any organized presentation of data are included in the results. Every Figure must have a title (1 points deducted without title) (**5 points**)

CONCLUSIONS: 3-4 sentences that address whether the hypothesis(es) was/were supported or refuted. (**3 points**)

Common Properties of Life

Learning Goals: After completing this laboratory exercise you will be able to:

1. To understand the different kinds of cell categorization
2. To build molecular models of various biomolecules
3. To recognize DNA as a common molecule to all life

Introduction

Energy utilization, reproduction, maintenance of homeostasis, and heredity are a few of the common properties of life. The properties are a result of cells, chemistry, and DNA. Cells are the basic form of life and are composed of atoms. Basic chemical properties of the biomolecules that make up these cells dictate how a cell can interact and adapt to the surrounding environment. One of the biomolecules that is common to all forms of life, is DNA. In fact, the tree of life is determined by studying DNA sequences.

Cells

Historically, the initial distinction made between cell types was dependent on either the presence or absence of a nucleus. Those containing a nucleus are eucaryotic and those lacking a nucleus are procaryotic – though remember both cell types contain DNA. Cells can also be categorized based on their function. For example, a single organism can contain many different types of eucaryotic cells. Tissues are groups of cells that have the same function within a single organism. As you will learn in your study of Biology, the types of genes that are expressed in a tissue or cell type help to establish its function.

In this lab you will examine examples of cells from at least 2 of the 3 domains of life (Bacteria, Archaea, and Eucaryotes). Also, you will examine tissues from at least 3 kingdoms within the Eucaryote domain (Protists, Plants, Fungi, Animal). **For a thorough explanation of bright-field microscopy refer to "How to use a Microscope" found in the Appendix.**

Chemistry

Matter is made up of approximately l00 elements. Of these, only carbon, hydrogen, oxygen, nitrogen, sulfur and about a dozen others are found in living organisms. Electrons from different atoms and molecules can interact with one another to form complex biomolecular structure. In this lab you will use chemical models to explore different biomolecules. There are four types of interactions that are important to us in Bio 111: l. covalent bonds; 2. dipole-dipole interactions (hydrogen bonds); 3. ionic interactions; 4. hydrophobic interaction

Today, we will focus on **covalent bonds**, which are the result of a sharing of electrons between two or more atoms. In this case the electrons of the atoms forming the bond occupy the space between each others' nuclei. Molecules can be made up of atoms of different elements, such as the gas methane (CH_4), in which one atom of carbon shares electrons with four atoms of hydrogen, or the molecule can be made up of atoms of the same element (O_2). Carbon atoms are unusual in that they will bond together to form long chains of carbons (-C-C-C-C-) thus making possible very elaborate molecules with carbon "backbones".

Dipole-Dipole interactions (Hydrogen bonds) are weaker than covalent bonds and tend to form between a hydrogen atom covalently bonded to a nitrogen or oxygen atom and another oxygen or nitrogen atom (in the same molecule or in another molecule). These are particularly important in the structure of proteins and nucleic acids, and will be discussed later in the course.

Ionic interactions are the attraction between positive (+) and negative (–) charges. Ionic bonds are the result of the transfer of one or more electrons between atoms. In Bio 111, we will encounter interactions between ions in which "unlike charges attract" and "like charges repel".

Hydrophobic interactions occur between regions of molecules that are not soluble in water (hydrophobic). In water, hydrophobic regions tend to cluster together (like an oil drop on water) to exclude the surrounding water.

DNA

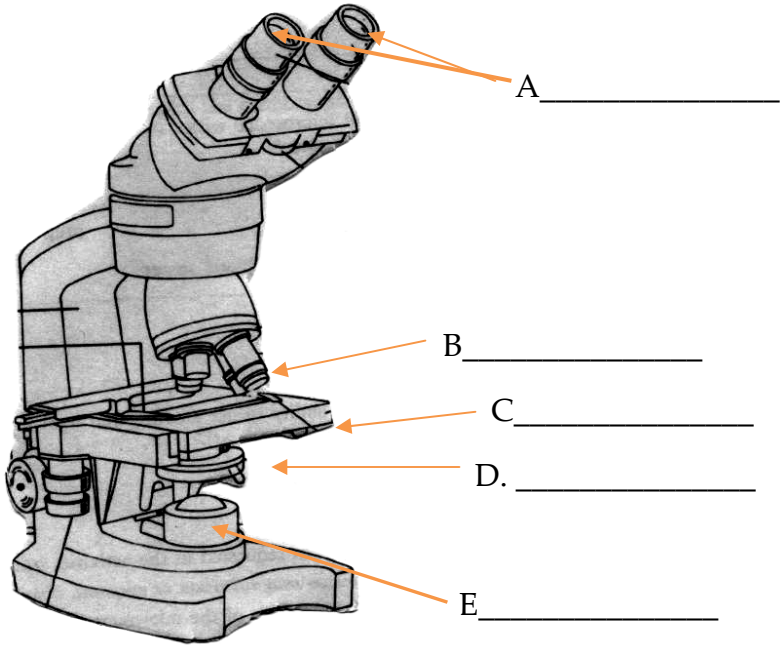
Deoxyribonucleic acid is found in all living organisms. The ability of a cell to utilize energy, reproduce, maintain homeostasis, and pass on genetic material necessitates DNA. In this lab you will isolate DNA from plant cells to be run on the gel along with other experimental samples. In order to isolate DNA from the other cell components, you will need to physically break up the plant tissue by grinding it in a salt solution. DNA is soluble and stable in salt solution due to its ionic properties. DNA is also complexed with proteins; Sodium Dodecyl sulfate (SDS) is a detergent that is used to disrupt the ionic interactions between the postitive histones and the negative backbone of the DNA molecule. It will also help to denature enzymes that would otherwise chop up the DNA (deoxyribonucleases). Adding a cold organic layer (alcohol) causes the surface of the aqueous layer to become less polar resulting in DNA precipitation. Despite the fact that it may not be totally intact, large thread like portions will still exist and can be collected or spooled out of the upper alcohol layer with a glass rod or transferred with a pipet. This can be repeated to further clean up the DNA sample by suspending the DNA in more of the salt solution and precipitating the DNA again. The aggregates are large enough to be seen without the aid of a microscope.

You will collect the aggregates and prepare them for storage so that in a future lab you can run them on the gel to visualize the isolated DNA.

In Class preparatory questions

The answers to the following questions will be reviewed at the beginning of each Lab. Refer to the appendix for more microscopy information. It is in your best interest as a student to attempt them prior to each Lab, though not required.

1. Label the specified parts of the microscope



2. The lab manual emphasizes that you should always start focusing with the stage up close to the lens and *always start to focus by moving the lens **away** from the stage.* Why is this wise to do?

3. Total magnification is the product of the: (***circle one***)

a.) objective X ocular

b.) objective X stage

c.) condenser X ocular

d.) body tube X ocular

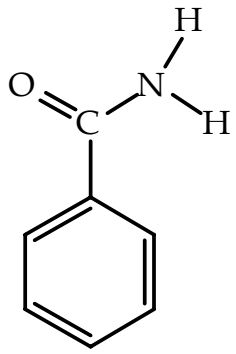
1) Draw two different molecules, all having the same formula: C_3H_5NO. That is, each molecule should follow all the bonding rules and consist of 3 carbon, 5 hydrogen, one nitrogen, and one oxygen atom(s).

a) b)

2) Consider the molecule below.

1. Draw a circle around one part of the molecule that is hydrophilic.
2. Draw a dotted line around one part of the molecule that is hydrophobic.

There are multiple correct answers to this problem.



3) Draw glycine and several water molecules making hydrogen bonds with the appropriate parts of it.

Laboratory Excercise

A. Introduction to Cells

In the first exercise you will use a microscope and prepared slides to visualize cells from different domains and explore different tissue types. Always treat the microscope with great care. Make certain that you do not touch any part of the lens system with anything abrasive (such as a slide or dirty water) or greasy (such as even the cleanest fingers). Never clean a lens with anything except clean lens paper! If the view gets foggy (as it probably will sometime during the semester), and lens paper will not clean it, call your laboratory instructor.

I. Comparison of Eucaryotic and Procaryotic cells – Find slides containing cells from 2 different domains. **How do you expect them to differ?** ***Formulate a hypothesis***

Make observations: include sketches, estimates of size and labels. Are cell structures recognizable? Are there differences in size? Are there differences other than just the presence or absence of a nucleus? Can you identify specialized structures within the cells?

II. Comparision of Eucaryotic Tissue Types – Find slides from at least 3 different kingdoms**. How do you expect them to differ?** ***Formulate a hypothesis***

Make observations: include sketches, estimates of size and label which Kingdom it represents. Are there differences in size? Can you identify specialized structures within the cells? Can you determine the function of the cell?

B. Chemistry

The molecular model kits have five different types of atoms. Carbon (black), Oxygen (red), Nitrogen (blue), Chlorine (green), and Hydrogen (light blue sphere). Each of these represents an atom, composed of its nucleus and the surrounding electrons. These atoms can be connected to each other by inserting the white rods into the holes. It will become apparent to you that different atoms have different capacities for bonding with other atoms. The holes in the plastic "atoms" indicate the number of electrons that the atom is able to share with another atom.

Note that there are two types of black atoms. One type has 4 holes - you should use these ones. Others may have 5 holes; don't use them since the geometry will be wrong.

A reminder of the number of bonds each atom makes & the corresponding charge:

	Number of bonds					
Element	0	1	2	3	4	5
H	+	neutral				
O		–	Neutral			
N				neutral	+	
C					neutral	
S		–	Neutral			
P						neutral

Also a reminder of the relative electro-negativities of a few relevant elements:

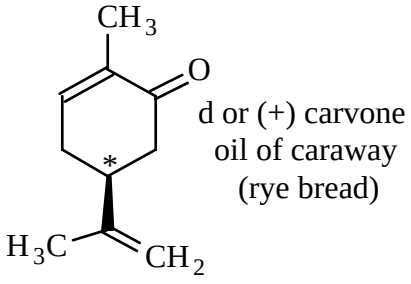
Low: C, S, P, and H

High: N, O and Cl

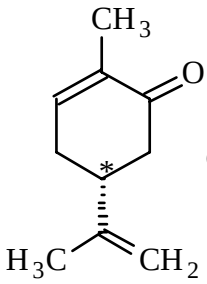
These properties can all be explained in terms of the electronic structures of the elements involved. You may want to take time to discuss this as a class. See periodic table at the end of this section for details.

The short rods are used to indicate the covalent bond involving hydrogen, since hydrogen, being the smallest atom, has a smaller distance between it and a carbon atom. Similarly, the curved rods are used to show double and triple bonds and have the effect of bringing the atoms closer together, which reflects the true situation. The nuclei of carbon atoms in a C=C (double) bond are closer together than in a C-C (single) bond, but not so close as in a C≡C (triple) bond.

Chirality is a very important feature of biological molecules because their exact 3-dimensional shape determines their function. An interesting example of this are the two forms of the molecule carvone:



d or (+) carvone
oil of caraway
(rye bread)



l or (-) carvone
oil of spearmint
(mint)

Both have the identical formulas ($C_{10}H_{14}O$) and identical structures except for the arrangement of the atoms at the *ed carbon. With the ring of carbons lying flat on the table and the C=O on the right, as shown, the dashed bond points down into the table and the triangle bond points up from the table.

I. Using the model kits

Working in groups of three, build these molecules using the stick models kits.

Based on the description of chirality above, do you think there will be many ways to build a complex biomolecule? Will the order or location of atoms make a difference?

Formulate a hypothesis

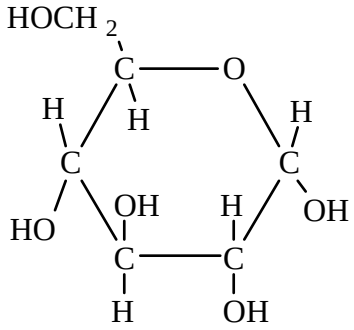
1) Alcohols

butanol C_4H_9-O-H

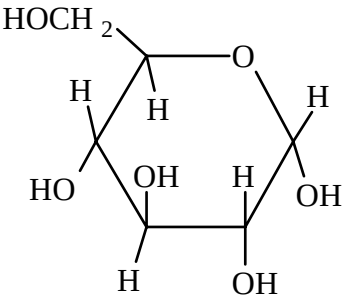
Note: there are 5 isomers of butanol. Three are structural isomers. Two are enantiomers - that is, they are mirror-image isomers (see your course textbook for explanation of the three types of isomers). Draw the three structural isomers and build models of the two enantiomers.

2) Sugars:

glucose

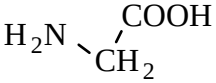


or:

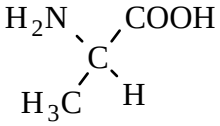


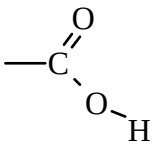
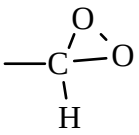
3) Amino acids

glycine

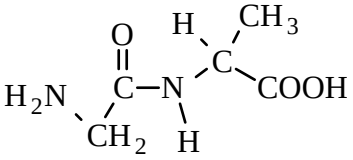


alanine



note that -COOH is  not 

Take two amino acids and join them to make a dipeptide, such as the one below: what did you have to remove to make this molecule? You have made a peptide bond.

a dipeptide (glycine-alanine) 

II. Computer Models

Jmol is a molecular viewing application. It lets you rotate, highlight, zoom in on, etc. a two-dimensional image of a three-dimensional molecule. It shows molecules in a simplified format, specifically:

- unless noted, hydrogen atoms are not shown
- all covalent bonds are shown as a single rod, whether the bond is single, double, or triple
- atoms are shown as colored spheres; the colors identify each type of atom

Procedure

A) You will work in groups of three people per computer

B) To find the program, open a browser on the computer and go to www.bio.umb.edu and look for the **Biology 111: Hawthorne** link and click on it. (scroll down to the middle of the web page, under spring 2010 course info)

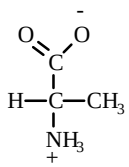
C) Once at the Spring Biology 111 web page, look at the lab syllabus in the row for this week's exercise. You will find in the links and downloads column "Chemical Models, Properties & Structures " click on it in order to proceed through this lab. Follow the exercise there and fill in the worksheet.

Your TA will give you two randomly-selected numbers which corresponds to two amino acids in a protein. One-by-one, choose the number assigned to you by your TA from the list provided on the web site for problem 8. The program will display the amino acid with the hydrogen atoms omitted. It will also show the adjacent two amino acids to help you find the right parts. Draw the complete structure of this amino acid, including the hydrogen atoms. Using the chart in the lab manual, identify the amino acid you have been assigned.

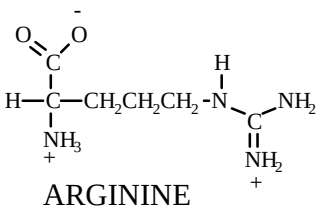
a) Numbers given by TA _____ _____

Identities of amino acids _____ _____

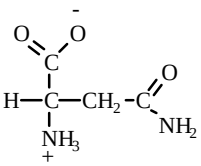
STRUCTURES OF AMINO ACIDS



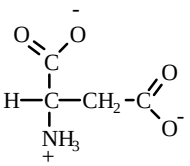
ALANINE
(ala)



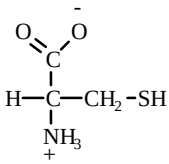
ARGININE
(arg)



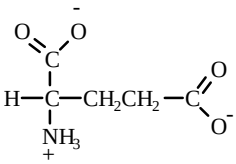
ASPARAGINE
(asN)



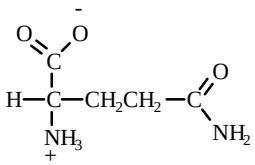
ASPARTIC ACID
(asp)



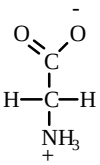
CYSTEINE
(cys)



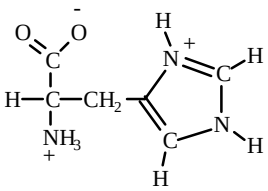
GLUTAMIC ACID
(glu)



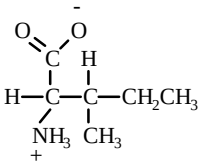
GLUTAMINE
(glN)



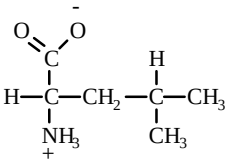
GLYCINE
(gly)



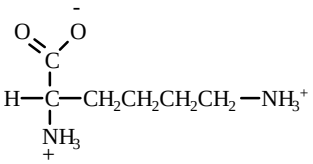
HISTIDINE
(his)



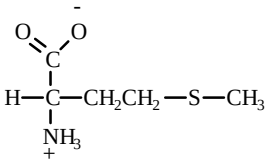
ISOLEUCINE
(ile)



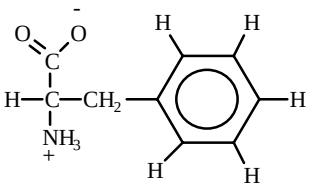
LEUCINE
(leu)



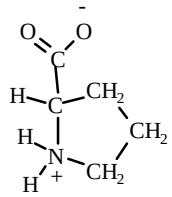
LYSINE
(lys)



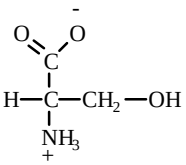
METHIONINE
(met)



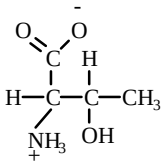
PHENYLALANINE
(phe)



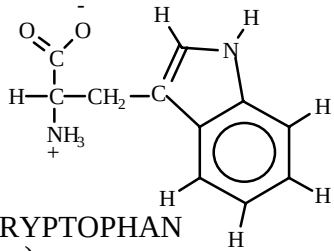
PROLINE
(pro)



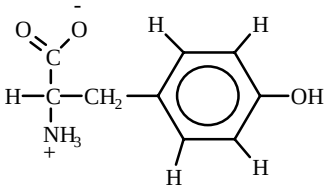
SERINE
(ser)



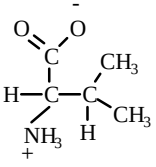
THREONINE
(thr)



TRYPTOPHAN
(trp)



TYROSINE
(tyr)



VALINE
(val)

Partial Periodic Table

H Hydrogen $1p^+$ $0n^0$ $1e^-$ 1(1 open) En=2.1							**He** Helium $2p^+$ $2n^0$ $2e^-$ 2(full) En=none
Li Lithium $3p^+$ $4n^0$ $3e^-$ 2(full) 1(7 open) En=1.0	**Be** Beryllium $4p^+$ $5n^0$ $4e^-$ 2(full) 2(6 open) En=1.5	**B** Boron $5p^+$ $6n^0$ $5e^-$ 2(full) 3(5 open) En=2.0	**C** Carbon $6p^+$ $6n^0$ $6e^-$ 2(full) 4(4 open) En=2.5	**N** Nitrogen $7p^+$ $7n^0$ $7e^-$ 2(full) 5(3 open) En=3.0	**O** Oxygen $8p^+$ $8n^0$ $8e^-$ 2(full) 6(2 open) En=3.5	**F** Fluorine $9p^+$ $10n^0$ $9e^-$ 2(full) 7(1 open) En=4.0	**Ne** Neon $10p^+$ $10n^0$ $10e^-$ 2(full) 8(full) En=none
				P Phosphorus $15p^+$ $16n^0$ $15e^-$ 2(full) 8(full) 5(3 open) En=2.1	**S** Sulfur $16p^+$ $16n^0$ $16e^-$ 2(full) 8(full) 6(2 open) En=2.5		

Elements in this column do not form any covalent bonds (at least, not in Bio 111)

Symbol

Name

Total number of electrons (e^-; (-1 charge). Balances protons.

P
Phosphorus
$15p^+$ $16n^0$
$15e^-$
2 (full)
8 (full)
5 (3 open)
En = 2.1

Contents of nucleus: p^+ = proton (+1 charge)
n^0 = neutron (0 charge)

electrons in innermost shell

electrons in next shell out

electrons in outer (valence) shell
("# open" = # required to fill outer shell)

Electronegativity. Increases as you go ⇑ and ⇒

C. DNA Extraction
How do you expect the DNA to look? *Formulate a hypothesis*

Materials for Plant DNA Extraction:

Raw plant material

- Salt solutions
- Graduated cylinders
- Ice cold ethanol on ice
- SDS solution
- microfuge tubes
- transfer pipets
- cheese cloth/funnel, flask
- 37 C waterbath
- microcentrifuge
- glass tube and rods
- TE buffer
- Nucleic acid sample buffer

Procedure

1. Grind the given plant material in 10ml of EDTA Salt Buffer, pH 8 using a mortar and pestle.
2. Strain the ground plant and salt solution with cheese cloth and a funnel, into a flask.
3. Add 5ml sodium dodecyl sulfate (SDS) solution, swirl and transfer to the glass cylinder.
4. Dribble ice cold ethanol very slowly down the side of the glass tube so that it forms a layer on top of the liquid in the tube. Continue until the volume of the liquid is doubled.
5. With a glass rod, stir very slowly and watch the DNA precipitate and float up into the alcohol layer. Threads/strands of DNA may wind up onto the glass rod. Once extracted by this method they can be purified, dissolved and used for many types of experiments.
6. Stop stirring, and let it sit for a few minutes (5) while you label a microfuge tube, "*Plant DNA"* with your name or a symbol to recognize. Carefully pipette some of the area between the top and bottom phase, transfer it into labeled microfuge tube. This is your crude, plant DNA extract, you will run it on the gel along with your experimental samples, but first you must get rid of the alcohol it is in.
7. Be sure your tube is labeled and that you record in your notebook how you labeled it so that you can identify it later on.
8. Spin down a pellet of DNA in the micro centrifuge (TA will collect everyone's samples and do this-top speed for 3 minutes). This pulls the DNA to the bottom, and you can then carefully pipette off as much of the liquid as possible without touching the pellet. Leave the tubes caps open for the ethanol to evaporate in one rack on the instructor's table for about 30 minutes.
9. Add 50µl of TE buffer to the DNA pellet and resuspend.
10. Add **15 µl** of sample buffer to your sample, mix and freeze until next lab.

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Biological Chemistry

Learning Goals: After this lab students should have a better understanding of

- Differences between hydrophobic and hydrophilic
- Enzymatic digestion
- Diffusion

Introduction

Artificial membranes can be chemically manufactured. Membranes are useful in the biotechnology field because they can serve as a model for biological membranes. For example, during the drug screening phase in some pharmaceutical companies, artificial membranes serve as models of the digestion system in order to determine if a drug would enter a person's blood stream if digested. We will use artificial hydrophilic and hydrophobic membranes in order to examine diffusion, or the movement of molecules.

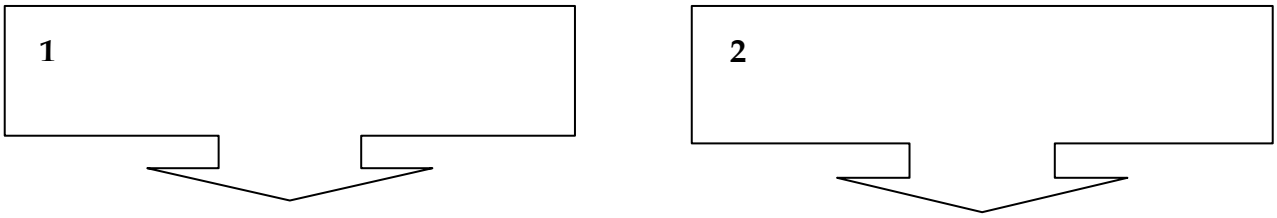
Cells can transport enzymes across membranes. Specific types of enzyme with the specific ability to break up proteins into smaller units are termed proteases. Major proteases in humans are pepsin, trypsin and chymotrypsin. Pepsin is found in the stomach of animals and works best at pH 2. Its catalytic site is a pair of aspartic acid residues. Trypsin and chymotrypsin have a catalytic triad of serine, histadine and aspartic acid residues. These proteases are found in the small intestine and have pH maximums near pH 8.0. Plant proteases are essentially found in tropical plants such as papaya (*Carica papaya*) and pineapple (*Anana sativa*). The active site of these proteases is marked by sulfhydryl groups. From papaya, a heavily used and important industrial enzyme, papain, is obtained. Pineapple fruit or its stalk provides us with bromelain. Meat tenderizer contains papain, a protein-digesting enzyme derived from papaya. In today's experiment we will measure the activity of several proteases sold on the commercial market. We will test these proteases on the protein gelatin.

Because of its unique properties, gelatin is widely used in the food industry to stabilize desserts, and to thicken, emulsify, and bind products. Gelatin is a very important protein nutrient with no cholesterol. Gelatin is extracted from dead pork and beef skins and bones as collagenous material, which is then treated for the final gelatin. Being a protein, gelatin has a unique sequence of amino acids. However, it has a high content of the amino acids glycine, proline, and hydroxyproline. These three amino acids frequently bind to form repeating sequence of the triplet glycine-proline-hydroxyproline which in reality give gelatin its triple helical structure. This helical structure is responsible for the trapping of water molecules and forming gelatin structure.

In Class preparatory questions

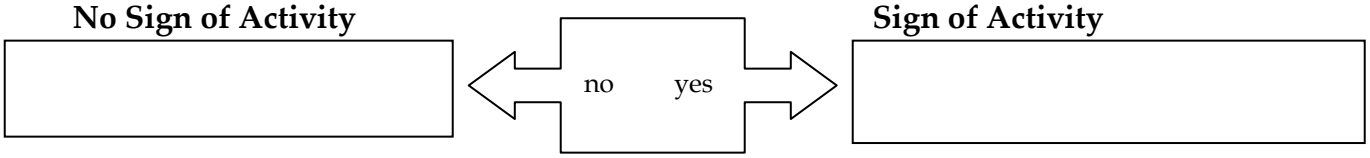
The answers to the following questions will be reviewed at the beginning of each Lab. It is in your best interest as a student to attempt them prior to each Lab, though not required.

Create a flow chart for Part C: Jell-O
(Describe each treatment sample that you will mix up in lab.)

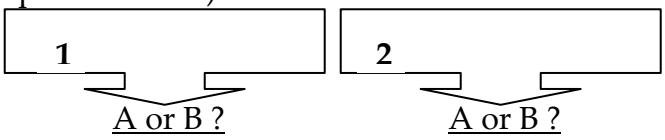


Concisely describe stepwise what you will do with the samples after you mix them up, for how long and the equipment you will use:

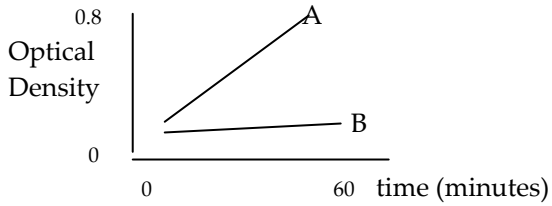
How will you know if protease enzymatic activity is present or not in the treatments ?



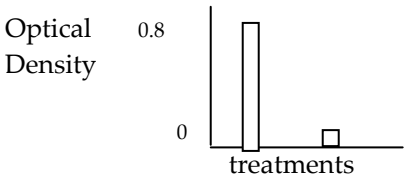
For each treatment do you expect to have activity? What would its graph most likely look similar to over time: line A or B from the hypothetical line graph below (which is of just two samples A and B) ?



Hypothetical Line Graph



Bar Graph: indicate which bar represents A and B from the nearby line graph.



Laboratory Excercises

A. Hydrophobic and Hydrophilic membranes

****Membranes should NOT be touched. Handle all membranes with FORCEPS or tweezers****

Depending on the composition of a membrane, it can **wet out**, as can be observed by a slight color change in the membrane. You will determine the "wet-ability" of one hydrophilic and one hydrophobic membrane in water, oil, ethanol, soap, and glycerol.

Question: Will hydrophilic membranes wet in water? Oil? Ethanol? Soap? Glycerol?
Hypothesis:

	Water	Oil	Ethanol	Soap	Glycerol
Wet? (yes/no)					

Experimental Design:
Place a different piece of hydrophilic membrane in water, oil, ethanol, soap, glycerol.
Record Observations:

Conclusion:

What other questions do the experimental results generate?

Question: Will hydrophobic membranes wet in water? Oil? Ethanol? Soap? Glycerol?
Hypothesis:

	Water	Oil	Ethanol	Soap	Glycerol
Wet? (yes/no)					

Experimental Design:
Place a different piece of hydrophobic membrane in water, oil, ethanol, soap, glycerol.
Record Observations:

Conclusion:

What other questions do the experimental results generate?

Formulate your own experiment using membranes:
Question:
Hypothesis:

Experimental Design:

Record Observations:

Conclusion:

What other questions do the experimental results generate?

B. Hydrolysis of Glucose from JELL-O

Hydrolysis reactions result in the breakdown of polymers into monomers. We will use Glucose test strips in order to monitor release of Glucose from sugar as a result of water, oil, ethanol, salt water, sugar water, soap, and glycerol.

Question: Will glucose release from JELL-O as a result of water? Oil? Ethanol? Salt water? sugar water? Soap? Glycerol?
Hypothesis:

	Water	Oil	Ethanol	Salt water	Sugar water	Soap	Glycerol
Hydrolysis?(yes/no)							

Experimental Design:
Place a piece of JELL-O in water, oil, ethanol, salt water, sugar water, soap, glycerol. Use a glucose test strip to monitor the presence/absence of glucose after 1 hour and again after 2 hours.
Record Observations:

Conclusion:

What other questions do the experimental results generate? Is there any way to be sure the glucose being measured is from hydrolysis?

C. Digestion of JELL-O with Proteases in Pineapple Extract

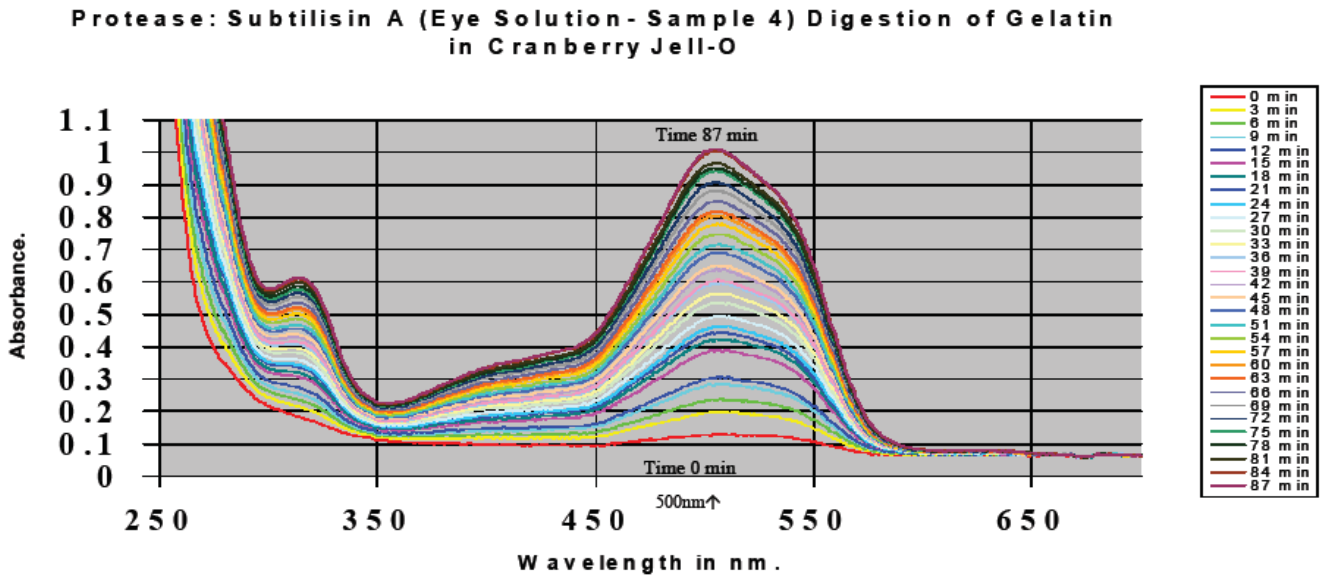
(start after you set up Part B)

The following is a list of the materials that are needed for this experiment:

JELL-O Brand Cranberry Flavor Gelatin Dessert.
Pineapple Extract
Weighing scale, various beakers, Pasteur pipettes, Petri dishes,
Spectrophotometer tubes
Spectrophotometer for absorption readings

The Spectrophotometer is a device for measuring the absorption of a particular wavelength or color of light. This absorption of light energy is proportional to the amount of absorbing material. Beer's law states that the absorbance (A) is dependent upon the concentration (c) of the solution, the light path (b), and a constant (a) which depends upon the absorbing compound. Absorbance is as easy as abc! A = log (Io/Ii) = abc. In this experiment we will use a Genesis Spectrophotometer to measure the color released when proteases are added to Jell-O samples. As the amount of Jell-O is being digested the food coloring within the gel structure is liberated and released into solution. The more color released the higher its solution concentration and the greater the Absorbance reading.

Absorption spectrum of dye released from protease digestion of cranberry Jell-O.



Preparation of cranberry flavored Jell-O: (This will already be done for you)

To make the gelatin molds, stir 350 ml of hot water into one package of cranberry gelatin in a large bowl. When all of the gelatin particles are completely dissolved, the solution is allowed to cool for 10 minutes. (You also may want to measure the absorption spectrum of the solution the liquid Jell-0 solution before it sets- especially if you are using another colored gelatin for your experiment). The warm solution of colored gelatin is poured into petri dishes up to 1 cm deep. This is then left overnight to dry and thicken. This is almost twice the concentration listed in the direction on the package, but the thicker concentration is easier to cut into small pieces of gelatin.

You will use a moistened spectrophotometer tube to core out individual gelatin fragments for consistency. This is like using a cookie cutter to produce different shapes of cookies you are about to bake. The mass might change a little with drying time, but each piece will nearly be the same. The absorption spectrum of the sample will be that of the cranberry flavoring dye added to the gelatin mixture.

The cranberry Jell-O solution absorbs light energy around 500 nm. The method we will be using to determine the activity of proteases is to measure the release of colored flavoring from the solid gel. When cranberry flavored Jell-O is digested by proteases a red color will be liberated from the gelatin structure and released into the solution. In this experiment, solid aliquots of cranberry Jell-O are placed in the bottom of a beaker and covered with solution. The pineapple extract will be added to the beaker and the release of the colored dye will be measured with a spectrophotometer.

Are there proteases in pineapple extract? ***~Formulate a hypothesis~***

Experimental Set up

1. Label 4 fifty milliliter beakers with numbers from 1 to 4 and indicate the treatment based on the chart below.
2. Place one solid core of cranberry red Jell-O into beakers 1 and 2. You should use a moistened spectrometer tube for cutting.

<u>Create the solutions :</u>

Use the other two beakers to mix and store the prepared solutions in as you make them, so they can be added to the appropriate beaker with jell-O at time zero.

3. Add 7 mls of distilled water to beaker 3 and add 5ml of distilled water to beaker 4.
4. Add 2mls of pineapple solution to beaker 4 only. (this solution is the juice of 1/3 cup chopped and squeezed fresh pineapple).

Begin Experimental Measurements

5. When you are ready to start the experiment, pour each solution over the jell-o in the corresponding beaker and start the timer.
6. Every five minutes, mix the solution by swirling the Jell-O containing beakers. Pour off around 4mls of this swirled solution into a spectrophotometer tube; be cautious not to get bits of the Jell-O in the tube, just liquid. Place the tube in a spectrophotometer and measure the absorbance at 500 nm.
7. When each measurement is completed pour the solution back into the respective beaker and swirl to mix.
8. Repeat for 40 minutes or until one treatment dissolves all of the jell-O.

Final Experimental Treatments.

	Jell-O	Water	Pineapple
Beaker # 1 Control Treatment	2 cc	7 ml	0
Beaker # 2 Pineapple Treatment	2 cc	5 ml	2 ml

Record Data:

	0′	5′	10′	15′	20′	25′	30′	35′	40′
#1 Control Treatment									
# 2 Pineapple Treatment									

D. Diffusion of Protease through Membranes

Each person in a group will design an experiment to monitor diffusion through hydrophilic membranes. Be sure to communicate with your group members to diversify the questions being addressed. Together you will design a set of experiments to address the following question: Will protease diffuse through a membrane and digest JELL-O? You will have 2 different membranes and 2 different proteases to test. Your set up will have the JELL-O on one side of the membrane and the protease on the other. You will be provided with petri dishes of JELL-O, 2 proteases, and 2 types of membrane. One factor that may influence diffusion, could be the state of the membrane. **Does the 'wetness' of the membrane effect diffusion?** How will you measure this?

Perform an initial experiment and monitor the results after 1 hour. Use the results from this experiment to formulate a new experiment. The results from the second round will

be observed during the next lab. ****Membranes should NOT be touched. Handle all membranes with FORCEPS or tweezers****

Question:
Hypothesis:

Experimental Design:

Question:
Hypothesis:

Experimental Design:

Record Observations in 1 hour:
Hint: What should you be looking for based on part B?

Conclusion:

What other questions do the experimental results generate?

Formulate an experiment to answer this question (next page)

Hypothesis:

Experimental Design:

Record Observations next week:

Conclusion:

What other questions do the experimental results generate?

References:

Hagar, William & Lornie Bullerwell (2003),"Supermarket Proteases" *The Science Teacher October 2003 pp26-30*

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Cell Communication in Planarians

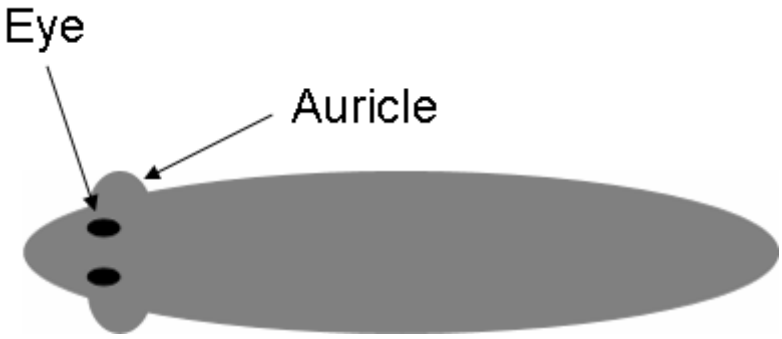
Written by Sabrina Hawthorne, PhD

Learning Goals:

- The role of signal transduction in an organism responding to its environment
- Role of Gap junctions in cell communication

Introduction:

Planarians are model organisms that are regaining popularity amongst scientists due to their regenerative abilities and its relevance towards stem cell research. They are also valuable in the study of organismal responses to environmental signals. The capacity of an organism to respond to its environment is dependent on signal transduction pathways within and between cells of the organism. These signal transduction pathways are series of biochemical reactions that initiate with a signal and end with a response.



One of the most basic responses is the ability to move. Such taxis (movement) is an organismal response to signals in the environment resultant from many signal transduction pathways involving many cell types. Gap Junctions (or gap channels) are intercellular junctions that enable signals to pass between cells. For example, the coordination of all the cells of a heart in the heart beat is the result of electrical signals passing through Gap Channels.

This lab contains a series of hypothesis driven exercises that will allow the student to reveal some basic features of the planarians ability to respond to environmental cues.

In Class preparatory questions

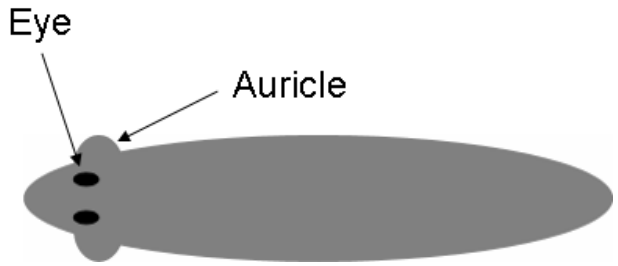
The answers to the following questions will be reviewed at the beginning of each Lab. It is in your best interest as a student to attempt them prior to each Lab, though not required.

1. What type of research are planarians currently being used as a model organism for? Why

2. List 4 examples of signals that a planarian can receive from the environment.

1)
2)
3)
4)

3. Indicate on the diagram below where a planarian might contain Protein Receptors that allow it to interact with the environment.



4. Would you expect that the Protein Receptors found in planarians also be found in other organisms? Why or why not? What would this also mean about potential genes shared between planarians and other organisms?

Laboratory Excercise

I. Mechanism of Locomotion in Planarians.

A specific question can be addressed with an experimental design that elicits an explicit result.

Question: Are cilia **or** muscles responsible for the movement of planarians?

Hypothesis:

Experimental Design:

A solution of Lithium Chloride inhibits ciliary action whereas a solution of Magnesium Chloride inhibits muscle action. Label 3 wells of a 6 well plate 1) spring water; 2) 1% lithium chloride; and 3) 1% magnesium chloride. Place 3 separate planaria into one of the 3 wells. Observe specimens with a dissection microscope.

Record Observations:.

Conclusion:

What other questions do the experimental results generate?

II. Planarians ability to respond to water currents.

Question: Can planarians respond to water currents? Or: Where do planarians contain receptors that enable them to respond to water currents?

Hypothesis:

Methods:

Use a pipette to apply a current in each of the following directions. Record your results.

1. the head (anterior)
2. the tail (posterior)
3. the top (dorsal) side
4. the bottom (ventral) side

Observations:.

Conclusion:

What other questions do the experimental results generate?

III. Phototaxis in Planarians.

In order to address a broad question, multiple specific questions can be answered.
Question: How do planarians respond to light?
Devise 4 specific questions that can be easily tested in order to elucidate how planarians respond to light. Write them in the boxes below:

Question 1:
Hypothesis 1:

Experimental Design 1:
Record Results:
Conclusion:

Question 2:
Hypothesis 2:
Experimental Design 2:
Record Results:
Conclusion:

Question 3:
Hypothesis 3:
Experimental Design 3:
Record Results:
Conclusion:

Question 4:
Hypothesis 4:
Experimental Design 4:
Record Results:
Conclusion:

IV. Chemotaxis in Planarians.

In order to address a broad question, multiple specific questions can be answered.
Question: How do planarians respond to food?
Devise 4 specific questions that can be easily tested in order to elucidate how planarians respond to food.

Question 1:
Hypothesis 1:
Experimental Design 1:
Record Results:
Conclusion:

Question 2:
Hypothesis 2:
Experimental Design 2:
Record Results:
Conclusion:

Question 3:
Hypothesis 3:
Experimental Design 3:
Record Results:
Conclusion:

Question 4:
Hypothesis 4:
Experimental Design 4:
Record Results:
Conclusion:

References:

Caroline E. Stringer (1917) The Means of Locomotion in Planarians. Proc Natl Acad Sci U S A. December; 3(12): 691–692.

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Cellular Respiration

Learning Goals:

After completing these laboratory experiments you will be able to:

1. Describe in detail the process of glycolysis.
2. Compare and contrast anaerobic and aerobic metabolism of glucose.
3. Design experiments to determine effective substrates for metabolic processes.

Introduction

All living organisms require an input of energy for their basic needs. Energy is used for the synthesis of biomolecules, maintaining osmotic gradients, and movement. The chemical changes and processes that occur in living cells are called metabolism. Metabolic activity usually results in the production of and/or uptake of a gas; for example, carbon dioxide evolution in fermenting organisms and oxygen uptake in respiring organisms.

It is very likely that early living cells evolved under anaerobic conditions -- that is, with no molecular oxygen (O_2) in the atmosphere. All living cells today have mechanisms for obtaining energy from organic molecules <u>without</u> O_2 (anaerobic metabolism). In some organisms the process results in the production of ethyl alcohol (used in wine & beer manufacture).

$$\text{Glucose } (C_6H_{12}O_6) \xrightarrow{\text{(Many enzymes)}} 2CO_2 + 2 \text{ Ethyl Alcohol } (C_2H_5OH) \text{ (plus energy)}$$

The organism traps some of the released energy in ATP molecules which then supply that energy to various energy-consuming activities necessary for life.

Later on oxygen began to accumulate in the atmosphere, as a result of photosynthesis, from the newly evolved photosynthetic organisms, and many new organisms developed metabolic pathways which degraded the molecules produced by anaerobic metabolism, utilizing O_2 as a "dump" for hydrogen atoms. The overall process (aerobic respiration), which utilizes oxygen, extracts much more energy from the original food molecules than does anaerobic respiration. When glucose is the starting molecule:

$$\text{Glucose } (C_6H_{12}O_6) + 6O_2 \xrightarrow{\text{(many, many enzymes)}} 6CO_2 + 6\,H_2O \text{ (plus energy trapped in ATP)}$$

In Class preparatory questions

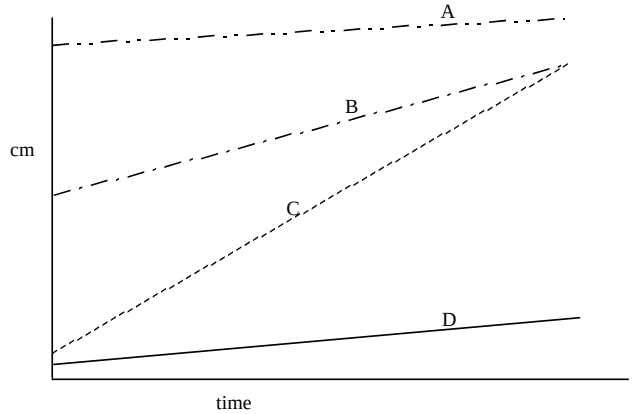
The answers to the following questions will be reviewed at the beginning of each Lab. It is in your best interest as a student to attempt them prior to each Lab, though not required.

Create a flow chart of your lab experiment. State the general comparison of today's experiment in the large rectangle below and describe each one of the treatment mixtures in a box beneath it.

Concisely describe stepwise in the rectangle below what you are measuring and the method of measure across your treatments. Include the equipment you will use and time intervals.

How will you recognize a high rate of activity?

The graph below shows some hypothetical results from four Smith tubes: A, B, C, and D. Which reaction has the greatest rate? Explain.



Laboratory Excercise

Part I: Effect of pH on the Rate of Fermentation in Yeast.

In this lab exercise, you will study the effect of pH on the rate of the overall process of anaerobic respiration, using a solution of glucose and yeast organisms in various buffers each with a different pH. As a measure of the rate of the reaction, you will be measuring the amount of CO_2 produced. **How will anaerobic respiration be effected by pH?** ***Formulate a hypothesis.***

l. Examine the Smith fermentation tubes and figure out how you will measure the CO_2 produced by the yeast. Label 5 tubes as listed below with numbers (l to 5 and correlating pH), and calibrate the closed portions of each tube by taping a ruler to it or marking off l mm intervals (or 5 mm, whatever seems feasible) with a waterproof marking pen.

Tube 1 pH 4
Tube 2 pH 5
Tube 3 pH 6 Fill the tubes after you mix up each treatment in a beaker.
Tube 4 pH 7
Tube 5 pH 8

2. Your Lab Instructor will mix the yeast solution (5% glucose + 55g yeast/1000ml). Students will pour off approximately 50ml of active yeast solution for their experiment. Set up 5 beakers, each labeled for each buffer you have. Add 5 ml of your yeast solution to each beaker. Wear gloves and add l5 ml of the appropriate buffer solution to each labeled beaker and mix the yeast and buffer by swirling the beaker, be careful with the more acidic (lower pH) solutions since they may damage clothes (Be sure to wipe up spills and use care handling).

Beaker 1 5ml yeast solution and l5 ml pH 4 buffer
Beaker 2 5ml yeast solution and l5 ml pH 5 buffer
Beaker 3 5ml yeast solution and l5 ml pH 6 buffer
Beaker 4 5ml yeast solution and l5 ml pH 7 buffer
Beaker 5 5ml yeast solution and l5 ml pH 8 buffer

3. Now transfer the solution in each beaker to the appropriately labeled smith tube.

4. Mix the solutions in each tube very well. Then test the pH with pH paper, record results in table I below. Two partners should each independently test each pH, and if results do not agree with each other repeat pH determinations.

5. Let the entire set of Smith tubes equilibrate for about l0 minutes. Invert the tubes as simultaneous as possible, so that only the future CO_2 gas generated is trapped. Record the time (this is the beginning of the experiment). If reaction rate is too slow place the tubes in a 37°C water bath to speed it up.

6. Measure the length of the tube occupied by gas at l0 minute intervals for 40 minutes (or 60 minutes, if time permits). Record measurements in table II below.

7. At the end of the experiment the pH of each solution should be measured again (record in table I). Yeast cells produce acids as well as alcohol, and the buffering used may not quite be able to keep the pH constant. If the pH of any solution has changed during the experiment, use the average pH of that solution when you plot your results (Graph 2, below).

8. All glassware must be thoroughly washed in hot soapy solution and rinsed well. Replace tubes and beakers where you found them at the start of the lab. Pipets can go in the dirty pipet bin on the instructor's table.

Table I -- pH Measurements

Tube #	pH of buffer	pH of mixture at the start	pH of mixture at the end	average pH of mixture (beginning+end/2)
1	4			
2	5			
3	6			
4	7			
5	8			

Table II -- Amount of Gas in Tubes. (Length of tube filled with gas, in mm)

Tube #	0 min.	l0 min.	20 min.	30 min.	40 min.
1					
2					
3					
4					
5					

Plotting results for Part I:

l. Graph l: each student should plot on one graph the amount of gas produced in each tube (dependent variable) vs. time of incubation (independent variable). The slope of the curve indicates the rate of the reaction -- the steeper the curve, the faster the reaction. Which tube had the fastest rate of reaction? Which had the slowest?

2. Graph 2: each student should plot on a second graph the amount of gas produced after 40 minutes incubation (or 60 minutes - whatever the maximum time of your experiment was) versus the average pH of the solution. In this graph the independent variable is pH, so it is to be plotted along the x axis.

3. Based on your graphed data what is the effect of pH on yeast enzymes? Would you expect human enzymes to have the same pH optimum as yeast enzymes? (Hint: the intracellular pH of most living cells is 6.8. Extra cellular pHs may vary: for instance, the pH in the stomach is about 2.0 or so.)

Part II: Oxygen Consumption

In this laboratory you will observe the respiration rate of a culture of algae, *Chlorella* (or a suitable substitute) using an oxygen electrode to monitor any changes in the oxygen concentration that occur during metabolism. Organisms that are aerobic or aerobically grown facultative organisms, such as *E. coli*, utilize oxygen as their terminal electron acceptor. Photosynthetic organisms respire as well.

Equipment

The oxygen electrode is a device that is used to measure the concentration of oxygen in solution. The Clark-type oxygen electrode used in this class consists of a silver-platinum electrode combination immersed in a saturated solution of potassium chloride covered by a teflon membrane - permeable only to gases. When a polarizing voltage is applied to this electrode, the silver at the anode goes into solution forming silver chloride. The electrons liberated at the anode from this half-cell reaction are then used to reduce oxygen at the platinum cathode. These oxidation-reduction reactions generate a small amount of current when oxygen is electro-reduced to water at the platinum electrode which is proportional to the oxygen concentration of the solution. Only oxygen diffusing through the teflon membrane from the solution above it will produce this current. The oxygen electrode you will be using has a digital readout meter which directly gives you the percentage of oxygen remaining in the solution.

Methods

An actively growing culture of *Chlorella* suspended in water has been added to each oxygen electrode sample chamber, and is uncovered.

<u>Start of Experiment for Students</u>

1. Cover the sample with the provided box, this will limit light from reaching the algae.

How will aerobic respiration be light? ***Formulate a hypothesis.***

2. Record an initial reading from the digital output.
3. Every 2 minutes, record a reading from the digital output again.
4. Monitor and record the rate of oxygen uptake for fifteen to twenty minutes.
5. A decent slope is eventually obtained (steady rate of oxygen uptake) determine where that is in your data, what is the difference between the rate of the first 8-10 minutes and the last 8-10 minutes?

Photosynthesis

Learning Goals: After completing this laboratory exercise you will be able to:

1. Observe the absorption of photosynthetic pigments at different parts of the visible light spectrum.
2. Describe the relationship between photosynthetic rate and light intensity.
3. Discuss the effectiveness of various colors of light for photosynthesis.

Introduction

Photosynthesis is the process whereby the radiant energy of the sun is converted into chemical potential energy of organic molecules. This process is responsible for present life on this planet - it provides food, and therefore the energy source for all living things. The utilization of light energy as an energy source is found only in certain photosynthetic organisms; a few bacteria and, of course, plants. Plants in addition to their formation of high-energy foodstuffs from light energy, water, and carbon dioxide also produce oxygen, a gas essential for our life. In today's experiments you will measure the formation of this "waste product" of plants using an oxygen electrode. The equation :

$$CO_2 + H_2O + \text{energy} \rightarrow (CH_2O)_n + O_2$$

can be used to represent the equation for photosynthesis (if read from left to right or for respiration (if read from right to left). The two processes have many similarities in their details but differ basically in the following ways: respiration is a way of getting useful energy from organic molecules $(CH_2O)_n$. Respiration occurs mainly in mitochondria in the cell, photosynthesis in chloroplasts**.** **Plant cells may be respiring and photosynthesizing at the same time.** Animals are dependent upon the photosynthetic activity of plants and only respire.

At least three factors limit the rate of photosynthesis: temperature, light intensity and CO_2 concentration. Water ordinarily does not limit the rate of photosynthesis. Why? For light to be used, it must be absorbed by a pigment. A pigment reflects some colors of light and absorbs other wavelengths of light. It is the energy that is absorbed by the pigment, which is useful biologically. Chlorophyll is the pigment involved directly in photosynthesis. It reflects green light, which is why it looks green, and absorbs at the red and blue ends of the spectrum. Other pigments in plants absorb other wavelengths of light and, though they don't participate directly in the photosynthetic reaction, they can apparently pass on the energy gained to chlorophyll molecules. Thus these accessory pigments are also important in photosynthesis.

Demonstration of Light Absorption by Pigments.

(Demonstration by Laboratory Instructor) Light that is a mixture of all the wavelengths we can see, appears white to us. A spectroscope separates light of different wavelengths into a spectrum. View the white light of a tungsten lamp through the spectroscope. Make a sketch of the spectrum. Solutions of leaf pigments which have been extracted and separated are available in test tubes next to the spectroscope. Now place a tube of pigment between light source and spectroscope. Hold the tube in place, view and make a sketch of the new spectrum. Are there any bands of color missing from the original spectrum? These are absorption bands. Where are these bands located?

What conclusions can you make regarding the visible color of a pigment and the color of light it absorbs?

The light absorbed by chlorophyll in an intact leaf is used to drive the chemical reaction shown previously. That is, light energy is converted to chemical energy. This conversion requires an assemblage of enzymes and other substances built into the chloroplast. When chlorophyll has been extracted into solution, it still absorbs light, but the energy cannot be captured as chemical energy. The light absorbed by the chlorophyll is re-emitted as light of a longer wavelength. This is called fluorescence. Hold a tube of chlorophyll extract so that bright light enters from its side with respect to you, and you will see its fluorescence. What color is the re-emitted light?

You may use this property of fluorescence to determine the absorption spectrum of a particular pigment molecule. Note that before a molecule that fluoresces can fluoresce light, it must first absorb light.

1. Place a colored filter between the light source and the pigment sample. Does the sample still fluoresce? If so, give it a relative rating such as +5 .
2. Now try another color and record its relative fluorescence response on the illuminated sample.
3. Continue with your measurements of "relative" fluorescence emission and the color of light illuminating the sample until you have tried and "rated" every color filter available.
4. Draw a graph of your data with the relative fluorescence as the dependent variable and the color of light (Red, Orange, Yellow, Green, Blue, Violet, (ROYGBV) as the independent variable. This type of plot is called an absorption spectrum. (You should be aware that the concentration of pigment in this sample was carefully controlled. Too little pigment, no visible fluorescence. Too much pigment, no fluorescence because of self absorption).

In Class preparatory questions

The answers to the following questions will be reviewed at the beginning of each Lab. It is in your best interest as a student to attempt them prior to each Lab, though not required.

Create a flow chart of your lab experiment.

Concisely describe stepwise what you will do in part I of your experiment:
(**the first part of today's experiment** / not the demonstrations)

Part I

Concisely describe stepwise what you will do in part II of your experiment:
(**the second part of today's experiment** / not the demonstrations)

Part II

Laboratory Excercise

Experimentation : Measurement of Oxygen Evolution.

You will determine what are the important physical factors involved in the production of oxygen paying particular attention to the effects of light intensity and light color on the photosynthetic rate of plants.

Materials and Methods.

In this experiment you will be using an oxygen electrode to monitor the oxygen concentration of an algal culture. This electrode continually measures the oxygen concentration in the solution. The higher the concentration of oxygen in solution the higher the voltage reading of this reduction. A thin teflon membrane, only permeable to oxygen, prevents any metal ions from altering this voltage reading. As long as this membrane is intact, the voltage output from the oxygen electrode is proportional to the oxygen concentration. In addition to the oxygen electrode we will also use a light source, and color filters (plastic) for selecting the color of illumination light.

Part I. Light intensity and photosynthesis Experimental Procedure

Get familiar with the equipment

(l) The oxygen electrode system should be turned on already and working properly. The chamber containing the chlorella sample is covered with a cardboard box. Take the box off and **without moving the top of the chamber**, check visually that there are no air bubbles trapped under the cover or in the sample chamber (one air bubble contains as much oxygen as there is in a liter of water). If you find one call your instructor to take care of it. This is crucial.

(2) Arrange the light source so that even, high intensity illumination impinges upon the sample chamber of the oxygen electrode. The intensity of light may be varied by varying the distance the light source is from the sample chamber. Intensity is proportional to 1000 divided by distance squared, if the light is a point source.

(3) Cover the sample chamber with a box. Only respiration should now be occurring.

Respiration rate (do not spend more than 15 minutes on this, if any machines do not show a decrease check again for air bubbles: there is probably one in the sample)

(4) Measure the respiration rate of your algal culture by recording oxygen concentration values every minute. Continue with these measurements until there are steady state readings of oxygen change per time (same change in oxygen concentration per time increment). This respiration rate will be added to your photosynthetic rate for a better evaluation of the actual photosynthetic rate of your culture. Record data in the table below.

Typical Table

Use the tables below to record your data for each filter treatment.

To calculate the rate from the tables:
Calculations:

Change in O_2 = (O_2-O_1) units
Change in Time = (T_2-T_1) minutes
Thus **RATE** will equal **±O/±T** = units of oxygen per minute

Part I, step 4 Respiration Rate

Time (min)	**± Time**	**Oxygen meter**	**± Oxygen**	**Rate ±O/±T**
0				
2				
4				
6				
8				
10				
12				
14				

Light intensity (10 cm, 5cm, 2cm, 1cm & 2nd respiration rate)

(5) Room lights may be turned off for all photosynthetic rate versus light intensity measurements, to guard against the room lights adding to your controlled light. To begin your light intensity measurements, start with the light source very far from the chamber (10cm). Turn on the light source and monitor any changes in oxygen concentration as the distance is shortened. As before, continue for 15 minutes or until a steady rate is reached.

(6) Measure the photosynthetic rate at several light intensities by varying the distance between the sample chamber and the light source. Light intensity is inversely proportional to the square of this distance (Relative intensity = $1/d^2$.) Start your measurements with the lowest light intensity (furthest distance) and continue your measurements with increasing amounts of light intensity until you reach 100% light saturation (photosynthetic rate remains the same even with higher light intensities).

(7) Be sure to measure the respiration rate of this culture again in the dark.

Part I, step 5
Light intensity 10cm

Time (min)	± Time	Oxygen meter	± Oxygen	Rate ±O/±T
0				
2				
4				
6				
8				
10				
12				
14				

Part I, step 6 Light intensity 5cm

Time (min)	± Time	Oxygen meter	± Oxygen	Rate ±O/±T
0				
2				
4				
6				
8				
10				
12				
14				

Part I, step 6 Light intensity 2cm

Time (min)	± Time	Oxygen meter	± Oxygen	Rate ±O/±T
0				
2				
4				
6				
8				
10				
12				
14				

Part I, step 6 Light intensity 1cm

Time (min)	± Time	Oxygen meter	± Oxygen	Rate ±O/±T
0				
2				
4				
6				
8				
10				
12				
14				

Part I, step 7 second Respiration Rate

Time (min)	± Time	Oxygen meter	± Oxygen	Rate ±O/±T
0				
2				
4				
6				
8				
10				
12				
14				

Part II. Wavelength and Photosynthesis Experimental Procedure

(1) Measure the rate of photosynthesis using the colored filters. The actual light intensity transmitted to the sample should be the same for each filter and the one intensity with the most effect on photosynthetic rate. Color filters are used to provide uniform illumination of specific wavelengths (colors) of light in the light quality experiments (crude action spectrum).

(2) Start with the red filter and cover the sample chamber with it. Do this in such a way that when you illuminate the sample, the light path passes through the filter. Take a reading every minute until you see a steady rate of photosynthesis. Record your data in the red filter table below.

(3) In between each filter treatment, cover the sample chamber with the respiration box for 5-10 minutes. This is to reduce any effect from the previous filter on the photosynthetic rate you are recording.

(4) Repeat with the blue filter and finally with the green.

Part II, Red Filter

Time (min)	± Time	Oxygen meter	± Oxygen	Rate ±O/±T
0				
2				
4				
6				
8				
10				
12				
14				
16				

Part II, Blue Filter

Time (min)	± Time	Oxygen meter	± Oxygen	Rate ±O/±T
0				
2				
4				
6				
8				
10				
12				
14				
16				

Part II, Green Filter

Time (min)	± Time	Oxygen meter	± Oxygen	Rate ±O/±T
0				
2				
4				
6				
8				
10				
12				
14				
16				

Photosynthesis Lab Report

Refer to the appendix section of the lab manual to find the section called "How to Write a Lab Report", in order to know how to write the report. It must be typed.
Be sure to address the issues highlighted in the lab manual and by your instructor in the appropriate sections of your lab report. Include the following :

1. The Results section will have 2 different graphs:
   - Plot the results from Part I, # 6-7 as the rate of true photosynthesis (Y axis) versus light intensity (X axis).
   - Plot the results from Part II**, Wavelength and Photosynthesis.** This type of plot is a crude "action spectrum" the rate of true photosynthesis (Y axis) versus wavelength (X axis). The approximate wavelengths to use for each broad band filter (which transmit more than one wavelength) are:
     Red: 660-760nm Blue: 350-450nm Green: 460-560nm

Notes for determining rates of photosynthesis:

The steady state rate (consistent rate) of oxygen evolution with the light on plus the previously measured respiration rate (sample was in the dark) is the "true" rate of photosynthesis (assuming that respiration is occurring at the same rate in the light as it does in the dark!). In all of these measurements you must determine the rate of change of oxygen concentration. For example, a change of l0 units of oxygen in a minute is a rate of 600 units of oxygen per hour. Your best estimate of any rate will be the slope of the oxygen concentration versus time plot.

2. Discussion should address these issues:
   - How these factors limit the rate of photosynthesis: light intensity and wavelength; as supported by your data.
   - Water ordinarily does not limit the rate of photosynthesis. Why?
   - What conclusions can you make regarding the visible color of a pigment and the color of light it absorbs?
   - Chlorophyll is the pigment involved directly in photosynthesis. How does it differ from some of the other typical photosynthetic pigments found in plants? (accessory pigments)

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The Cell Cycle

Learning Goals: After completing this laboratory exercise you will be able to:

1. Identify the stages of the cell cycle.
2. Follow the duplication and separation of chromosomes in cell division.
3. Follow genes, alleles, and chromosomes through mitosis, meiosis, and fertilization.
4. Get a clearer picture of gene/allele and how they connect to chromosomes.

Introduction:

Eucaryotic cell division is used in the process of growth, development, and reproduction and is controlled by the Cell Cycle. The purpose of cell division during growth and development is to create two daughter cells with the exact genetic makeup of the parent cell. In the creation of reproductive cells, the daughter gametic cells contain half of the amount of genetic material as the parent cell. Two gametes then fuse and contain a full set of genetic material.

The Cell Cycle: The cell cycle is 4 successive steps of Growth (G1), Synthesis (S), Growth (G2), and the M phase (M). The G1, S, and G2 phases collectively are referred to as Interphase. If a cell is not dividing, it is in a state of rest, also termed G0. The G1 and G2 phases are growth phases in which the cell acquires all the necessary equipment for division. During the S phase the entire genome is duplicated, each chromosome now has 2 sister chromatids. Sister chromatids are exactly the same DNA held together at the middle. In the M phase each one will go to a daughter cell. The M phase can be either Mitosis or Meiosis. Mitosis is used in growth and development whereas meiosis is used in reproduction for the creation of gametes (egg or sperm).

The cell cycle is regulated by two groups of proteins: cyclins and cyclin-dependent kinases (CDK). In order for the cell to progress into the next phase of the cell cycle, a specific cyclin must interact with a specific cyclin-dependent kinase. This cyclin-CDK protein complex can then phosphorylate or activate the appropriate proteins necessary for the functioning of the next phase. This is an example of signal transduction happening within a cell. Specific cyclins and CDKs exist for each phase of the cell cycle.

Mitosis

A) Prophase – The DNA begins to coil up into the 3-D structure that we visualize as chromosomes. The centrosome (made of 2 centrioles) duplicates and each begins to migrate towards opposite ends of the cell. The centrosome is a microtubule organizing center, it is responsible for the creation of microtubules using the protein tubulin.

B) Prometaphase – Microtubules break down the nuclear envelope and begin the search and capture of the kinetochores of each chromosome.
C) Metaphase- Each chromosome is lined up at the metaphase plate.
D) Anaphase- Each sister chromatid is pulled to the respective end of the daughter cells.
E) Telophase- the process of cell division finishes through the pinching off or division of the two daughter cells.

Meiosis – Is two rounds of division similar to that of mitosis except that in the first division the homologous chromosomes are separated and in the second division the sister chromatids are separated.

Demonstrations

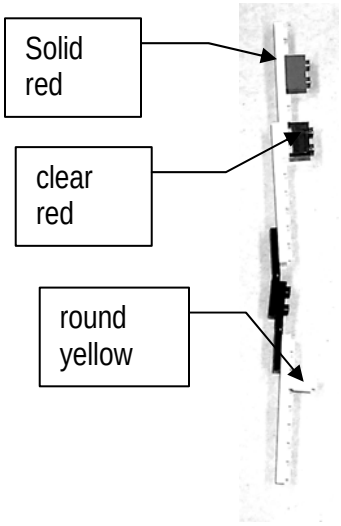
Set up under the microscopes on the counter tops.
(Do not enter in lab notebook)

Phases of Mitosis.
Today you will observe the stages of mitosis in animal (white-fish blastula) and plant (onion root tip) cells, using prepared slides.
Plants
A series of slides will be set up showing various stages of meiosis in the anther of a lily flower. See if you can identify the stages available.
Animals
A series of slides will be set up showing various stages of meiosis in animals using Rat testis. See if you can identify the stages available.
Giant Chromosomes of *Drosophila*, (fruit fly). In several tissues of *Drosophila* and other Dipteran insects (flies and such) unique giant chromosomes can be seen. Following synapsis the chromosomes replicate about ten times with no division of the nucleus or cell. The result is a chromosome consisting of about one thousand strands which become very rigid and tightly aligned with each other. These giant chromosomes are seen to have alternating light and dark bands of varying widths. The bands have been mapped and it has been demonstrated that the positions of specific bands can be related to chromosome abnormalities and are often reflected in visually apparent rearrangements or deletions of parts of the banding patterns in the giant chromosomes.
Human Chromosomes Separate microscopes will be set up with slides showing human male and female chromosomes. Count the number of chromosomes in one or two cells. Why are they doubled? What do we mean by karyotype? Would it be possible to distinguish between male and female sets of chromosomes? Observe the demonstration of human karyotypes. Count the number of chromosomes in the photomicrograph, also on demonstration.

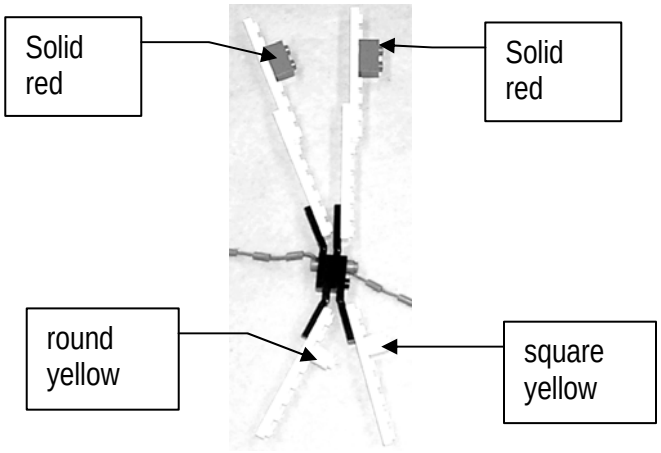
In Class preparatory questions

The answers to the following questions will be reviewed at the beginning of each Lab. It is in your best interest as a student to attempt them prior to each Lab, though not required.

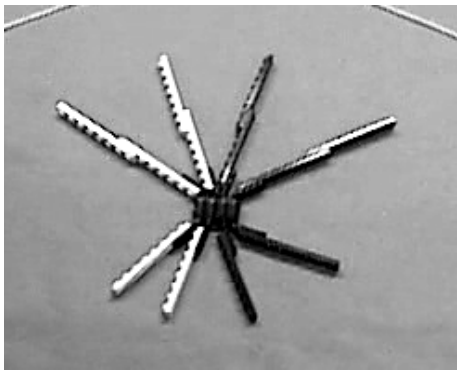
1) What is wrong with the genes on this chromosome? Why?



2) What is wrong with the genes on this pair of sister chromatids? Why?



3) From which process (mitosis, meiosis, or fertilization) was this picture taken?



Laboratory Excercise

I. A. Formulate a hypothesis and indicate in the diagrams below where you think the greatest amount of cell division is found in the onion root.

Portion of the root, close to the onion bulb.

Tip of the root.

Test your hypothesis by viewing a prepared slide beneath the microscope. Refer to the Mitosis stages described in part B above if you must.

B. Was your prediction correct? Describe below how you were able to distinguish the area of most activity:

II. Lego Genetic System

The three genes we will study in the Furbies are as follows:

(1) The gene for Height

allele	**contribution to phenotype**	**Lego**		**Piece**
H	tall (dominant)	square		yellow "one"
h	short (recessive)	round		yellow "one"

(2) The gene for Eye color

allele	**contribution to phenotype**	**Lego**		**Piece**
B	black (dominant)	black		flat "two"
b	blue (recessive)	blue		flat "two"

(3) The gene for Blood-type

allele	**contribution to phenotype**	**Lego Piece**	
Q^X	type X blood (co-dominant)	solid red	 "two"
Q^Y	type Y blood (co-dominant)	clear red	 "two"
Q^Z	type Z blood (co-dominant)	clear pink	 "two"

Note that the Lego pieces that represent different alleles of the same gene are *very similar* – for example, all the alleles of the blood-type gene are the same shape. This is deliberate – the different alleles of a particular gene are very similar; much more similar to each other than they are to alleles of a different gene.

The genes are located on the chromosomes as follows:

Note: that, no latter which alleles are present, each gene is *always* in the same place on its particular chromosome.

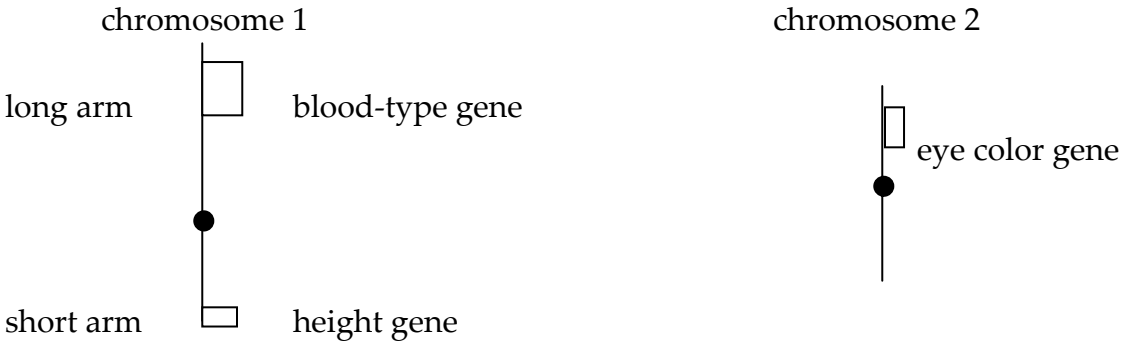


Figure 3

Notes:

1. The chromosomes are different because of their different lengths and the different genes on them; the different colors are just to track the maternal and paternal copies.
2. While the exact position of the alleles along the chromosomes is not critical, they should be in similar positions.

The picture below shows the Lego model of the chromosomes of a diploid cell with the genotype: Q^XQ^X Hh bb. It would be from a tall, blue-eyed, Furby with blood type X.

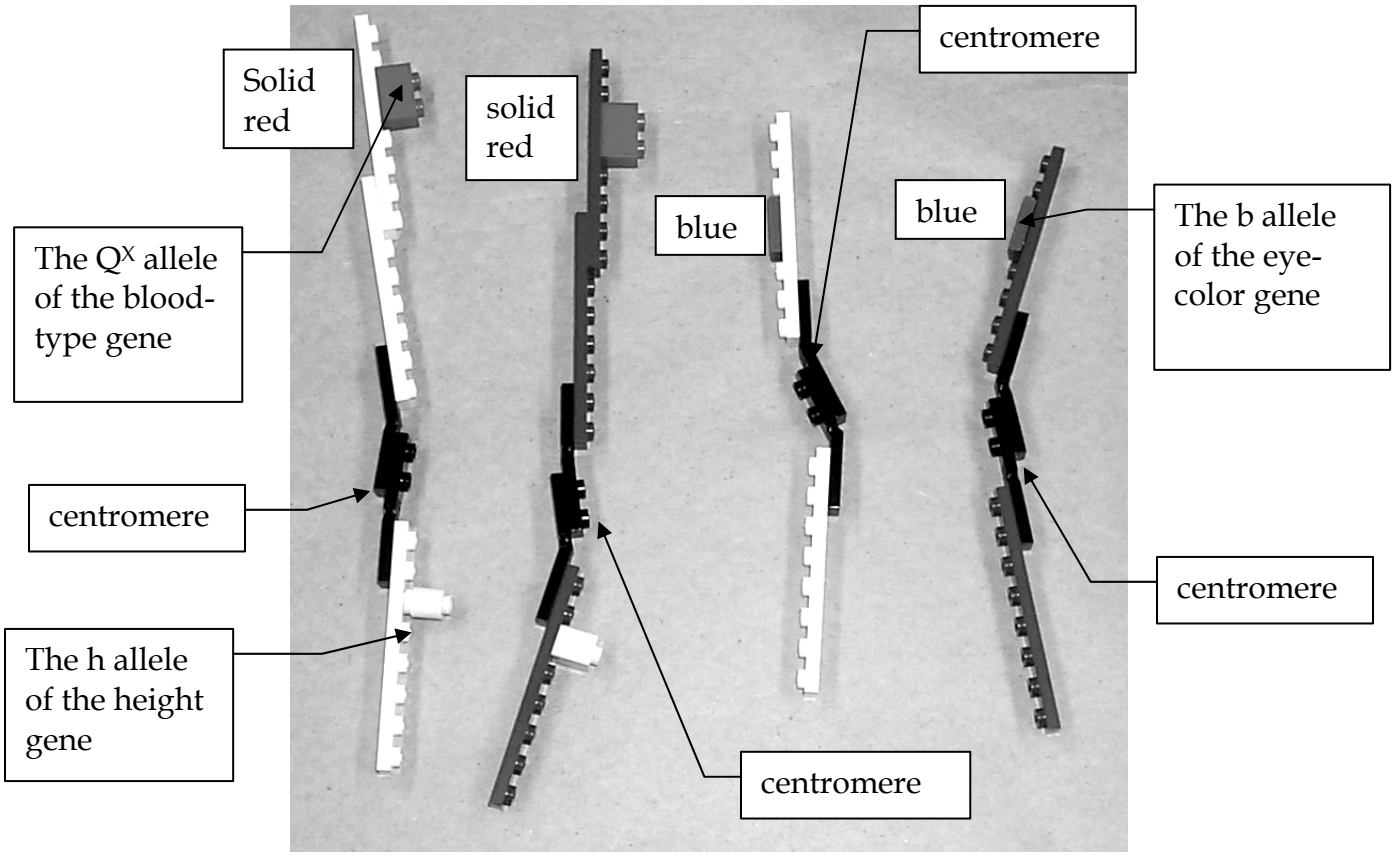


Figure 4

The maternal copy of each chromosome (the copy this Furby got in the egg from its mother) is shown in white. Therefore, the egg had the genotype: Q^X h b.

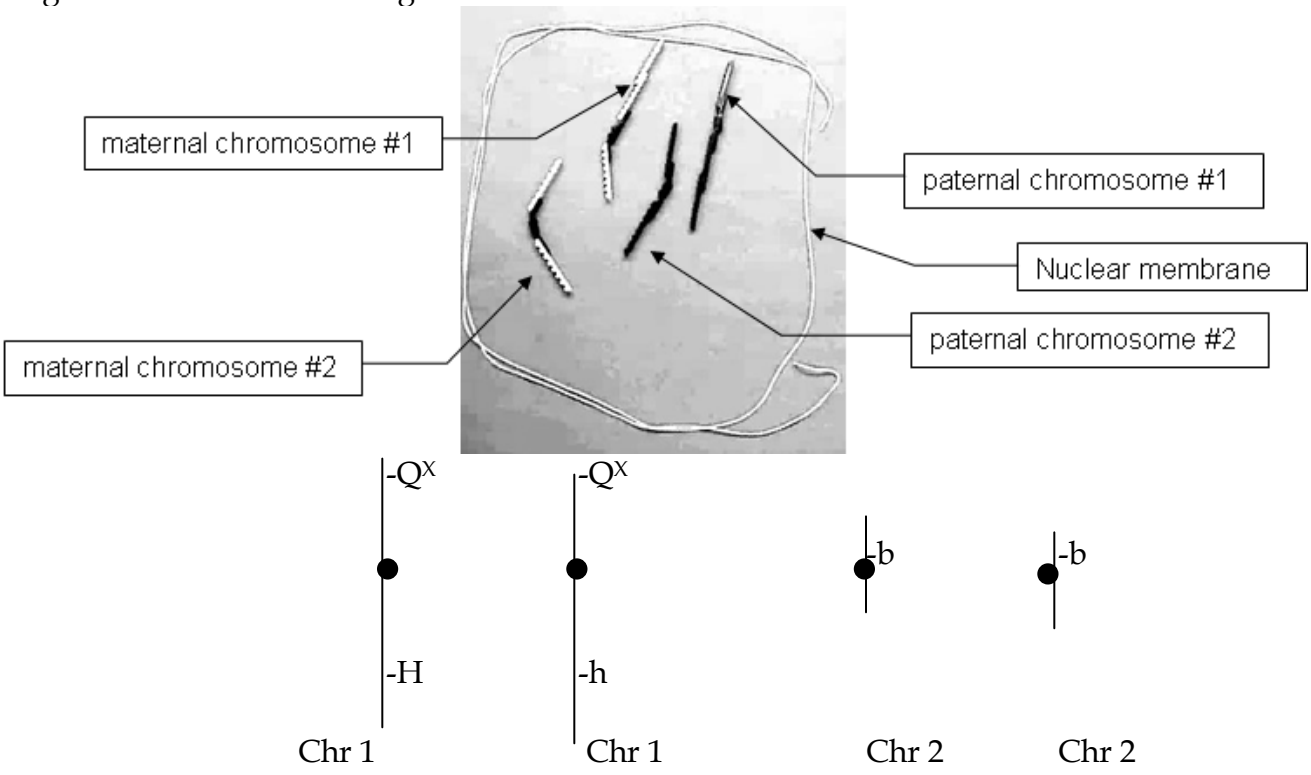
The paternal copy of each chromosome (the copy this Furby got in the sperm from its father) is shown in purple. Therefore, the sperm had the genotype: Q^X H b.

Procedure

1. Choose a genotype for your starting cell.
Write this genotype here_____. (**your genotype must include all three genes**)
2. Build a set of diploid chromosomes that models the genotype you picked in step (1).
3. Use the Legos to model the mitosis of a Furby cell with the genotype you picked in step (2). You should use the figures that follow as a guide. Note that these figures do not show the genes and alleles; your models must include the genes and alleles. In order to get checked off, you must demonstrate all the stages shown below to a member of another lab group.

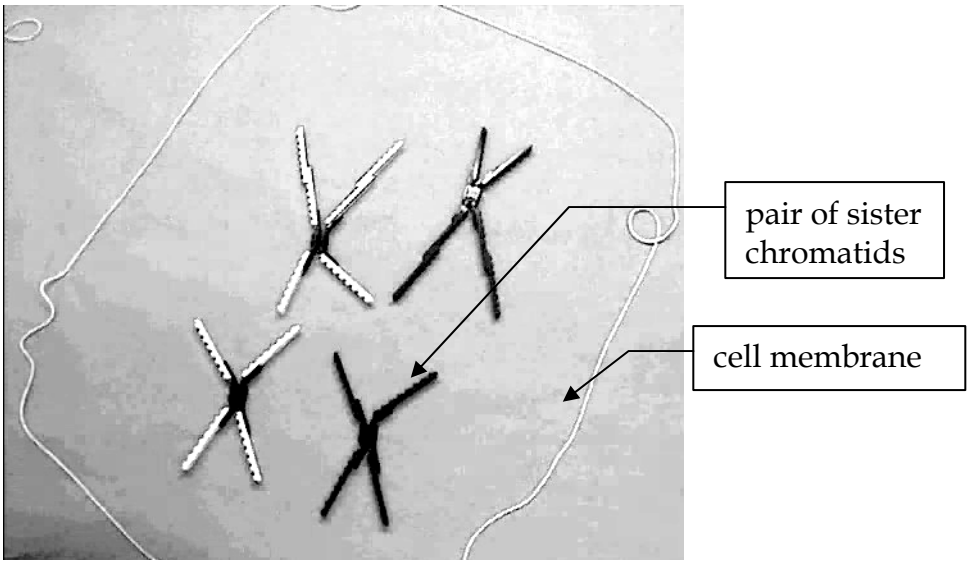
a) A resting cell (G0 and G1 phase)

(**Note** the chromosomes are not visible in a real cell at this stage; we are showing them for the purposes of illustration only)
The drawing below shows a schematic of the chromosomes at this stage assuming a genotype of Q^XQ^XHhbb. Use this as a model for the drawings you will make of the later stages of mitosis and the stages of meiosis.



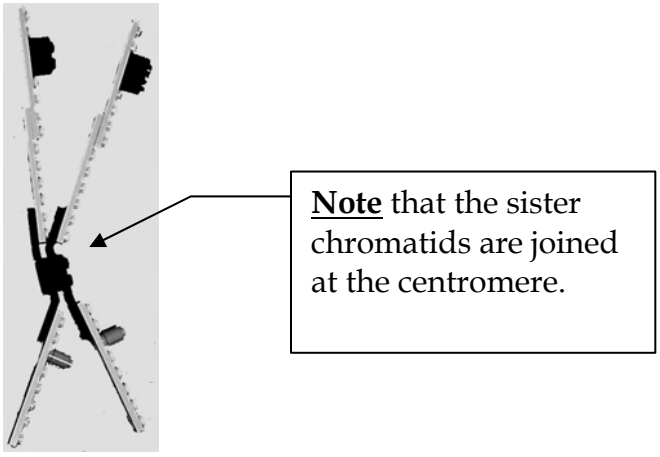
Draw a similar diagram for the genotype you chose in part (2) as they would appear in a resting cell:

b) Prophase (**chromosomes have duplicated in S phase**; the "X"s here

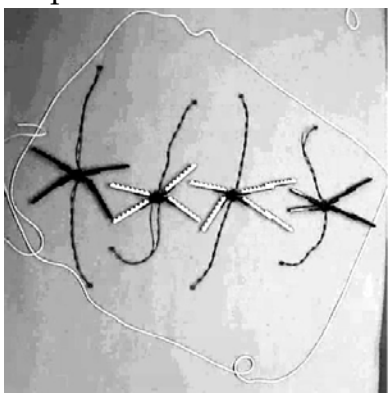


correspond to the "X"s in the figure that shows human chromosomes at the start of this section of the lab manual)

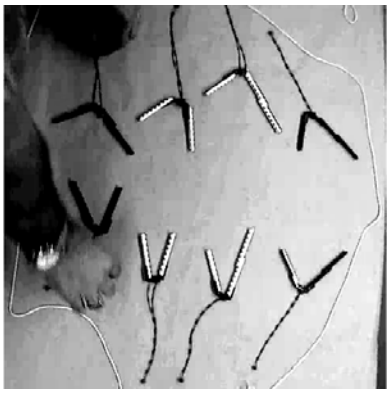
Note that the sister chromatids are exact duplicates. Therefore the alleles should be identical for all genes in a given pair. This is shown below: Draw a schematic diagram of the chromosomes for the genotype you chose in part (2) as they would appear in a cell at prophase:



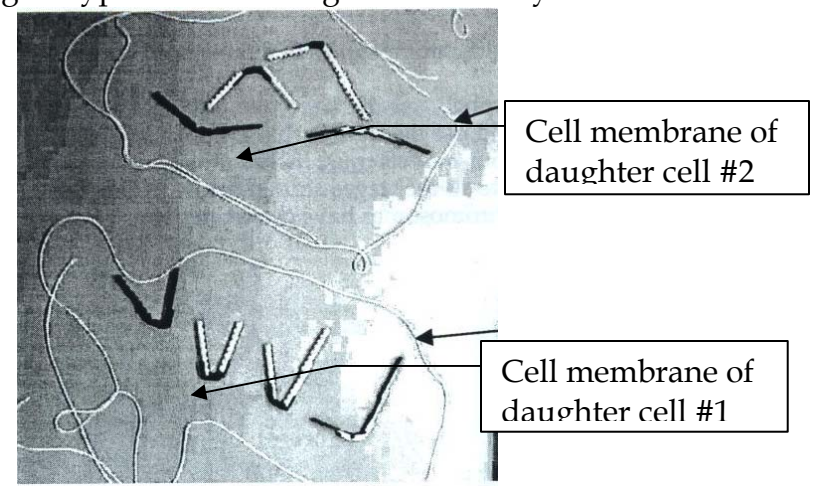
c) Metaphase (chromosomes have lined up) Draw a schematic diagram of the chromosomes for the genotype you chose in part (2) as they would appear in a cell at metaphase:



d) Anaphase (sister chromatids pulled apart by kinetochore microtubules) Draw a schematic diagram of the chromosomes for the genotype you chose in part (2) as they would appear in a cell at anaphase:



e) Telophase & Cytokinesis (cell divides) **Note** that both cells now have the same genotype as the starting cell. Draw your cells

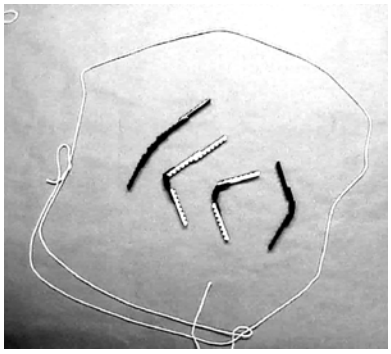


Part II: Meiosis

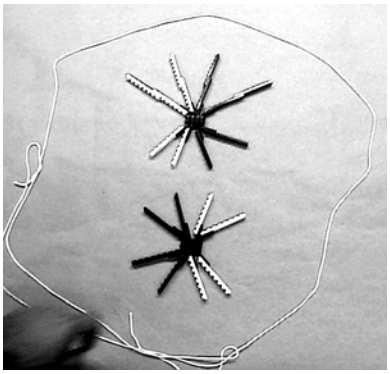
(5) Construct a model of the diploid chromosomes of a Furby cell with the genotype Q^XQ^X Hh Bb.

(6) Use the Lego to model the meiosis of a Furby cell with the genotype from step (5). You should use the figures that follow as a guide. Note that these figures do not show the genes and alleles; your models must include the genes and alleles. In order to get checked off, you must demonstrate all the stages shown below to a member of another lab group.

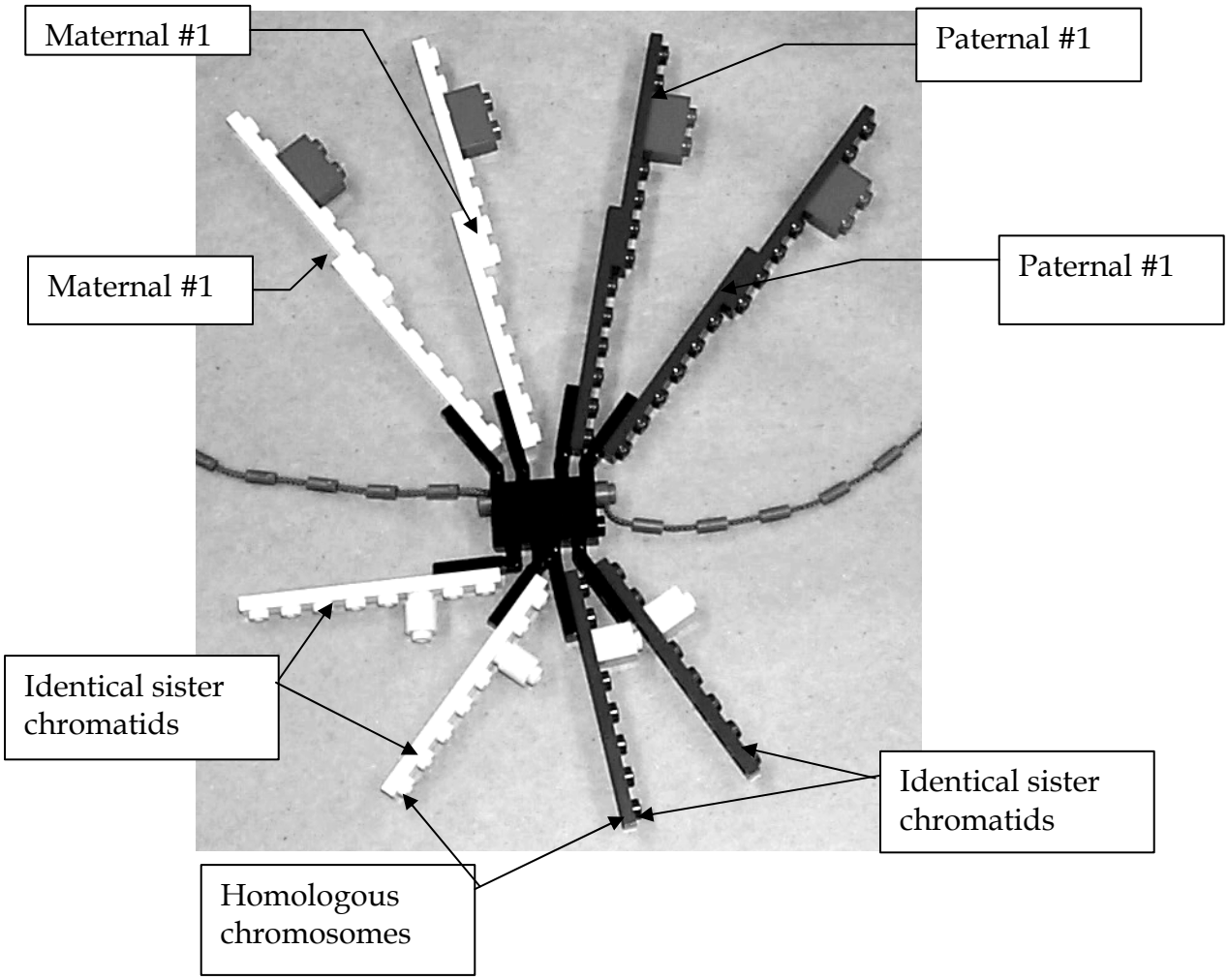
a) resting cell (G0 or G1) Draw a schematic diagram of the chromosomes for the genotype Q^XQ^X Hh Bb as they would appear in a resting cell:



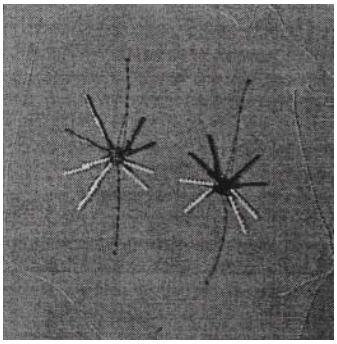
b) Prophase I (After S phase) Draw a schematic diagram of the chromosomes for the genotype Q^XQ^X Hh Bb as they would appear in a cell at prophase I of meiosis (see photo on next page for hints):



close-up of a homologs pairing in a tetrad:



c) Metaphase I (tetrads have lined up) **Note** that there are two possible configurations here. You should work all the way through meiosis with one configuration and then go back and do the other.

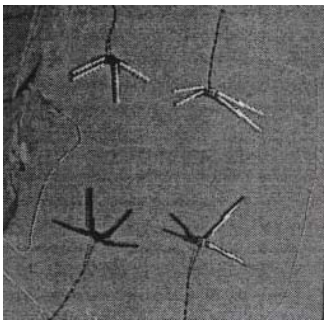


Draw a schematic diagram of the chromosomes for the genotype Q^XQ^X Hh Bb as they would appear in a cell at Metaphase I of meiosis:

configuration 1:

configuration 2:

d) Anaphase I (tetrads split between homologs - sisters remain attached)

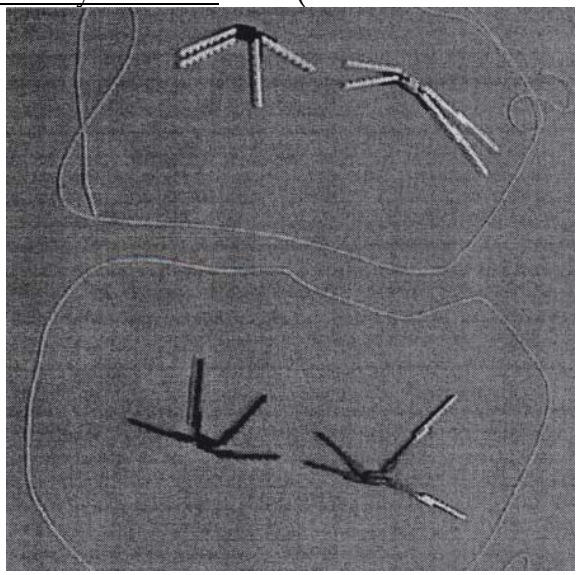


Draw a schematic diagram of the chromosomes for the genotype Q^XQ^X Hh Bb as they would appear in a cell at Anaphase I of meiosis:

configuration 1:

configuration 2:

e) Telophase I & Cytokinesis (cells have divided for the first time)

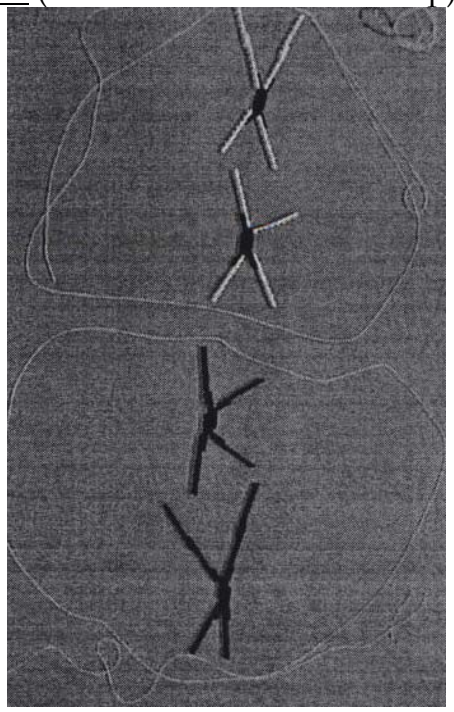


Draw a schematic diagram of the chromosomes for the genotype Q^XQ^X Hh Bb as they would appear in a cell at the end of Cytokinesis I of meiosis:

configuration 1:

configuration 2:

f) Metaphase II (chromosomes have lined up)

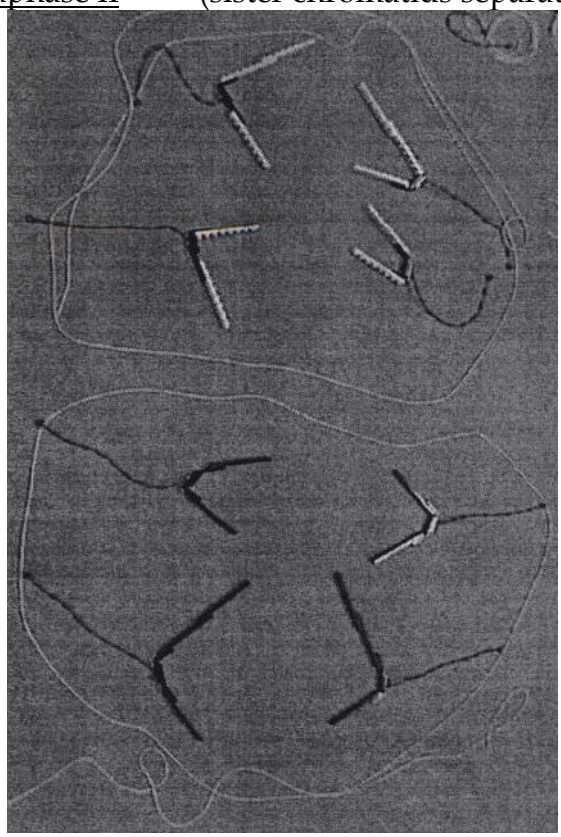


Draw a schematic diagram of the chromosomes for the genotype Q^XQ^X Hh Bb as they would appear in a cell at Metaphase II of meiosis:

configuration 1:

configuration 2:

g) Anaphase II (sister chromatids separate)

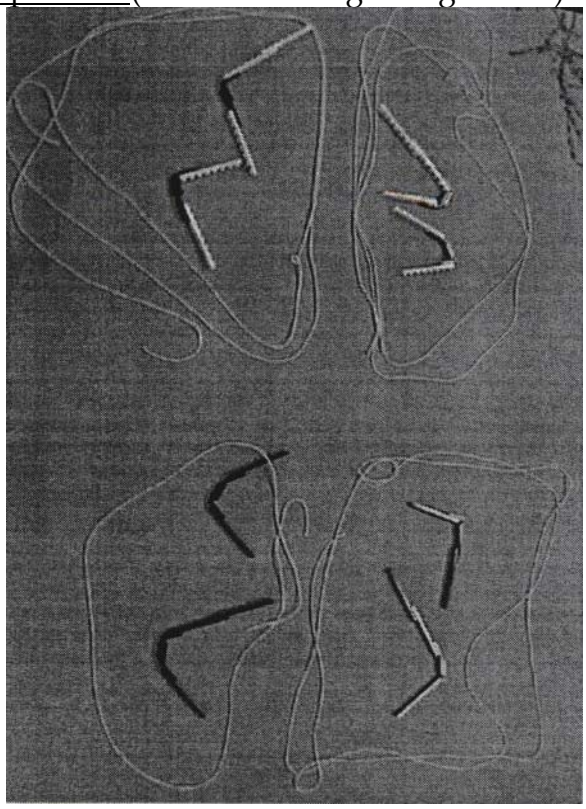


Draw a schematic diagram of the chromosomes for the genotype Q^XQ^X Hh Bb as they would appear in a cell at Anaphase II of meiosis:

configuration 1:

configuration 2:

h) Telophase II (cells divide to give 4 gametes)



Draw a schematic diagram of the chromosomes for the genotype Q^XQ^X Hh Bb as they would appear in a cell at the end of Cytokinesis II of meiosis:

configuration 1:

configuration 2:

Note that there are four possible gametes that a Q^XQ^X Hh Bb cell can produce:

Q^X H B Q^X H b Q^X h B Q^X h b

you must show how all four can be made.

Fertilization

(6) Take one gamete that you made in step (5) and combine it with a gamete produced by another group. What is the genotype of the resulting offspring?

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Virtual Genetics Lab (VGL)

Learning Goals

I. To use your knowledge of genetics to design and interpret crosses to figure out which allele of a gene has a dominant phenotype and which has a recessive phenotype. This simulates a real lab in that there is no way to see if you've got the answer right. You will have to convince yourselves when you've done enough experiments to be sure.

II. To use your knowledge of genetics to design and interpret crosses to figure out the inheritance of very complex characters (these can include: sex-linkage, and incomplete dominance). This should bring together all parts of the genetics portion of the course.

Introduction:

This lab will develop hypothesis building skills while learning the basics of genetics. Keep notes in your lab manual as you make crosses at the different levels. The general progression of science is:

hypothesis → *model* → *theory*

In the beginning, scientists test many hypotheses in order to establish a model. A scientific model is a representation of many different experiments. This model can be tested by creating new hypotheses: "If this model is true, then hypothesis XYZ must also be true". By continuing to challenge a model through hypothesis driven experimentation, scientific theory can be established. With VGL, each cross you perform should test a hypothesis. As you perform multiple crosses you will begin to develop a model. Once you have a model, formulate a few more hypothesis to test it until you are convinced. Scientists practice peer review, where models are continually challenged.

You will use, VGL, a computer program that simulate genetics. The algorithm randomly picks a character with two traits. It then randomly chooses which form of the character will be dominant and which will be recessive. That way, each time you start the program, you get a different problem (also, every group will get a different problem). Finally, it creates a population of insects with random genotypes called the Field Population.

As in a real genetics lab, the insects are kept in cages; Cage 1 contains the Field Population. You can select any two insects (one must be male and the other female) and cross them; the computer automatically puts their offspring in a new cage.

Please note VGL was developed last year by a group of Computer Science graduate students at UMB. It is a new program and, although it was extensively tested, it may contain bugs. In addition, it may run slowly and be a little 'touchy'. Therefore, please be patient and let the computer work for you. If you are impatient and click rapidly all over the screen when nothing seems to be happening, the program will likely crash. So please treat it gently and report all bugs to your TA or Brian White so they can be fixed.

The Genetic Models found in VGL

You test multiple hypotheses by performing crosses in order to build a model of the genetic system portrayed by the phenotypes you are monitoring. All the problems in VGL involve genetic models with one gene that has two alleles. Based on this, there are several features that can vary:

The interaction between the alleles; this can be either:

Simple Dominance: (Models 1, 3, & 5) The heterozygote has the same phenotype as the dominant homozygote. That is, with two alleles A and a:

- AA = tall
- Aa = tall
- aa = short

Incomplete Dominance: (Models 2, 4, & 6) The heterozygote has a different phenotype than either homozygote. In nature, this is usually intermediate; in VGL it need not be.

- AA = tall
- Aa = medium
- aa = short

Whether the character is sex-linked or not; this can be either:

Not sex-linked - the gene for the character is carried on an autosome so it is inherited identically in both sexes. (Models 1 & 2)

Sex-linked - the gene for the character is located on a sex-chromosome so it is inherited differently in different sexes. This can be either:

- XX/XY Females are XX; males are XY (Models 3 & 4). Here, Y carries no genes except those needed to make the organism male.
- ZZ/ZW Females are ZW; males are ZZ (Models 5 & 6). Here, W carries no genes except those needed to make the organism female.

This leads to six possible genetic models.

Model 1: Simple Dominance; Autosomal. For example:

Genotype	Phenotype
Green; Green	Green Body
Green; Red	Green Body
Red; Red	Red Body

Model 2: Incomplete Dominance; Autosomal.
For example:

Genotype	Phenotype
Green; Green	Green Body
Green; Red	Purple Body
Red; Red	Red Body

Model 3: Simple Dominance; XX/XY Sex-linked. For example:

Genotype	Phenotype
X-Green; X-Green	Green Body Female
X-Green; X-Red	Green Body Female
X-Red; X-Red	Red Body Female
X-Green; Y	Green Body Male
X-Red; Y	Red Body Male

Model 4: Incomplete Dominance; XX/XY Sex-linked. For example:

Genotype	Phenotype
X-Green; X-Green	Green Body Female
X-Green; X-Red	Purple Body Female
X-Red; X-Red	Red Body Female
X-Green; Y	Green Body Male
X-Red; Y	Red Body Male

Model 5: Simple Dominance; ZZ/ZW Sex-linked. For example:

Genotype	Phenotype
Z-Green; Z-Green	Green Body Male
Z-Green; Z-Red	Green Body Male
Z-Red; Z-Red	Red Body Male
Z-Green; W	Green Body Female
Z-Red; W	Red Body Female

Model 6: Incomplete Dominance; ZZ/ZW Sex-linked. For example:

Genotype	Phenotype
Z-Green; Z-Green	Green Body Male
Z-Green; Z-Red	Purple Body Male
Z-Red; Z-Red	Red Body Male
Z-Green; W	Green Body Female
Z-Red; W	Red Body Female

In Class preparatory questions The answers to the following questions will be reviewed at the beginning of each Lab. It is in your best interest as a student to attempt them prior to each Lab, though not required.

Consider the cage below; it was generated by a VGL problem like one that you will do in lab this week. In this problem, the Body Color phenotype was either Pink or Blue.

1) The cage above shows the parents and offspring of a particular cross. Based on the picture above:

i) What was the phenotype of the male parent of this cross?_____

ii) What was the phenotype of the female parent of this cross?_____

iii) What were the phenotype(s) of the offspring of this cross and how many of each phenotype were produced?

Consider the following trait:

allele	contribution to phenotype
X^R	red: incompletely dominant with white; dominant to green
X^W	white: incompletely dominant with red; dominant to green
X^g	green; recessive to red and white.
Y	none

2) Fill in the following chart with the phenotype that corresponds to each genotype. Include male or female as appropriate.

Genotype	Phenotype
X^WX^W	
X^RX^R	
X^gX^g	
X^WX^R	
X^WX^g	
X^RX^g	
X^RY	
X^WY	
X^gY	

Laboratory Excercise:

To solve two different problems generated by VGL. A solution is a genetic model that accounts for all your data. Models will involve only one gene but may include incomplete dominance and/or sex-linkage (either XX/XY or ZZ/ZW).

Starting up VGL:

1) You will work in groups of three people per computer. You may want to take turns using the computer. It is easy to fill the screen with cages of creatures and get totally confused so you should work slowly and deliberately and keep careful notes about the experiments you do and the contents of each cage. If you get very confused, you can quit the program and start fresh with a new problem.

2) The program runs on two kinds of computers in the lab: the iMacs (one-piece blue computers in W-2-030) and the PCs (two-piece tan computers around the sides of W-2-031); it does not run on the Macs on the center tables in W-2-031. You can also download the program and run it from your home computer; go to http://v-g-l.sourceforge.net/index.html.

3) To run the VGL program: follow the directions on the welcome page that appears on the screen; you either single-click (on the PCs) or double-click (on the iMacs) the VGL icon.

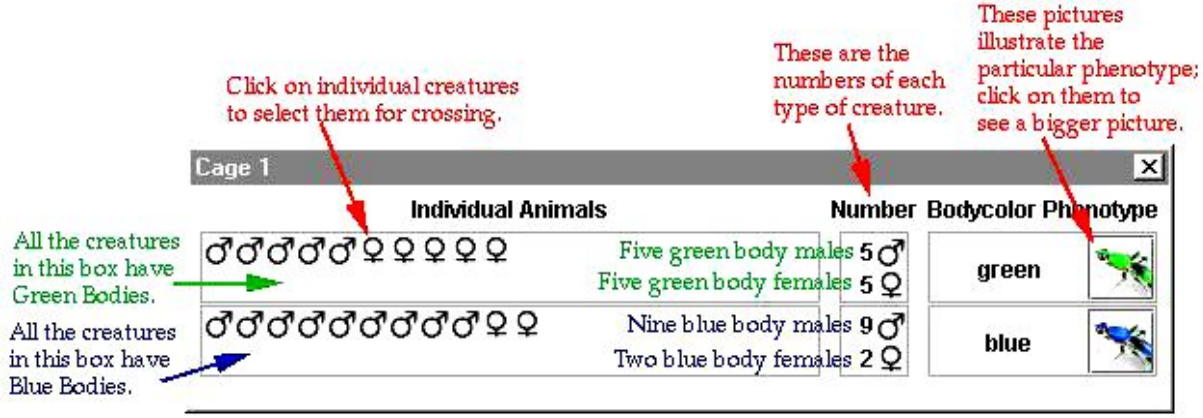
4) If you want to read the manual, click on the VGL Help bookmark at the top of the Welcome page. Note that the manual describes some features present in the version of VGL that you will not be using until later on in Bio 111.

5) Click on "New Problem" to begin. Each time you start a new problem, the computer will choose a new set of traits and characters as well as the underlying genetic model. Note that a character with the same name may have different properties in a different problem.

Once you click "New Problem", a window will appear for you to select the problem you will work on. Double-click on the "Problems" folder and select "Level 1".

6) You will then see a window that asks if you want to show the model and genotypes or not. Select the "Show model and genotypes" option and click "OK".

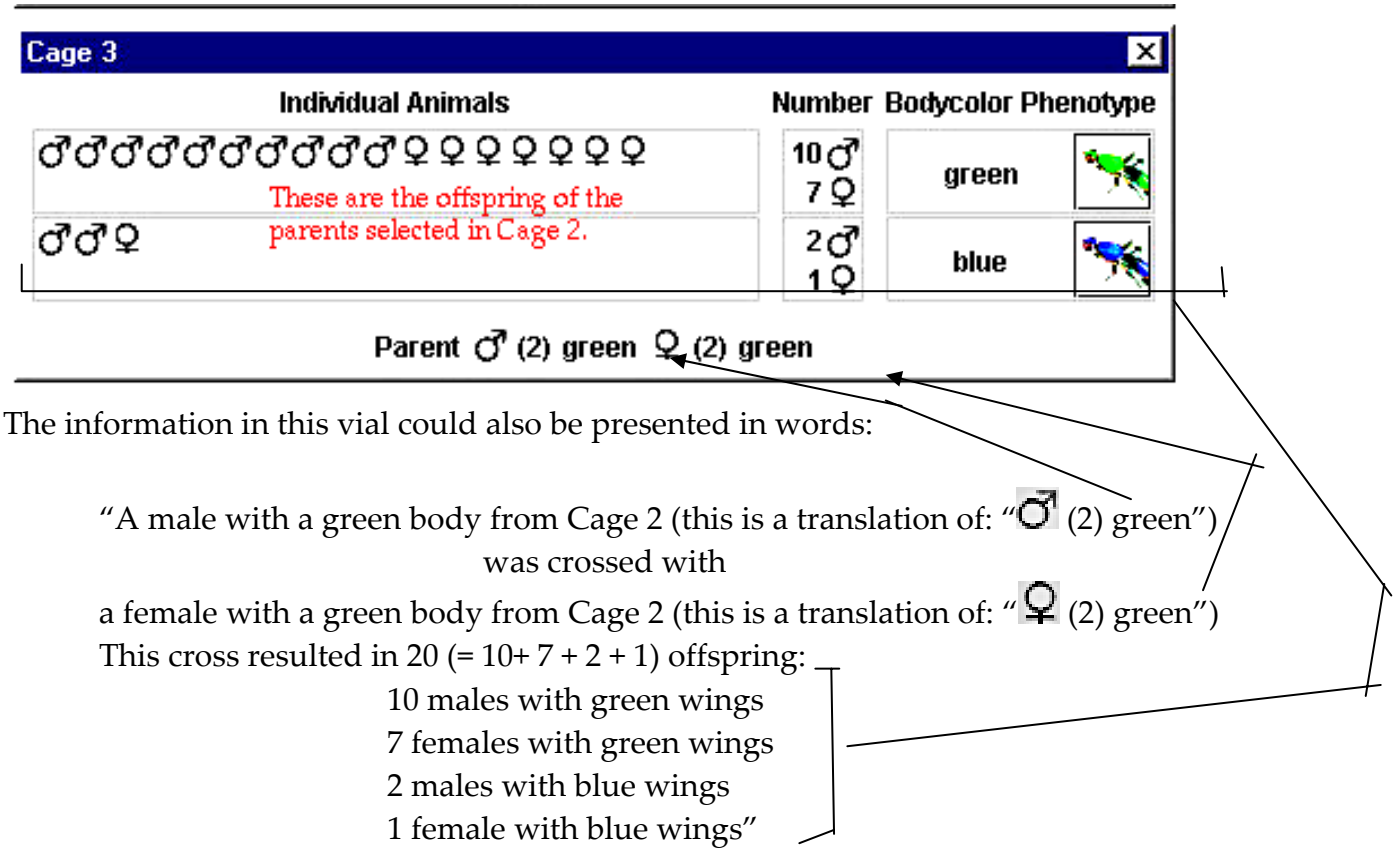
7) A cage will appear holding the "Field Population". It will look something like this:



In your case, the phenotypes involved will likely be different. Your task is to figure out which is dominant and which is recessive. You will determine this by designing crosses and analyzing the resulting offspring.

8) Begin your experiments. Select a male and a female fly to be the parents: click on one parent then click on the other parent. One parent must be male and the other female but they may come from different cages. Note that you can cross a given fly more than once.

To cross (also known as "mate") the selected flies, click the "Cross" button at the top of the VGL window. A cage will appear with the resulting offspring. A typical result is shown here:



The information in this vial could also be presented in words:

"A male with a green body from Cage 2 (this is a translation of: "♂ (2) green")
was crossed with
a female with a green body from Cage 2 (this is a translation of: "♀ (2) green")
This cross resulted in 20 (= 10+ 7 + 2 + 1) offspring:
10 males with green wings
7 females with green wings
2 males with blue wings
1 female with blue wings"

9) Continue crossing as needed; the objective is to make a genetic model to explain the inheritance of the traits you are studying. You decide whether you're convinced or not; if not, keep crossing until you are. A complete model would look like:

"The color of the body is controlled by one gene with two alleles:

allele	contribution to phenotype
G	green body (dominant)
g	blue body (recessive)

10) Once you and your partner are convinced of your model, you can check to see if you are right. Click on Cage 1 to bring it to the front of the screen. Click on the button

marked "Show Model and Genotypes". The window will expand to show the genetic model underlying the trait you are studying.

11) When you are done with this problem, click the "Close Work" button at the top of the VGL window. When it asks if you want to save your work, click "Don't save". 

12) Click the "New Problem" button and choose "**Level 2**". You should solve this problem with your partner as you did before. However, this time you will not be able to see the correct answer; you must decide for yourselves when you have it right. At this point, you will then present it to another pair of lab partners, called the **reviewers**. The reviewers will then perform a cross with your creatures to test your model. The final step is to have your TA check off that the reviewers' cross results agree with the prediction.

13) **You should choose Level 6** problems for this lab session. For practice, you can choose Level 5, which will allow you to see the underlying genetic model and the genotype of each fly (to see the genotypes, just leave the cursor over a creature's symbol and a window will pop up that shows its genotype). This problem will be used for your lab notebook (see below).

14) The objective is to make a genetic model to explain the inheritance of the traits you are studying. There is no way to find the "right answer"; you decide whether you're convinced or not. A complete model would look like:

"The shape of the thorax is controlled by one gene with two alleles:

T - tetraltera (dominant)

t - grooveless (recessive)"

or:

"In this creature, XX are female; XY are male. The color of the eyes is controlled by one gene, located on the X-chromosome with 2 alleles:

X^B - black (dominant)

X^b - blue (recessive)

Y - no contribution to phenotype"

16) Once you and your partners are convinced of your model, you will then present it to your TA for **review**. Your TA will then perform a cross with your creatures to test your model. You must do this until you have solved one sex-linked and one non-sex-linked problem.

17) Finish by cleaning up the computer screen. Quit VGL by clicking the box in the upper right corner of the window; you do not need to save your work. Please leave the welcome page up on the browser window.

Lab Notebook Write Up Notes:

The lab exercises 13-16 will be used for your lab notebook entry. Please be sure to include the following in the appropriate sections.

1) Your model of the inheritance of a particular trait. For example:

The shape of the thorax is controlled by one gene with two alleles:

T - tetraltera (dominant)

t - grooveless (recessive)

2) The results of one cross which are consistent with your model. For example:

Cross 27: Male Tetraltera X Female Grooveless gave these offspring:

41 Tetraltera

3) The genotypes of all the individuals involved. For example:

Parents: Male Tetraltera (TT) X female grooveless (tt) gave offspring:

41 tetraltera (Tt)

4) A Punnett Square and a brief explanation showing that these are the expected results. For example:

	T	T
t	Tt	Tt
t	Tt	Tt

All the offspring should be Tt - tetraltera as was observed.

5) A Punnett Square and explanation showing that **the alternative model is inconsistent with the data**. When a given parent could have more than one genotype, you must list all the possibilities and show that they do not match the data. For example:

The only alternative model is:

G - grooveless (dominant)

g - tetraltera (recessive)

If this were so, then the male tetraltera would have to be gg. The female grooveless could be GG or Gg. This leads to 2 possible cases:

Case 1: tetraltera male (gg) X grooveless female (GG)

	G	G
g	Gg	Gg
g	Gg	Gg

All the offspring should be Gg - grooveless. No grooveless offspring were observed, so this is not consistent with the data.

Case 2: tetraltera male (gg) X grooveless female (Gg)

	G	g
g	Gg	gg
g	Gg	gg

Half the offspring should be Gg - grooveless. No grooveless offspring were observed, so this is not consistent with the data.

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Molecular Biology I

Learning Goals:

To work with a physical model of DNA in order to help you to understand:

- rules for DNA structure
- base-pairing
- DNA replication
- restriction enzyme digestions

Introduction:

Molecular biology is centralized around working with DNA in the laboratory. Understanding the physical structure of DNA and its asymmetry is crucial for molecular biology. In order to understand more complex molecular biology problems for next week we will work through some simpler problems using LEGO DNA models this week. This week we will only look at DNA Replication, but the kit contains both DNA and RNA, to tell the pieces apart use the diagrams below.

Molecular biology is one of the core disciplines in biology and can offer the opportunity of technology, known as Biotechnology. An important example is that of DNA fingerprinting. It is one example of the manipulation of our biological knowledge in order to address a forensic question. It is a method that allows for identification of the source of an unknown DNA sample. This method has become very important in providing evidence in paternity as well as criminal cases.

DNA fingerprinting involves the uses of restriction enzymes which are found in certain bacteria as a defense against viruses. Restriction enzymes isolated from certain bacteria are used to break up the human DNA into smaller fragments due to the fact that smaller fragments will migrate through the gel faster than the larger. Those fragments are separated from each other using gel electrophoresis. The pattern of the separated DNA fragments (restriction fragment lengths) is used to identify the individual by comparing the two patterns of separate fragment lengths of DNA.

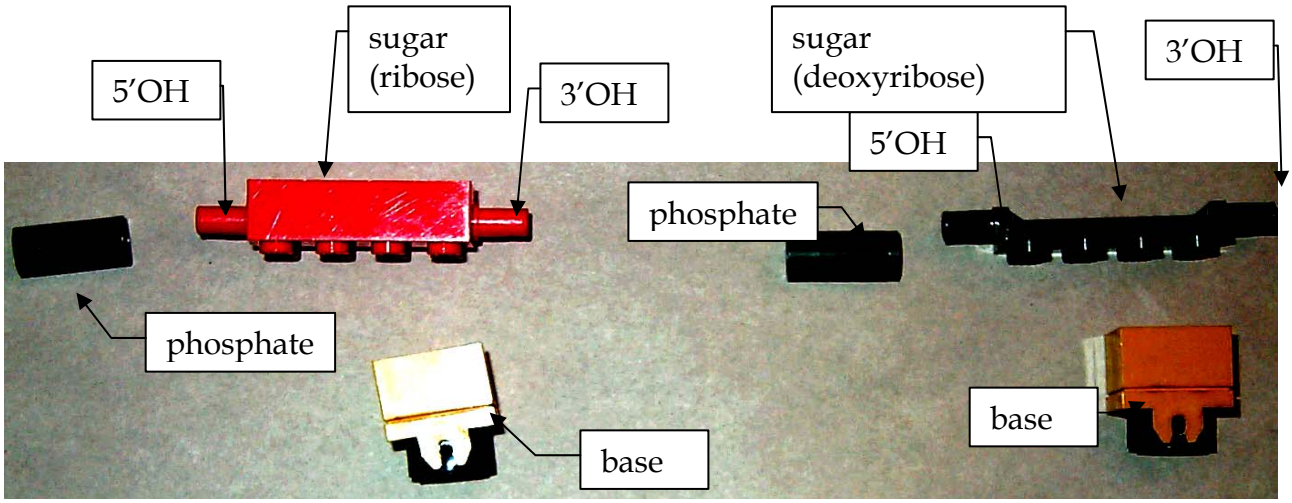
Restriction endonucleases catalyze the hydrolysis of specific bonds in the double-stranded DNA. This is useful to change the circular nature of plasmid DNA to one which is linear. This allows for inserts to be made into the DNA to construct a hybrid plasmid. Restriction enzyme activity and electrophoresis are basic tools used to characterize and manipulate DNA. These enzymes each have a name that starts with a three letter abbreviation of their source followed by a capitalized letter representing the strain and then a roman numeral of the order of discovery and characterization. For example EcoR I was the first restriction enzyme isolated from *E. coli* (strain R) whereas EcoR V was the fifth restriction enzyme to be isolated from *E. coli* (strain R) and EcoN I was the first to be isolated from *E. coli* (strain N).

Lambda (λ) is a well studied and manipulated bacterial virus, a bacteriophage. It infects bacteria but is not infectious to humans or other eukaryotic cells. This makes it a desirable tool for molecular studies. Today you will set up test tubes with uncut DNA of bacteriophage

lambda (λ) and the endonucleases EcoR I which cleaves DNA at the known sequence: G↓AATTC.

The LEGO models DNA and RNA as follows:
DNA and RNA are polymers of nucleotides. The LEGO models the nucleotides as follows:

RNA nucleotide



DNA nucleotide

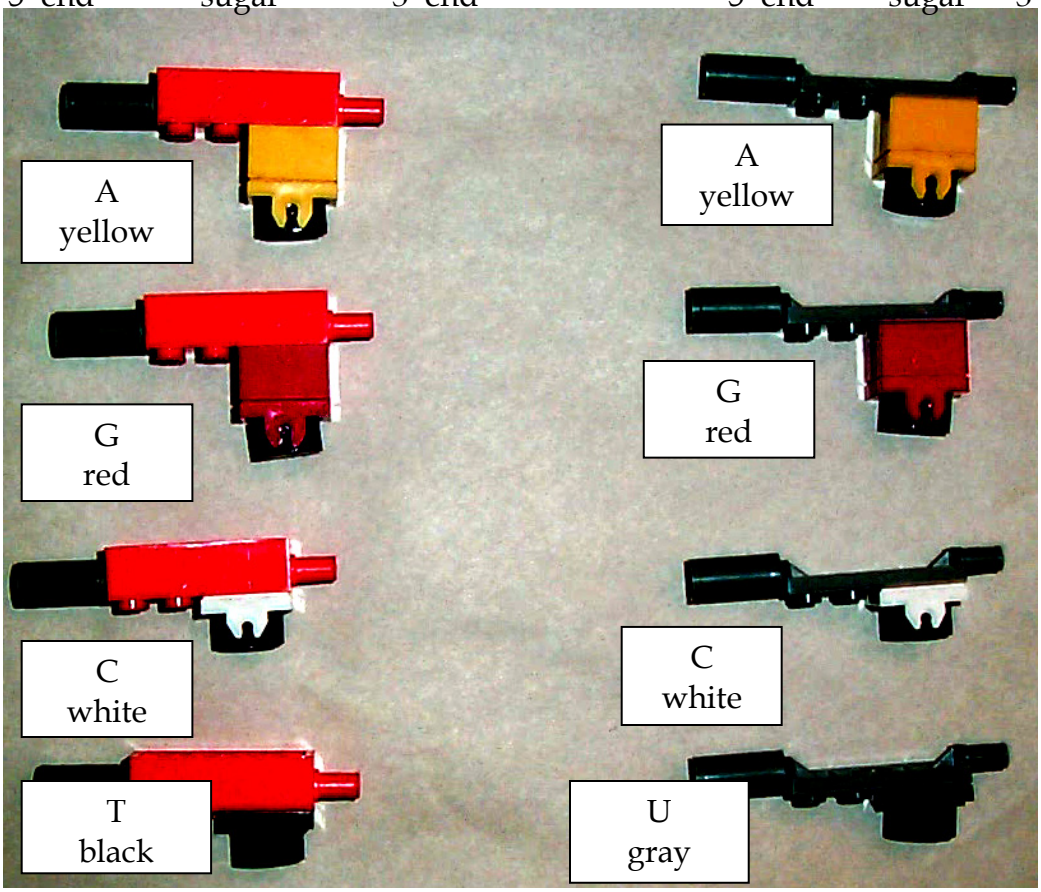
When nucleotides are assembled, they look like this:

RNA nucleotides

5′ end sugar 3′ end

DNA nucleotides

5′ end sugar 3′ end



In Class preparatory questions

The answers to the following questions will be reviewed at the beginning of each Lab. It is in your best interest as a student to attempt them prior to each Lab, though not required.
What are the three components of a nucleotide?

In DNA replication, what would be the base sequence that is complementary to:

ATCGGCTTAACGTAC

What type of bonds must be broken in DNA in order for replication to take place?

What reaction is catalyzed by a restriction enzyme (aka a restriction endonuclease)?

What is a restriction site (aka recognition sequence)?

Laboratory Excercise:

0. Build DNA model

1) Build a single-strand of DNA with the following sequence:

5′-ACGGTACGCTAT-3′

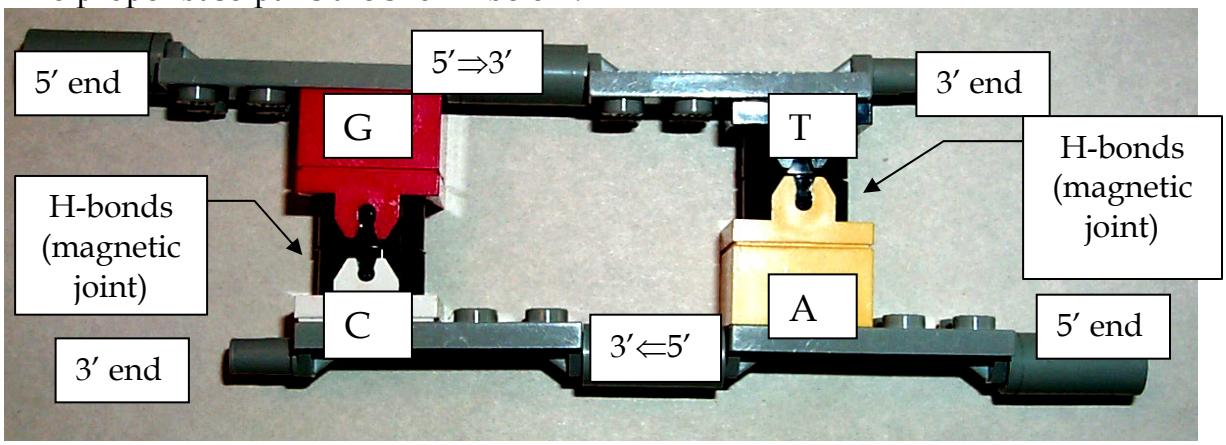
Notice that all the sugars run in the same direction.

2) Build another DNA strand properly base-paired to the one you made in step (1).

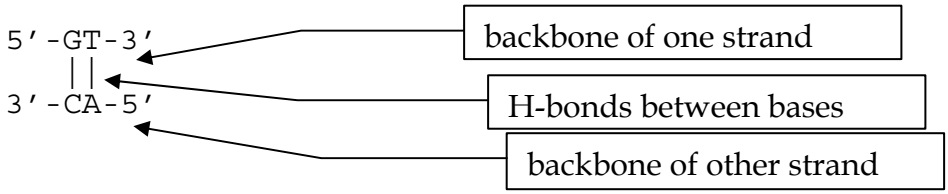
Note:

- the strands must be anti-parallel (run 5′ ⇒ 3′ in opposite directions)
- A pairs with T (yellow with black) the magnets won't let you pair it any other way
- G pairs with C (red with white) the magnets won't let you pair it any other way

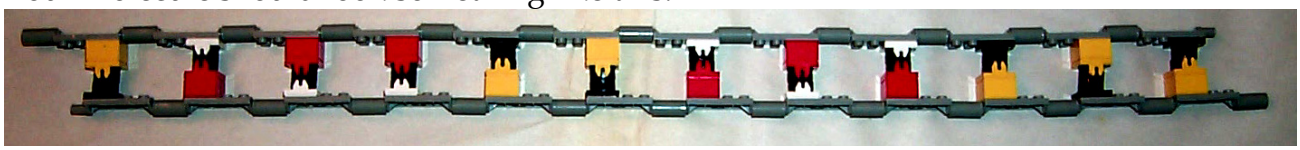
Two proper base-pairs are shown below:



The sequence of the DNA molecule in the picture above would be abbreviated like this:



Your molecule should look something like this:



What is the sequence of the DNA strand you just built?

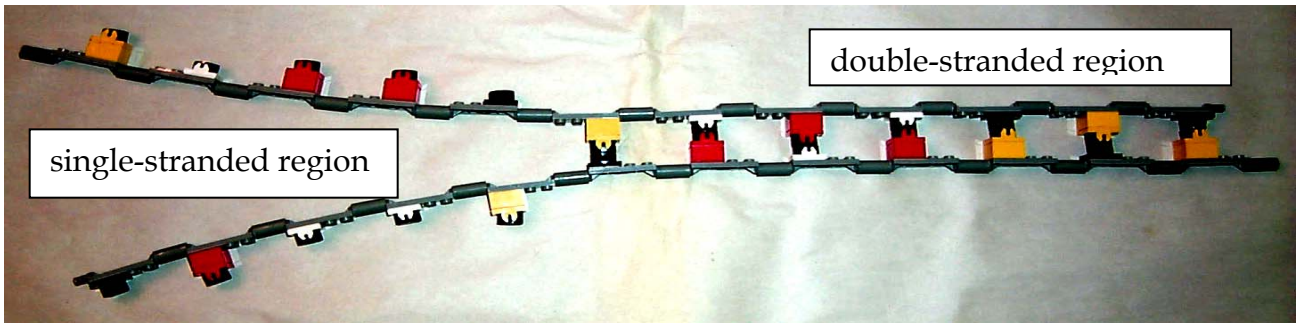
5′-_____-3′

What is the structure of the double-stranded DNA molecule you now have?

I. DNA Replication

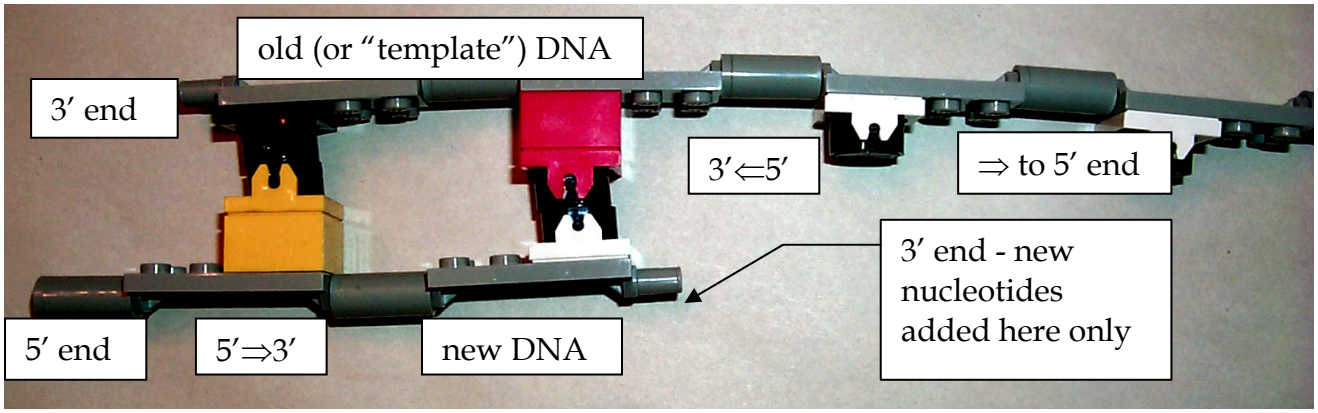
You will now simulate the replication of this DNA molecule.

3) Prepare the left-hand end of the molecule for replication. Un-zip (break the hydrogen bonds - simulated by separating the magnets) the 5 base-pairs at the left end of your DNA molecule to make a region of single-stranded DNA. You will have to turn the bases to face out from the center or they will stick back together. This is shown below:



4) Start replicating DNA on one of the single-stranded regions of your DNA molecule. Remember to follow the rules:

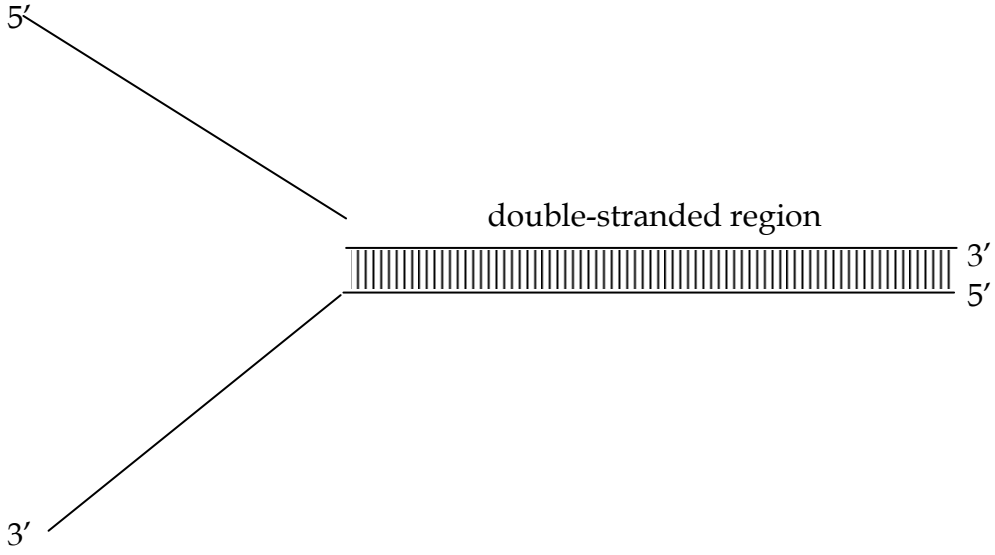
- the strands must be anti-parallel (run 5′ ⇒ 3′ in opposite directions)
- A pairs with T (yellow& black) the magnets won't let you pair it any other way
- G pairs with C (red & white) the magnets won't let you pair it any other way
- DNA polymerase can only add nucleotides to a 3′ end. This is shown below:



5) Continue replicating this strand until you have to stop-because you've reached the end of the template strand or you've run into the double-stranded region.

6) Replicate the other strand in the single-stranded region. Keep in mind the rules from step (4). You will notice an important difference between the two strands.

7) The lines in the diagram below represent the template DNA strands. On the diagram below, draw the two new DNA strands you made. Be sure to indicate their 5′ and 3′ ends. Put an arrowhead on the 3′ end to indicate that this is where the strand can grow. (5′ ⇒ 3′)



8) Unzip the remaining base-pairs in the double-stranded region and finish replicating the DNA strands. List the differences between the replication on the two strands:

Leading strand:

Lagging strand:

9) Disassemble the DNA molecules you made. Do this carefully so that the phosphates stay on the 5′ ends of the nucleotides (the end farthest from the base). This simulates the hydrolysis that occurs during digestion. This is shown below:

II. Restriction Enzyme Digest: G↓AATTC. You will subject Lambda DNA to the enzymatic reaction of EcoRI, a restriction enzyme. You will run your samples on a gel next week. Therefore, after lab we will freeze the samples from today's activity.

Materials

- Uncut Lambda DNA
- Restriction enzyme (EcoR I)
- Buffer
- Water bath at 37°C for the digest
- Floating racks
- microfuge tubes
- micropipetters (to accurately transfer liquids)
- micropipet tips (change between samples)
- beakers (for collection of used tips)
- microfuge tube racks
- sample buffer
- lab marker

Restriction Digest of Lambda using the Enzyme Eco R1.

- To prepare your group's samples**, follow the chart** below. (Notice that tube one will have Lambda DNA and no enzyme. Tube 2 will have Lambda DNA and ECOR I, both tubes have Buffer as well. Tubes 3 and 4 are control tubes with no DNA expected in them unless you have contamination!
- Clearly label all tubes, number them according to the chart or label with the contents that will go into each, and your name or a symbol that you will recognize. (Record in you notebook.)
- Your TA will show you how to transfer liquids with the micropipette. You are using microliters (µl), these are small quantities. 1µl=1/1,000ml. Pay careful attention to the instruction. Fill each tube with the DNA first, use 1 tip, change it and add the next component to the appropriate tubes, change the tip and go to the next. (this avoids contamination)
- Place all tubes in the 37°C water-bath for at least 50 minutes, this is the digest incubation. We will probably leave them in a few hours or overnight to be sure that your digest is complete. Your samples will digest beyond lab today, they will get collected and placed into the freezer until next week.

Tube	**Treatment**	**Lambda DNA**	**Enzyme ECO RI**	**Buffer E**	Total volume
1	Lambda (no digest) *Positive control*	8 µl		42 µl	50 µl
2	ECOR I digest *Experimental tube*	8 µl	8 µl	34 µl	50 µl
3	Enzyme control *Negative control*		8 µl	42 µl	50 µl
4	Buffer control *Negative control*			50 µl	50 µl

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Molecular Biology II

Learning Goals:

To work with a physical model of DNA and RNA in order to help you to understand:

- rules for both DNA & RNA structure
- transcription including promoters & terminators
- translation including start & stop codons

To work with a computer program in order to help you to understand:

- how to begin to analyze nucleic acid sequences
- the structure of a gene and the effects of mutations

Introduction:

This week's topics in molecular biology will include gene expression and sequence analysis using a computer. The field of bioinformatics is based on sequence analysis and using computer algorithms to answer biological questions. Gene expression is a complex process that allows cells to respond to the environment through the genome. The genome contains the information necessary to produce proteins through the coupled process of transcription and translation. Transcription is the process in which an mRNA molecule is made by RNA polymerase from DNA. Translation is the process in which an mRNA molecule is read by ribosomes (a composite of rRNA and proteins) in order to place amino acids together in a chain to form a polypeptide. Currently, the sequences of genes are analyzed using computer tools, a process called sequence analysis.

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met START	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Figure 1: Codon Table

How to decode the genome using a simple eucaryotic gene:

Deciphering the Genetic code requires the following steps: 1) Start transcription, 2) Stop transcription, 3) mRNA processing, 4) Start translation, and 5) Stop translation. A eucaryotic gene contains sequences that specify each of these steps. Currently, research in biology is underway in order to elucidate these sequences. Analyzing the sequence of a protein coding gene sequence will allow you to decipher the resulting protein.

1) Start Transcription: What is the first base of the resulting mRNA?

The actual gene contains more bases than the resulting mRNA. This is because a gene contains non coding regions, such as the promoter. The function of the promoter region is to regulate the recruitment of the RNA polymerase. The TATAA box is sequence motif found in the promoter regions of many genes. For our purposes, the mRNA will begin at the base immediately following the TATAA box.

2) Stop Transcription: What is the last base in the resulting mRNA?

The terminator region of a gene helps to regulate termination of transcription by the RNA polymerase. For our purposes the mRNA will end at the base immediately preceding the GGGGG.

3) mRNA processing

The major processing events are: 1) Introns are spliced out, 2) a poly A tail is added to the 3′ end and, 3) a modified Guanosine triphosphate is added to the 5′ end. For our purposes, introns begin with GTGCG and end with CAAAG

4) Start Translation

Translation does not begin at the very first codon of an mRNA! The AUG codon initiates translation.

5) Amino Acid sequence produced

Each successive codon is read and translated using the codon table until a stop is reached

6) Stop Translation

The amino acid sequence stops after an UAA, UAG, or UGA is reached.

As many genomes have been sequenced and are currently being analyzed by scientists around the world, a standard has been set up for DNA sequences. When a double stranded DNA molecule is written down, it is assumed that the sequence is read from left to right as the 5′ to 3′.

For example: ATGATGCGTAG

Means 5′ ATGATGCGTAG 3′
3′ TACTACGCATC 5′

Remember, the RNA polymerase makes RNA in the 5′ to 3′ direction, thus reading the 3′ to 5′ strand (the BOTTOM strand as written above). This ultimately produces a nucleic acid that is the same as the "top" strand, only with Us replacing Ts.

In Class preparatory questions

The answers to the following questions will be reviewed at the beginning of each Lab. It is in your best interest as a student to attempt them prior to each Lab, though not required.

1. β-thalassemia is an autosomal recessive genetic disease. This question deals with three alleles of the β-globin gene: the normal allele and two β–thalassemia alleles. The three alleles are described below:

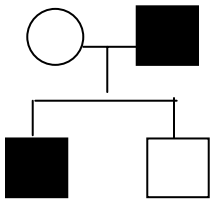
β-globin gene

Allele	**DNA sequence** (◎ means mutated to)	**contribution to phenotype**
H	normal	normal - dominant
h_1	codon 15 TGG ◎ TAG (stop)	β-thalassemia (recessive)
h_2	codon 17 AAG ◎ TAG (stop)	β -thalassemia (recessive)

1) What type of mutation is present in the h_1 and h_2 alleles (missense, nonsense, frameshift)?

2) Based on your knowledge of protein structure, provide a plausible explanation for why the β–globin protein encoded by the h_1 and h_2 alleles is non-functional.

3) Consider the following pedigree. Filled symbols represent individuals with β–thalassemia.



a) For each individual in the pedigree, write his or her genotype next to his or her symbol using the allele symbols defined above. Assume that the h_1 allele is causing β–thalassemia in this family.

b) What is the chance that the couple's next child will have β-thalassemia?

4) What would the phenotype (normal or β–thalassemia) of an individual of genotype h_1h_2 be? Explain your reasoning.

Laboratory Excercise

I. A Small Gene

In this part, you will build a small gene and simulate how it produces a protein.

1) Build the gene. Build a single-strand of DNA with this sequence (the spaces are to make it easier to keep your place in the sequence - they are not gaps in the backbone):

5′-CTATA AGCAT GCCCC TATGA GGGT-3′

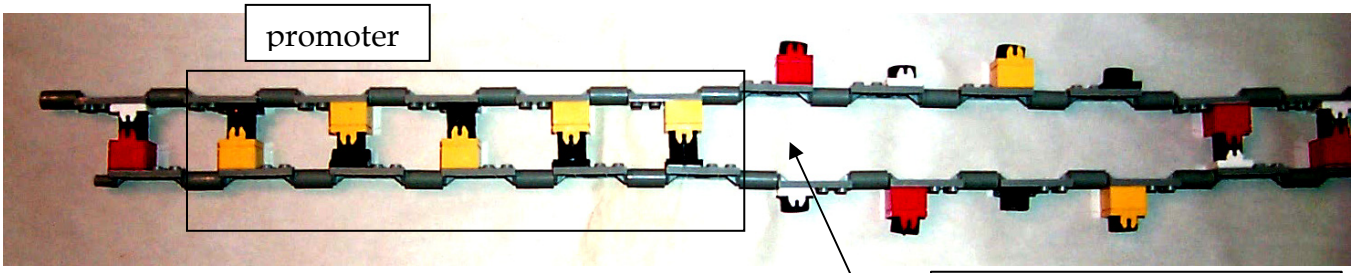
2) Build the corresponding other strand of DNA. If you have got the sequence exactly right, you will use up all of your DNA nucleotides.

II. Transcription

Transcription in this simulated organism starts at the first nucleotide after a promoter. In this organism, promoters have this sequence:

DNA bases	LEGO Colors (B = black (T), Y = yellow (A))
5′-TATAAx.....-3′ |||||| 3′-ATATTy.....-5′	5′-BYBYYx....-3′ |||||| 3′-YBYBBy....-5′

The 5′ end of the mRNA starts at base pair x-y. This is shown below:



5′	T	A	T	A	A
	|	|	|	|	|
3′	A	T	A	T	T

Transcription in this organism ends at the base-pair just before the terminator. In this simulated organism, terminators have the following sequence:

DNA bases (B = black (T), Y = yellow (A), R = red (G), W = white (C))

```
5′-xGGGT.....-3′
   |||||
3′-yCCCA.....-5′
```

LEGO Colors

```
5′-xRRRB...-3′
   |||||
3′-yWWWY...-5′
```

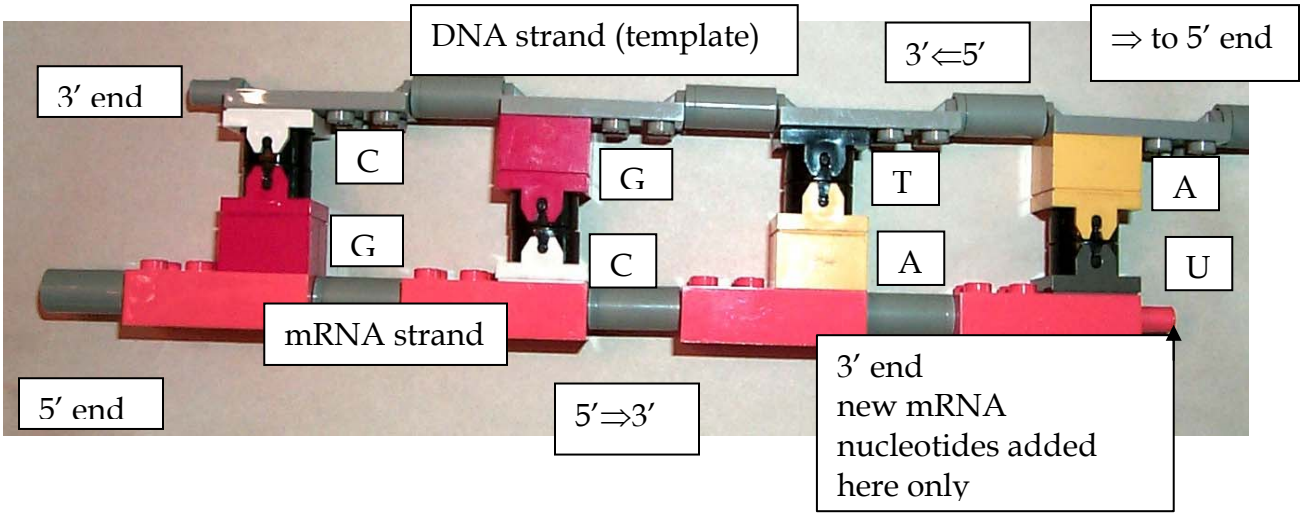
The 3′ end of the mRNA ends with base pair x-y.

3) Unzip the base-pairs from the end of the promoter to the start of the terminator - don't forget to flip the bases out or they will re-pair. This is shown in the picture on the previous page.

4) Make the mRNA using the following rules:

- the strands must be anti-parallel (run 5′ ⇒ 3′ in opposite directions)
- A pairs with U (yellow with gray) the magnets won't let you pair it any other way
- G pairs with C (red with white) the magnets won't let you pair it any other way
- RNA polymerase can only add nucleotides to a 3′ end.

Correct RNA-DNA base-pairs are shown below:



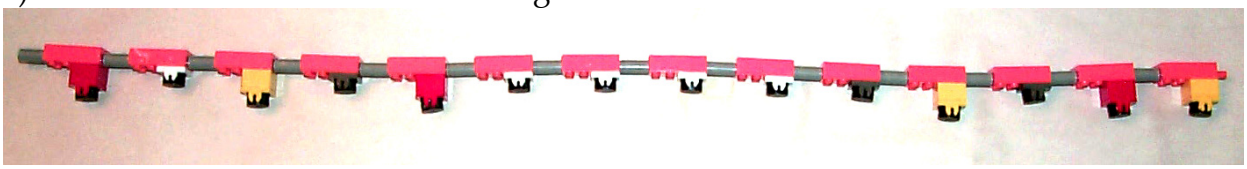
Notice that only one mRNA strand can be made that follows these rules.

What is the sequence of this mRNA?

5′-_____-3′

III. Translation

5) Your mRNA should look something like this:



Ribosomes in all organisms start at the 5′ end of the mRNA and look for the first start codon. This is 5′-AUG-3′ or 5′-yellow-gray-red-3′ and encodes the N-terminal methionine. Translation ends with a stop codon. A table of the genetic code can be found on page 125 of the lab manual.

Acting as a ribosome, translate this mRNA.

What is the resulting protein sequence?

N-_____-C

IV. A mutant gene

Mutations are alterations in DNA sequence. You can simulate their effects by changing the LEGO bases at a particular place in your simulated gene.

6) The 12th base-pair in your gene is a G-C base pair. Change it to a C-G base-pair. That is, the original DNA in that region **was**:

```
5′-...TGCCC...-3′
       |||||
3′-...ACGGG...-5′
```

Change it to (the altered base-air is **bold-underlined**)

```
5′-...TGGCC...-3′
       |||||
3′-...ACCGG...-5′
```

What is the resulting mRNA sequence? 5′-_____-3′

What is the resulting protein sequence? N-_____-C

Disassemble your DNA and mRNA molecules and sort them into the kits. Remember to keep the phosphates on the 5′ ends.

V. Sequence Analysis

Procedure :

1. Enter the following sequence into the Gene Explorer Program (Brian White, PhD)
TTGTATAACGTGATGAAACCCGATATTGCGTGCGTAGCTAGCTCAAAGCTTTTAAGATCGGGGGG
GCGATC

a b c d e f g h

2. Create a hypothesis for how each of the mutations (a-h) above effects the expression of our gene. Test your hypothesis by mutating your gene and analyzing the output from the Gene Explorer Program.

	HYPOTHESIS	RESULTS
a		
b		
c		
d		
e		
f		
g		
h		

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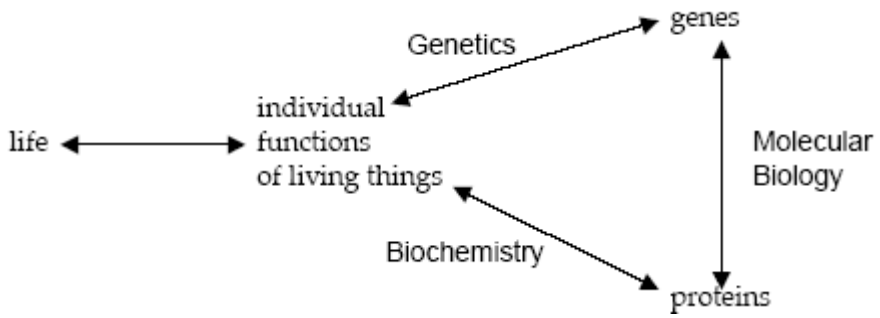
Function of Biochemistry

Learning Goals:

•To reinforce your understanding of Genetics, Biochemistry, and Molecular Biology
•To show the connections between these three disciplines
•To show how these three approaches can be combined to give a complete picture of a biological phenomenon
•To figure out a complete explanation of a biological phenomenon

Introduction:

This lab will unify Genetics, Biochemistry, and Molecular Biology.



•**Genetics** = explaining biological phenomena in terms of genes. *How is flower color inherited?*
•**Biochemistry** = explaining biological phenomena in terms of proteins and other molecules. *How does protein sequence determine protein structure and color?*
•**Molecular Biology** = explaining the connection between genes (DNA) and protein.
How does the DNA sequence of the color gene lead to a particular color?

The connections between these three fields of biology are shown below in a diagram credited to David Botstein (each of the arrows corresponds to "can be explained in terms of", the words in this font are the different disciplines of biology):

Software MGE:

In order to explore the relationship between Genetics, Biochemistry, and Molecular Biology you will simulate plant crosses to explore the underlying mechanisms that produce flower color. Three modules are available in MGE and are described below:

•**Genetics**. The flowers in this simulation are diploids. As with most flowers, they are all hermaphrodites (both male and female). With this tool, you can perform the following experiments:

- *Cross any two organisms*. A new window will appear with the offspring of this cross.
- *Self-cross any organism*. In this case, the single selected organism is both mother and father to the resulting offspring.
- *Mutate any organism*. A new window will appear with a set of flowers that are mutant versions of the selected organism.

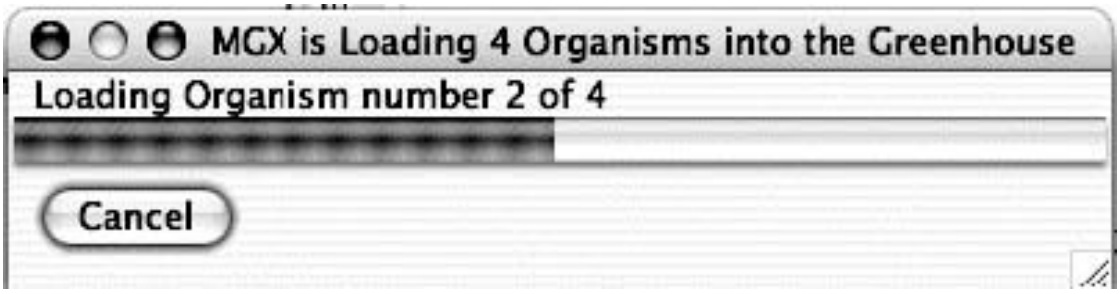
•**Biochemistry**. The color in these flowers results from the form(s) of pigment proteins present in an individual plant. With this tool, you can perform the following experiments:

- *Examine the pigment proteins present in a plant*. The tool shows you the amino acid sequence and two-dimensional structure of the pigment proteins present in a given plant.
- *Design your own proteins*. You can edit an existing protein sequence or type in an entirely new sequence. The program will then predict the two-dimensional structure of the resulting protein as well as its color. It will also predict the color resulting from the combination of any two proteins.

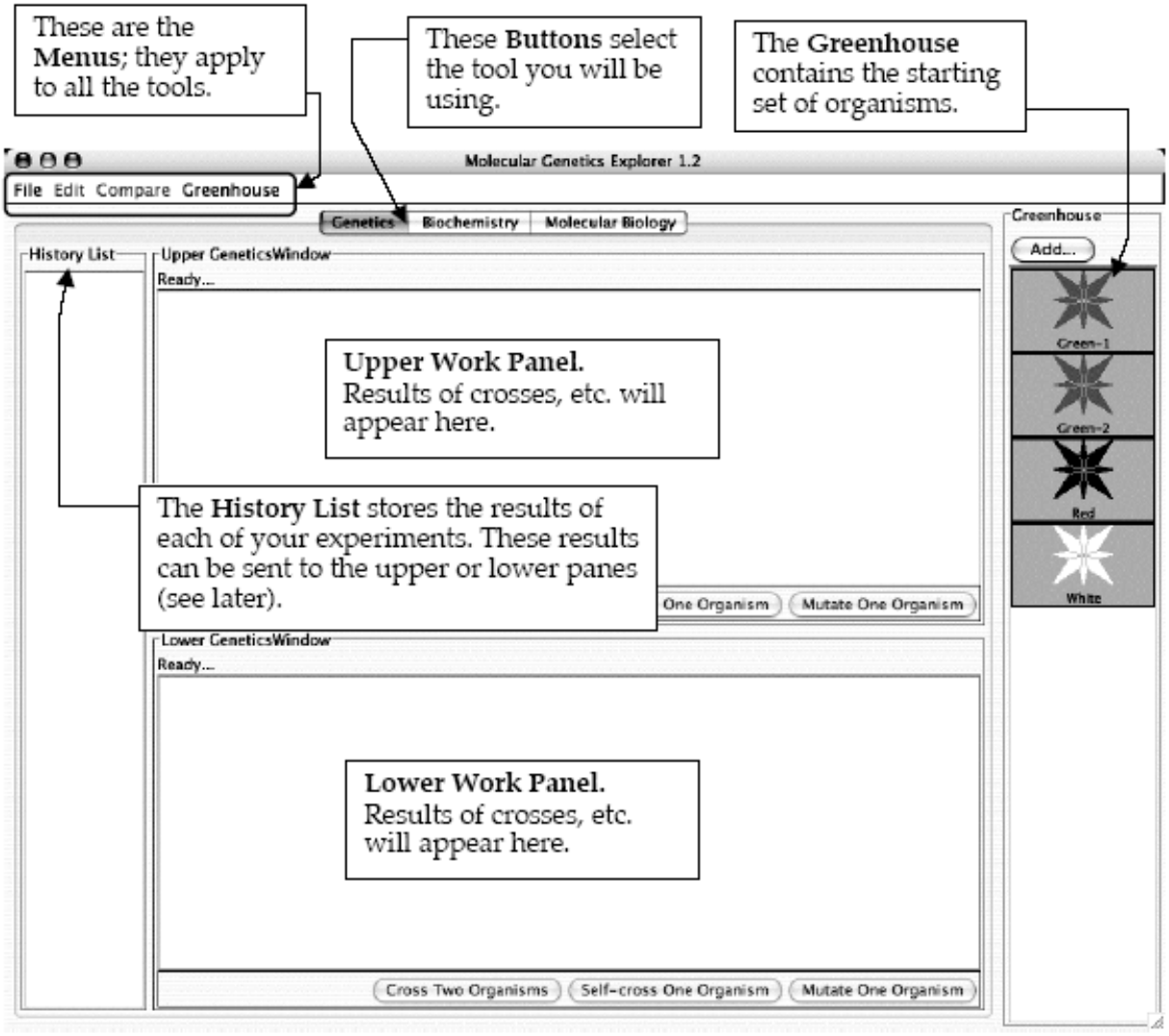
•**Molecular Biology**. The pigment proteins in these plants are produced by pigment protein genes. With this tool, you can perform the following experiments:

- *Examine the pigment protein genes present in a plant*. The tool shows the DNA, pre-mRNA, mature mRNA, and protein sequences present in a given plant. You can explore the introns, exons, etc. of these genes.
- *Design your own genes*. You can edit an existing DNA sequence or type in an entirely new sequence. The program will then predict the mRNA, protein sequence, two-dimensional structure of the resulting protein as well as its color. It will also predict the color resulting from the combination of any two proteins.
- *Design your own plants*. You can save edited DNA sequences as new organisms for further study.

When you start MGE, the program will load the four starting flower types into the Greenhouse; this takes a little while. You will see this:



When the program is ready, you will see a screen like this:



Each organism is shown as a flower:

The color of the picture shows the color of the flower. These flowers can be white, red, orange, yellow, green, blue, purple, or black. When any organism is selected, the black border turns green to show that it has been selected.
The next sections of this manual will show you the various tools and the tasks that you will need to carry out.

I. Demonstration: Genetics

The Biological Phenomenon Under Study

In this lab, you will explore the biological mechanisms behind the expression of flower color in a hypothetical plant. These flowers can be white, red, orange, yellow, green, blue, purple, or black.

Scenario:

You are the chief biologist for Flowers Unlimited, a breeder of fine flowers. Your company sells seeds that their customers plant in their gardens. Since most of your customers expect that the flowers will grow each year from seeds produced the previous year, you try to produce true-breeding plants whenever you can.

You've found a new species of flower with an attractive shape. You've collected four plants from the wild: two green, one red, and one white. Your customers would really like to have purple flowers from this plant. You set out to create a true-breeding purple flower.

Tasks:

- Determine how color is inherited in these flowers. NOTE: the color is controlled by one gene only.
  - Determine the colors of the alleles present in the original set of organisms.
  - Which alleles are dominant?
  - Which alleles are recessive?
  - How do the alleles combine to produce the overall color of the plant?

- Construct a purple organism to demonstrate your understanding of this process.

Using the tool:

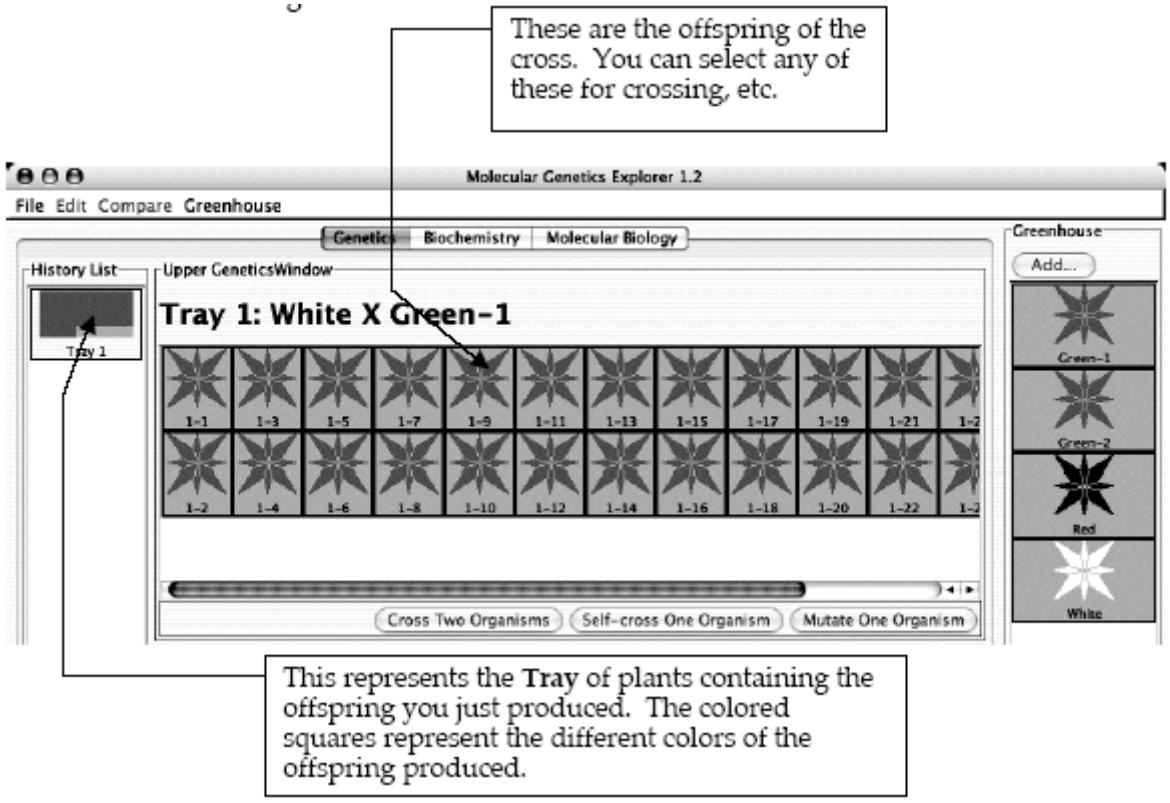
You can switch to this tool by clicking the "Genetics" tab near the top of the window.

There are three kinds of experiments you can perform with this tool. The following sections use examples to show you how to do each; you will need to devise your own experiments to carry out the tasks above.

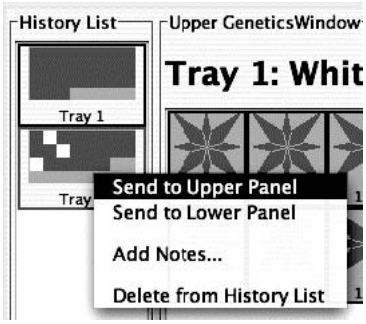
I) *Cross Two Organisms.* Suppose that you wanted to cross Red and White:

1) Click on Green-1 and then on White in the Greenhouse. The rectangular borders of both should turn green to show that they have been selected. The "Cross Two Organisms" buttons in the Upper and Lower Work Panels should be activated.

2) Click the "Cross Two Organisms" button in the Upper Work Panel. You should see something like this:



If you double-click on the **Tray** in the **History List**, you get a pop-up menu with a list of useful options:



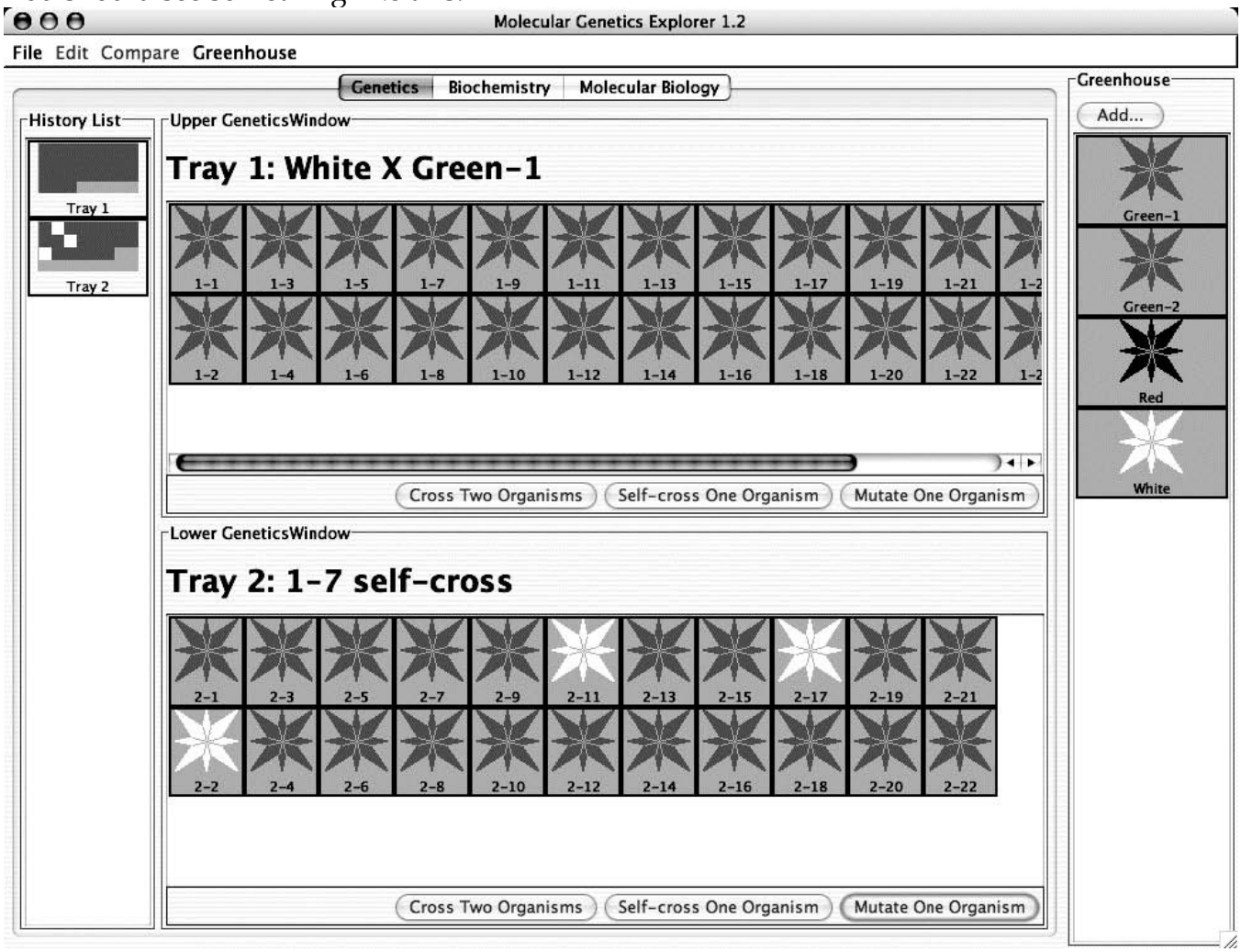
•**Send to Upper Panel**: Sends this **Tray** to the **Upper Panel** so you can cross those organisms.
•**Send to Lower Panel**: Sends this **Tray** to the **Lower Panel** so you can cross those organisms.
•**Add Notes...**: Allows you to add notes to the **Tray** in the **History List**. These notes will appear if you leave the cursor over the **Tray** for a few seconds.
•**Delete from History List**: deletes the **Tray** from the **History List**; this is cannot be undone.

II) Self-cross a single Organism. Suppose that you wanted to self-cross one of the offspring in Tray 1:

1) Select any one organism from Tray 1 in the **Upper Work Panel**. You can de-select an organism by clicking on it. When you have only one organism selected, the "Cross Two Organisms" buttons will be grayed out and the "Self-Cross One Organism" and "Mutate One Organism" buttons will be activated.

2) Click the "Self-Cross One Organism" button in the **Lower Work Panel**. You should see something like this (since offspring are generated by random choice of parental alleles, you will likely see slightly different numbers of red and white offspring):

You should see something like this:



The offspring of this self-cross are in the **Lower Work Panel**. Note the addition of Tray 2 to the **History List**.

III) Mutate any one organism. This simulates treating the cells of one organism with a strong dose of a mutagen and then growing each of those mutant cells into a separate plant. The flowers of the resulting mutant plants are displayed in a new **Tray**. This process can take a few minutes. Suppose that you wanted to mutate one of the white organisms in Tray 2:

1) Select a white organism from Tray 2. When you have only one organism selected, the "Cross Two Organisms" buttons will be grayed out and the "Self-Cross One Organism" and "Mutate One Organism" buttons will be activated.

2) Click the "Mutate One Organism" button in the Upper Work Panel. A pop-up will appear telling you that the program is busy making mutants. You can cancel this process if you like.

You will then see a new **Tray** with the mutants and a new entry in the **History List.**

At this point, there are several other things you can do:

A) If you find an interesting organism, you can save it to the **Greenhouse**:

1) Click the "Add…" button at the top of the **Greenhouse**.

2) You will be prompted to give the organism a name. Give it a descriptive name and click "OK". You will see your new organism appear in the **Greenhouse**. You can now access it using the other tools in this program.

3) At this point, the organism is saved in the program, but not on the disk. To save the contents of the **Greenhouse** to disk, click on the Greenhouse **Menu** and select "Save Greenhouse".

B) You can cross or mutate any of the organisms visible in the **Greenhouse**, **Upper Work Panel**, or **Lower Work Panel**.

C) You can bring any **Tray** from the **History List** to a **Work Panel** by double-clicking the **Tray** and selecting the appropriate item in the pop-up menu.

D) You can add notes to any **Tray** in the **History List** by double-clicking the **Tray** and choosing the "Add Notes..." item from the resulting pop-up menu. These notes will appear if you leave the cursor over the **Tray** for a few seconds.

Demonstration Task: Try to breed true breeding purple flowers.

II. Laboratory Excercise: Biochemistry

The Biological Phenomenon Under Study

In this lab, you will explore the biological mechanisms behind the expression of flower color in a hypothetical plant. These flowers can be white, red, orange, yellow, green, blue, purple, or black.

Scenario:

You are the chief biologist for Flowers Unlimited, a breeder of fine flowers. Your company sells seeds that customers plant in their gardens. Since most of your customers expect that the flowers will grow each year from seeds produced the previous year, you try to produce true-breeding plants whenever you can.

You've found a new species of flower with an attractive shape. You've collected four plants from the wild: two green, one red, and one white. Your customers would really like to have purple flowers from this plant. You set out to create a true-breeding purple flower.

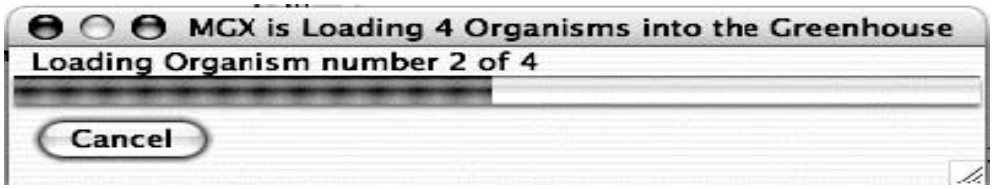
What is the biochemical basis for the different flower colors? *~Formulate a hypothesis~*

Now you will expand your understanding to how these colors are produced and how they interact at a Biochemical level.

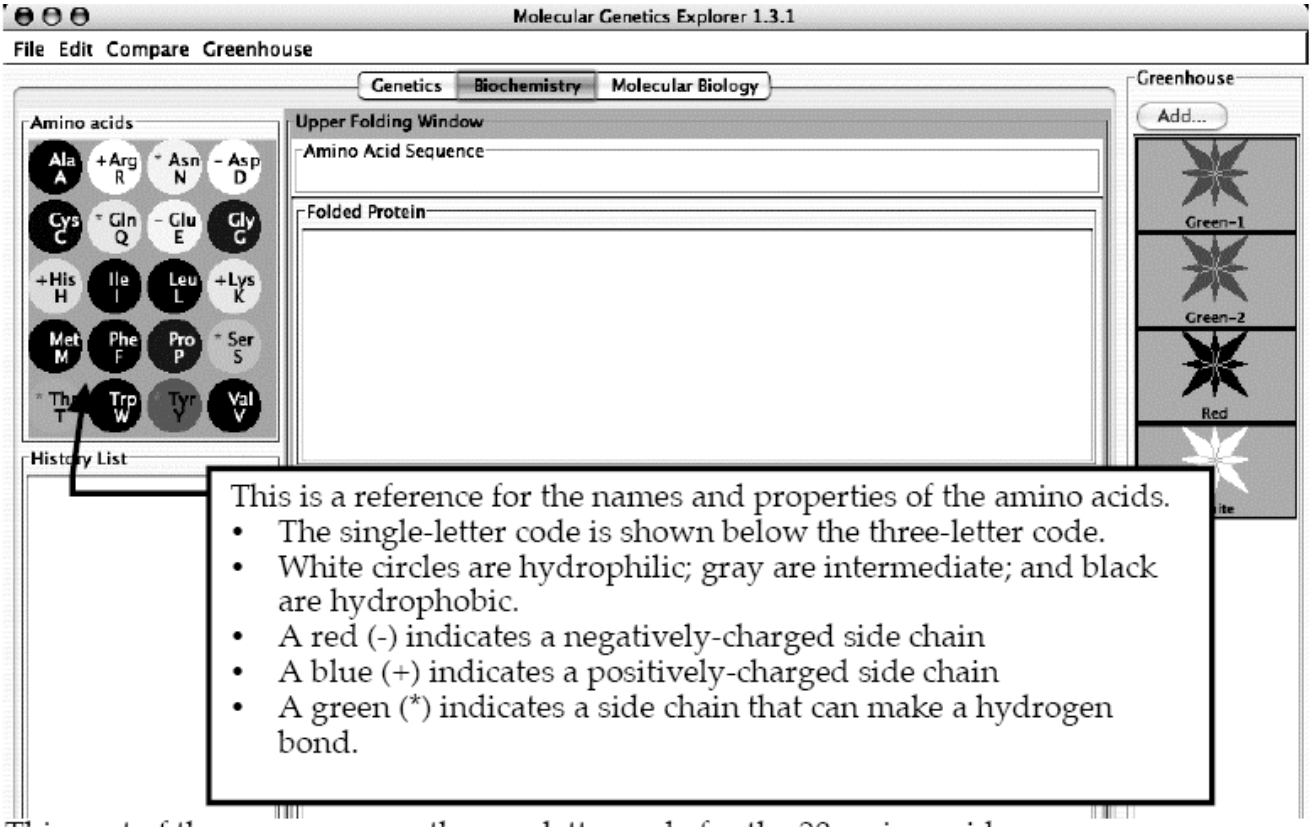
Tasks:

- Determine the differences in amino acid sequence between the proteins produced by the alleles you found in Part I.
- Determine how the amino acid sequence of a pigment protein determines its color.
- Explain, in terms of the proteins present, the interactions between the alleles you found in part I.
    - Why is the color phenotype of some pigment proteins dominant while others are recessive?
    - How do the pigment proteins combine to produce the overall color of the plant?
- Construct a purple protein to demonstrate your understanding of this process.

Using the tool:



When the program is ready, you can switch to the tool for this section by clicking the "Biochemistry" tab near the top of the window. You will see something like this:



This part of the program uses the one-letter code for the 20 amino acids:

Amino Acid	3-letter code	1-letter code	Mnemonic
Alanine	Ala	A	Alanine
Arginine	Arg	R	aRginine
Asparagine	Asn	N	asparagiNe
Aspartic acid	Asp	D	asparDic acid
Cystine	Cys	C	Cystine
Glutamine	Gln	Q	Q-tamine
Glutamic Acid	Glu	E	glu-tE-amic acid
Glycine	Gly	G	Glycine
Histidine	His	H	Histidine
Isoleucine	Ile	I	Isoleucine
Leucine	Leu	L	Leucine
Lysine	Lys	K	lysinK
Methionine	Met	M	Methionine
Phenylalanine	Phe	F	Fenylalanine
Proline	Pro	P	Proline
Serine	Ser	S	Serine
Threonine	Thr	T	Threonine
Tryptophan	Trp	W	tWptophan
Tyrosine	Tyr	Y	tYrosine
Valine	Val	V	Valine

Click in the Amino Acid Sequence Box at the top of the Upper Folding Window. Type a short sequence of letters and you will see a short amino acid sequence appear in the window. This tool converts the single-letter code to the three-letter code automatically.

Click the "FOLD" button and a two-dimensional version of your amino acid sequence will appear in the Folded Protein window.

There are several important things to note about this folding process:

This is a highly-simplified model of protein folding. It is not intended to predict the correct structures of any proteins; it is designed to illustrate the major principles involved in that process. The important features of proteins that this software retains are as follows:

- Amino acids have side-chains of varying hydrophobicity, charge, and hydrogen bonding capacity.
- The amino acids are connected in an un-branched chain that can bend.
- Hydrophobic amino acids will tend to avoid the water that surrounds the protein; hydrophilic amino acids will bind to the water.
- Amino acids that can form hydrogen bonds will tend to form hydrogen bonds if they can.
- Positively-charged amino acids will tend to form ionic bonds with negatively-charged amino acids if they can.
- Like-charged amino acids will repel each other if they can.
- Ionic interactions are stronger than hydrogen bonds, which are stronger than hydrophobic interactions.

Even though this software provides some important insights into protein folding, you should always keep in mind that this is an approximation. The most important "gotcha's" to be aware of are:

- This program folds proteins in 2-dimensions only.
- This program treats all amino acids as equal-sized circles.
- This program models an environment where disulfide bonds do not form.
- This program folds the protein based on the interactions between the side chains only.
- This program does not model secondary or quaternary structure.
- This program assumes that all side chains with hydrogen bonding capability can bond with each other.

These simplifications are necessary for two reasons. The first is technical: it turns out to be extremely difficult to predict the full 3-d folded structure of a protein given only its amino acid sequence. As of the writing of this lab manual, it takes a super-computer several days to predict the fully-folded shape of even a small protein like lysozyme. Even then, the predictions don't always match known structures. Given the computers we have in the Bio 111 labs, it might take years….

The second reason is educational. Proteins are complex 3-dimensional molecules; thus, it can be hard to find your way around when inside one. Likewise, it would be very difficult to visually compare two protein molecules to observe the effects of changes to their amino acid sequence. It would be easy to miss the forest (the forces that control protein structure) for the trees (the tiny details of the structures).

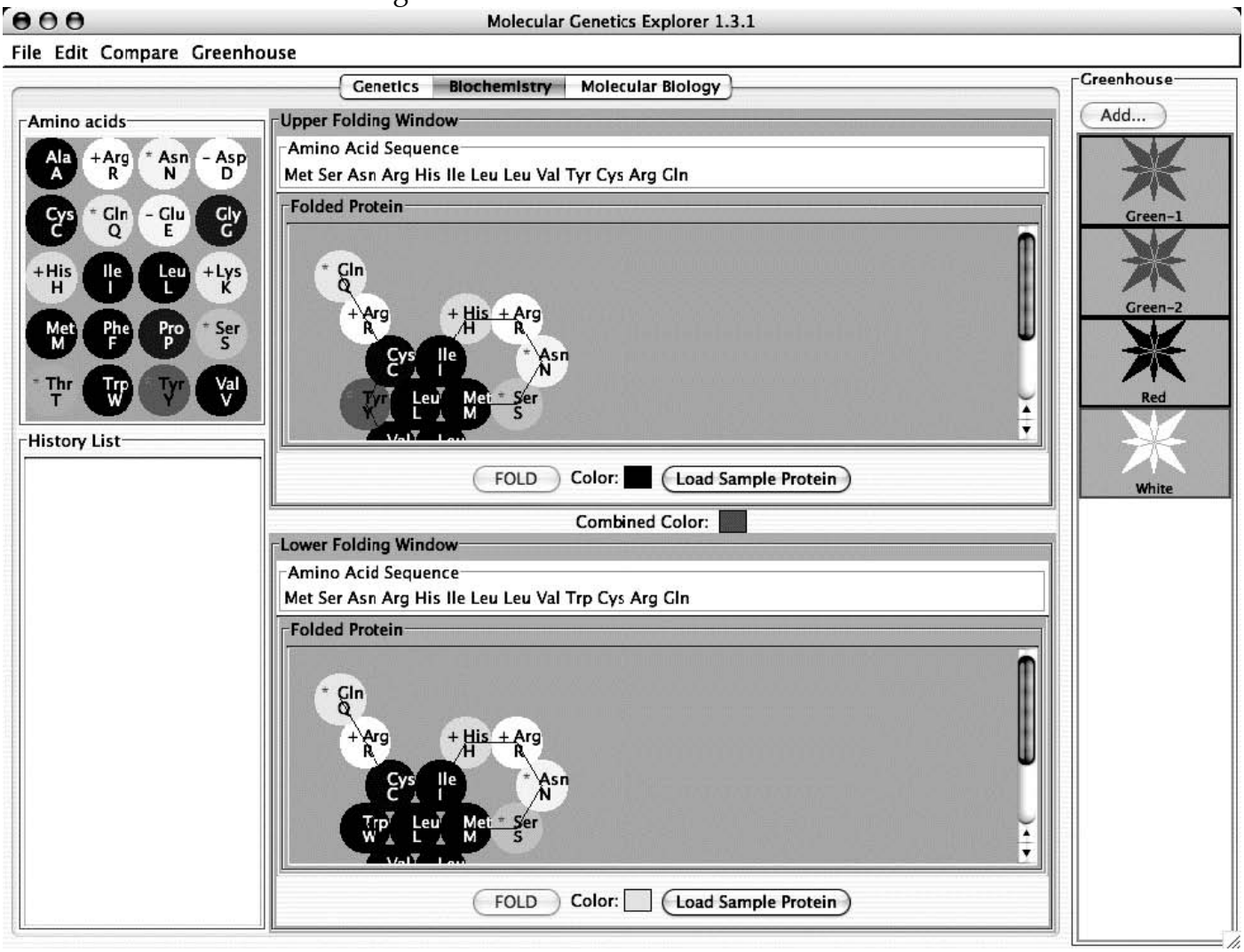
For these reasons, we will use this simplification. It retains the properties of amino acids that are important for this lab while being simple and fast.

There are three kinds of experiments you can perform with this tool. The following sections use

examples to show you how to do each; you will need to devise your own experiments to carry out the tasks above.

*I) Examine the Pigment Proteins Present in an Organism from the **Greenhouse***. This simulates extracting the pigment protein(s) produced by the two alleles of the pigment protein gene that an organism possesses, displaying their two-dimensional structures, and displaying their colors.

Double-click on the **Green-2** organism in the **Greenhouse**. You should see this:

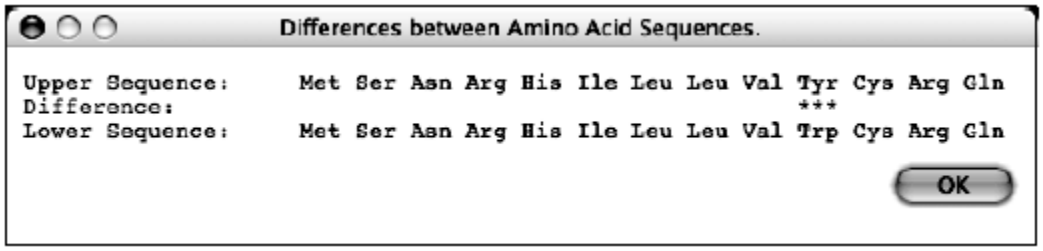


The **Green** organism contains two alleles of the pigment protein gene. Each of these alleles produces a different protein. One of these proteins is shown in the **Upper Folding Window**; it is a blue-colored protein as shown by the blue square next to the "Color:" label. The other protein is shown in the **Lower Folding Window**; this is yellow-colored protein. The combined color of the two proteins is green as shown by the **Combined Color** in between the two **Folding Window**s.

II) Compare the amino acid sequences of two pigment proteins. This aligns the two amino acid sequences so that the highest number of matching amino acids is obtained and then finds the remaining differences.

1) Double-click on the **Green** organism in the **Greenhouse**. You should see that the **Upper Folding Window** shows a blue protein and the **Lower Folding Window** shows a yellow protein.

2) You can compare the amino acid sequence of these two proteins by clicking on the "Compare" menu and choosing "Upper vs. Lower". A window will appear showing the differences between the two sequences. This is shown below:



This shows that the only difference is that, in the upper (blue) protein, amino acid 10 is tyrosine, while in the lower (yellow) protein, amino acid 10 is tryptophan.

III) Edit a Protein Sequence or Create a New Protein Sequence and Determine its Two-Dimensional Structure and Color. You can edit the sequence in either of the **Amino Acid Sequence** boxes and click the "Fold" button to predict the two-dimensional structure and color of the protein. The tool will also give the color that results from the combination of the colors in the Upper and Lower windows.

For example, click anywhere in the "Tyr" corresponding to amino acid 10 in the **Upper Amino Acid Sequence** box. Click the "delete" key and that amino acid will disappear. Type an "L" (the one letter code for leucine) and the amino acid sequence should be:

Met Ser Asn Arg His Ile Leu Leu Val Val Cys Arg Gln

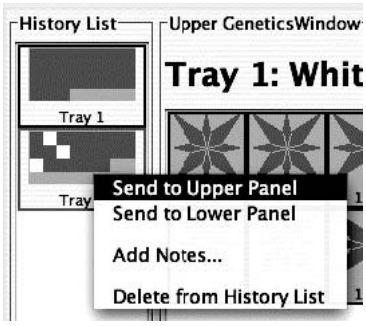
Click the "FOLD" button in the **Upper Folding Window**. You will see that the color of the new protein is white as shown by the "Color:" in the **Upper Folding Window**. You should also notice that:

- the "Combined Color" at the center of the window is now yellow.
- there is now an entry in the **History List** with your new protein. The background of

History List entry is white to show the color of this protein.

You can also click the "Load Sample Protein" button. This will load a sample amino acid sequence that folds to a white-colored protein with a shape that is similar to many colored proteins.

If you double-click an entry in the **History List**, you will you get a pop-up menu with a list of useful options:



- **Send to Upper Panel**: Sends this **Tray** to the **Upper Panel** so you can cross those organisms.
- **Send to Lower Panel**: Sends this **Tray** to the **Lower Panel** so you can cross those organisms.
- **Add Notes...**: Allows you to add notes to the **Tray** in the **History List**. These notes will appear if you leave the cursor over the **Tray** for a few seconds.
- **Delete from History List**: deletes the **Tray** from the **History List**; this is cannot be undone.

IMPORTANT NOTE: This software is under development. Please treat it gently and be patient. Please report any bugs to your TA. **You should save your Greenhouse regularly, especially if you save a large number of organisms.**

Specific Tasks for this section

a) What are the differences in the amino acid sequences of the proteins produced by the alleles you define in Part I? Hint: use the **Compare** menu to find the difference(s)between the amino acid sequences.

b) What features of the amino acid sequence make a protein pigmented?

c) What features of the amino acid sequence make a protein a particular color?

d) How do the colors combine to produce an overall color? How does this explain the genotype-phenotype rules you found in part (I)?

e) Which proteins are found in each of the four starting organisms?

f) Using this knowledge, construct a purple protein.

Put your data in the tables below:

(a) **allele** **color** **amino acid sequence** (highlight differences)

(b) What features of a protein make it colored?

(c) What features of the amino acid sequence make a protein a particular color?

(d) How do the colors combine to produce an overall color? How does this explain the genotype-phenotype rules you found in part (I)?

(e) Which proteins are found in each of the four starting organisms?

Green-1

Green-2

Red

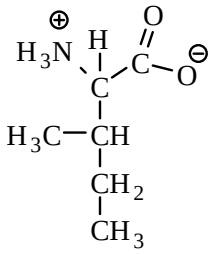
White

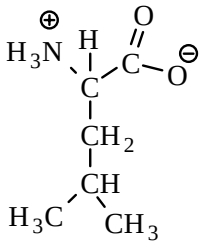
(f) Show your TA that you have made a purple protein. For full credit, you need to explain to your TA *why* it is purple.

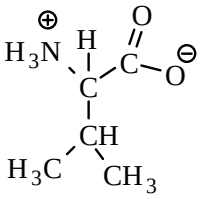
Amino Acids

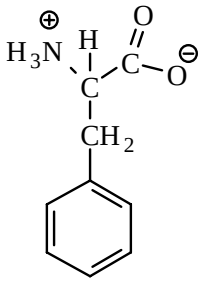
Non-polar

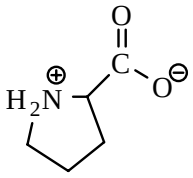
most hydrophobic →


isoleucine (ile)

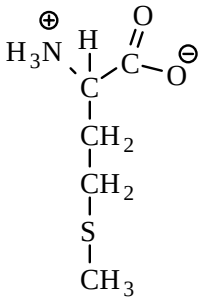

leucine (leu)

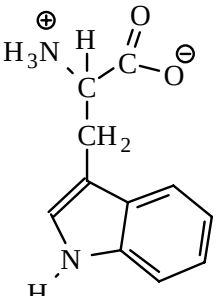

valine (val)

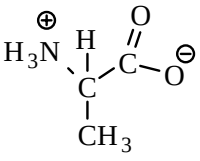

phenylalanine (phe)

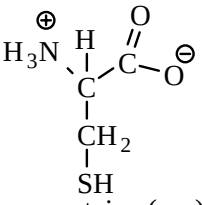

proline (pro)

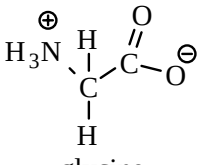
→ least hydrophobic (but still non-polar)


methionine (met)


tryptophan (trp)

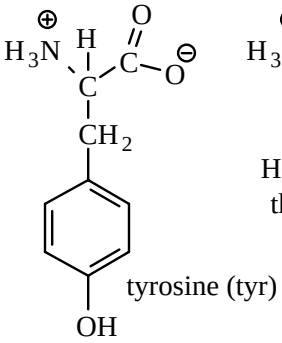

alanine (ala)

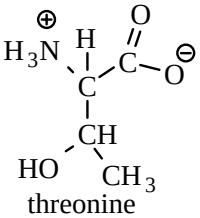

cysteine (cys)
(Campbell says that cysteine is polar, but it really isn't)

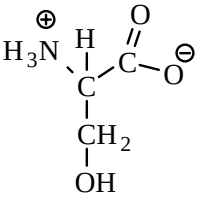

glycine (gly)

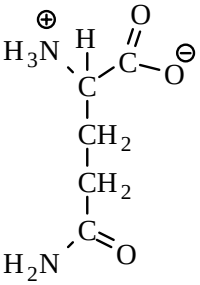
Polar (but uncharged)

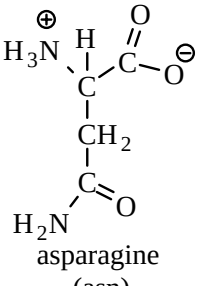
least hydrophilic → most hydrophilic


tyrosine (tyr)


threonine (thr)

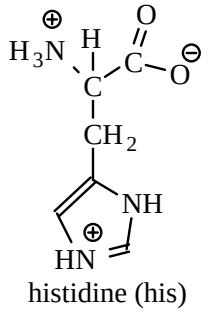

serine (ser)

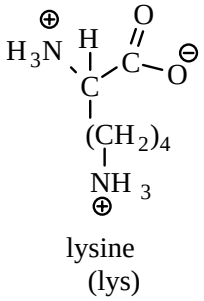

glutamine (gln)

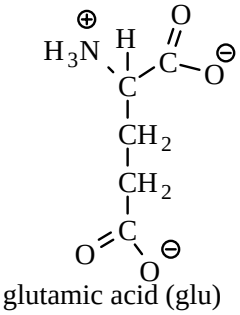

asparagine (asn)

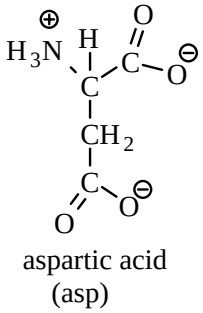
Charged

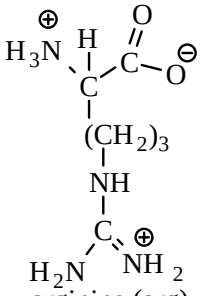
least hydrophilic → most hydrophilic


histidine (his)


lysine (lys)


glutamic acid (glu)


aspartic acid (asp)


arginine (arg)

Biotechnology Skills

Learning Goals: After completing this laboratory exercise you will be able to

- Discuss the use of restriction enzymes to characterize DNA.
- Describe gel electrophoresis
- Introduce the field of bioinformatics
- Familiarize the student with performing sequence alignments
- Understand the assembly process in genome sequencing

Introduction

Biotechnology is the use of biology to advance technology. Many businesses use biochemical and molecular applications for the progression of society. In the Molecular Biology I lab, you performed a restriction enzyme digestion. In one tube you had lambda DNA that was subjected to the activity of ECOR I. This should have produced a particular number of fragments of DNA, each a particular size. The positive control is your tube of Lambda by itself, which should remain uncut, a large fragment on the gel. There are also two negative controls that you will load on the gel. What is their role? After electrophoresiss of the samples you will realize if your expected results match the observed results. This application is a basic biotechnology skill.

Biochemical and molecular applications of biology use a variety of electrophoresis techniques for answering an assortment of questions. One referred to earlier in this manual was that of DNA fingerprinting. Restriction enzymes are used to break up the human DNA into smaller fragments which are separated from each other using gel electrophoresis. The gel is a matrix that the samples are loaded into and to which an electric current is then applied. This allows the fragments within a sample to migrate through the gel at various speeds based on the characteristics of the fragments. The type of gel varies by application. We will use a horizontal gel made of agarose which allows large molecules like DNA (200-50,000 base pairs) to migrate through it. Some applications use vertical gels or cylindrical gels made of other substances.

You will figure out the details of the expected results of your digest using a computer, the sequence of Lambda and the cleavage information of the enzyme you used. While you do this you will run your samples on a gel and observe the physical results of your digest, analyze it and compare to the hypothetical expectation.

In today's lab :

1. We will predict the expected results of last week's restriction digest using a computer to search the DNA sequence of Lambda for sites where the enzyme cut.
2. You will map these results on a Lambda sequence map.
3. You will count the number of fragments (bands) created on your agarose gel to see if it agrees with the prediction.
4. You will estimate the size of fragments (bands) in your sample lanes on your gel and compare them to your expected predictions using the known information of the standard lane. (also called the marker)

The analysis of the Lambda DNA sequence map is a precedent to understanding genome assembly, which could have a dramatic impact on modern medicine in the near future. Scientists are working towards a personalized medicine that would enable Doctors to tailor medicine to each patient. New technology can sequence entire genomes in a few days. The order of the nucleotides in a gene and the order of the amino acids in a protein are referred to as the sequence of the gene and sequence of the protein respectively. The process in which the sequence of a particular gene is determined is gene sequencing. It is possible to determine the sequence of a protein if the sequence of a gene is known by using the genetic code. If a gene has been sequenced, then its sequence is submitted to the public database at the National Center for Biotechnology Information.

http://www.ncbi.nlm.nih.gov

Determining a protein sequence is more difficult than determining a gene sequence and involves using Mass spectroscopy. Knowing a gene sequence is useful in analyzing the function of a protein as the amino acid composition and order determines the chemical properties of the protein.

Genome sequencing has become an essential tool in biology. By sequencing the genome of a species, the sequence of every gene is known. Knowing the sequence of a gene enables a biologist to develop primers for PCR and RT-PCR, important laboratory methods for analyzing gene expression and function. Furthermore, the sequence of a gene can provide information as to its function. If a doctor knows the exact mutation responsible for a patient's diagnosis, it would aid in treatment.

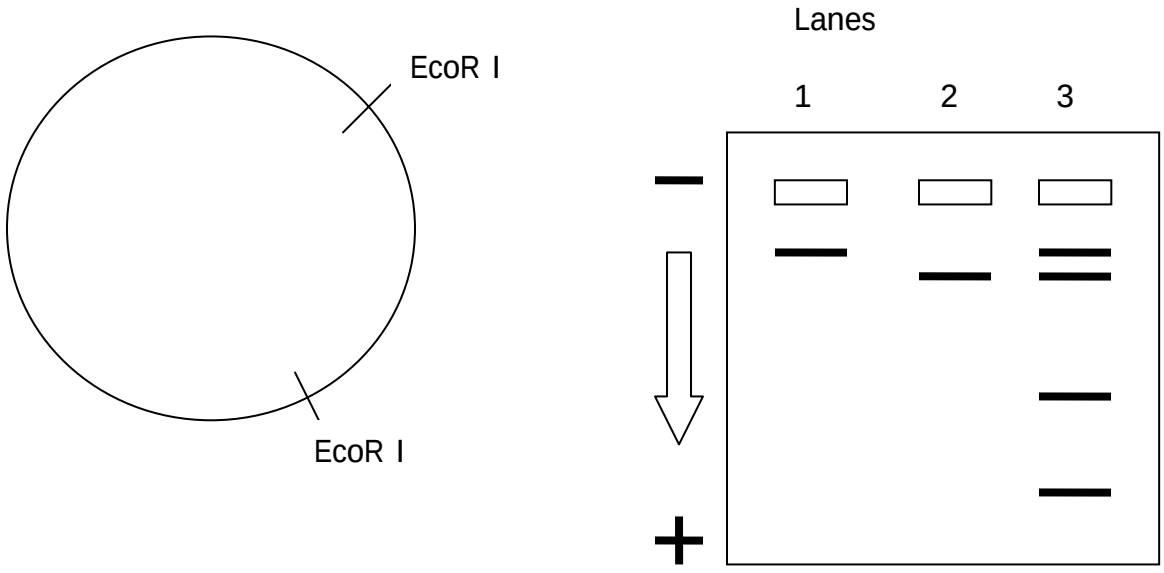
Genome sequencing is an enormous task and requires both laboratory and computational techniques. First, the genome is broken up into many smaller fragments using restriction enzymes. Each fragment is cloned using cloning vectors such as

plasmids, yeast artificial chromosomes, or bacterial artificial chromosomes and then sequenced. Ideally a large number of fragments are cloned and sequenced such that there are overlapping regions between the fragments. Once a sufficient number of fragments have been sequenced, the **assembly** process begins. Bioinformatic techniques allow for the production of an entire genome sequence by aligning the fragments and determining their order.

In Class preparatory questions

The answers to the following questions will be reviewed at the beginning of each Lab. It is in your best interest as a student to attempt them prior to each Lab, though not required.

1. In the incomplete diagram below (not drawn to scale), Lane 1 contains plasmid DNA that is UNCUT. Lane 2 contains the plasmid DNA cut with the Restriction enzyme EcoR I. Lane 3 contains a DNA ladder, where the bands represent the following sizes: 4,000 bp, 3000 bp, 500 bp, and 250 bp. Draw in the additional band missing from lane 2. (2 points)
(1 point)



2. How do dNTPs differ from the ddNTPs used for DNA sequencing? (2 points)

3. Briefly describe the whole genome shotgun approach to sequencing. (2 points)

Laboratory Excercise

A. Lambda Sequence Analysis

Materials

- Frozen samples from last week
- DNA standard (fragments of known size)
- sample buffer
- micropipetters (to accurately transfer liquids)
- micropipet tips (change between samples)
- beakers (for collection of used tips)
- Computer
- Lambda sequence file
- Enzyme cleavage information
- For gel electrophoresis:
- Fast Blast DNA stain
- Electrophoresis rigs, gels poured and filled with running buffer
- Power-packs
- containers for stain/destain
- Collection beaker for used dye
- Plastic ruler
- Semi-log graph paper

Your samples are:

lambda DNA undigested
EcoR I digest
Enzyme control
Buffer control

I. Electrophoretic Separation of Genomic DNA

Loading samples See diagram for a visual of the gel and gel rig.

- Your TA will load what is called a standard in the first well of your gel. The standard is a mix of DNA fragments of known size.
- The other wells are for your group to load your experimental samples: Lambda uncut DNA and Lambda cut with an enzyme.

Before you load the gels, practice once or twice:

- The agarose gels are prepared for you. Notice they are rectangular, and at one end a comb was used to create indented wells. They are located at the negative probe end of the apparatus.
- Running buffer is the liquid that the gel is standing in, and conducts electrical current. The sample buffer your DNA is suspended in is heavier than the running buffer and dyed. You can load it in buffer filled wells, the sample is visible and will sink.
- You will load your samples into the wells using a micropipette, put a clean tip onto it. You will use a new tip for each sample. Be sure the micropipette is set at 40**µl. Your TA will show you how to load the gel.**
- Use the practice gel outside of the gel rig, to load some dye into a few times before you actually load your samples into your gel. Place the pipette tip into the tube of practice dye, gently press down on the top, until the first stop, then **slowly** let it rise up, if you let it up too fast bubbles form and the volume is incorrect.
- Stabilize yourself by putting your elbow on the counter and rest your other hand towards the lower end of the pipet but not on the tip. Do not poke at the gel, either rest the tip just inside or hold it above the opening of the well and very slowly dispense your sample until the first stop. Hold it there and remove the micropipetter/tip from the gel rig before releasing the top. (Do not go past the first stop, this not only pushes out all of your sample but some air and will make bubbles in the well. Do not release the top of it until you pull the tip out of the well- otherwise you will draw your sample back up)
- Once you have practiced you can load your digest samples onto your gel in the gel rig.
- Be sure that your samples are fully thawed, hold them in your hand if they are still frozen. Draw up 40µl of the first sample. Place the tip just inside or above the very top of the well. VERY slowly depress the pipette and carefully expel the sample into the well, remove the tip from the well. Put the tip into the waste tip beaker and get a new clean tip for the next sample that will be loaded into the next well.
- Be sure to record in your notebook sample sequence that you loaded (which sample went into which well) , so you know what is in each well.

Running the gel:

- After all samples are loaded, your TA will cover the gel rig, and plug it into the power pack. Be sure that the negative (black) electrodes and the positive (red)

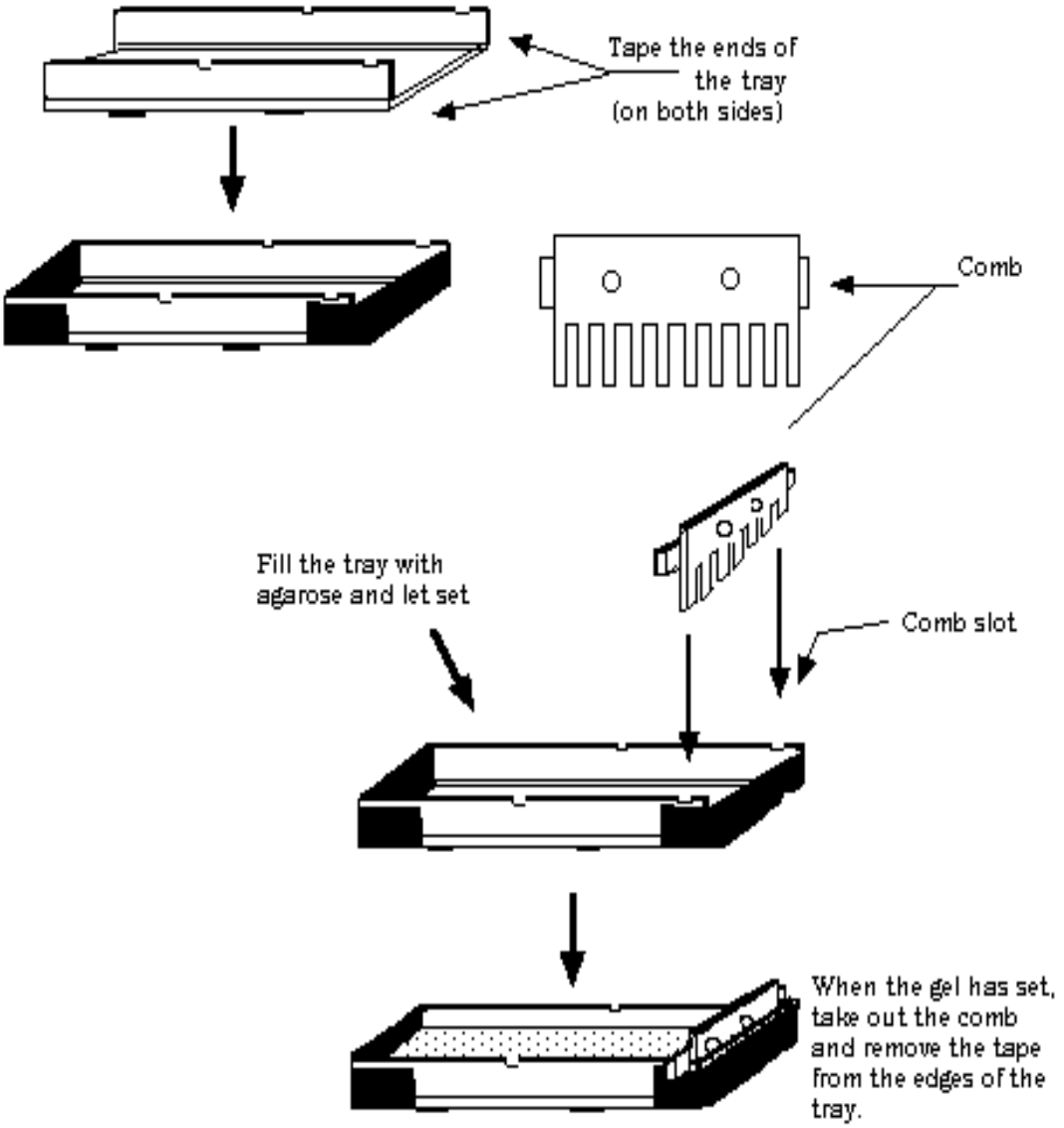
electrodes are matched up the wells should be on the negative end because the samples will move towards the positive end.

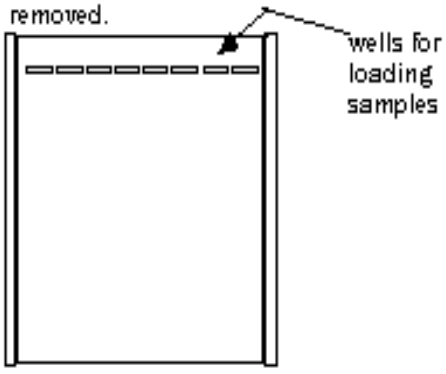
- Turn the power on and run at 80 volts for one hour and 30 minutes or until the dye front has moved at least 3/4ths or more through the gel.
- Turn the power off. Unplug power pack from the outlet. Remove the cover from the gel rig.
- Wear gloves, remove the tray that holds your gel, pour off any buffer into the gel rig and carry it to your table.

Staining and destaining the gel:

- Carefully push the gel into a staining container, pour enough stain over the gel to cover it by a few millimeters. Only stain for 3 minutes, gently shake the container to move the stain and gel around a bit.
- After 3 minutes, use the funnel as you pour the stain back into its container.
- To Destain: bring the container to the sink and carefully fill it with water, use a small stream of water as not to break the gel. Stay at the sink for about 15-20 minutes and continue to rinse, and refill the container, getting as much stain out of the gel as you can. The more background stain that you rinse out now the faster you will see the stained DNA that will remain in the gel.
- Bring the gel destaining in water back to your table, keep an eye on it rinsing and changing the water every 5-10 minutes and the bands will be easier to see.
- Once you can see the bands, record the results. Keep the gel in water until next lab, when it will be completely destained.

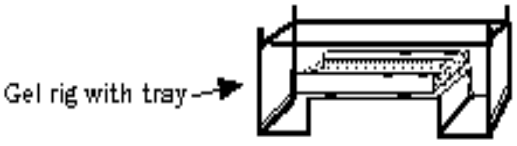
Gel tray





- Place tray in gel rig, wells on the negative end. (Samples will migrate towards the positive end with the application of an electrical current.)

-Add buffer, load samples and place cover on the gel rig, have instructor check, then start.



II. Expected Results (the sequence, the computer and mapping)

Determining the number and size of the DNA fragments using the computer.

On the desktop of the computers in the lab, is a folder named bio 111 computer labs.. Open the folder, and open the file inside named Lambda sequence. Notice that this is the full sequence of bacteriophage lambda. It was found on the National Center for Biotechnology Information (NCBI) website, (Entrez). Scroll down to where you see "origin". Beneath that is the sequence, the information at the top is additional and not needed to search.

1. Each line of the sequence is numbered and contains 71 bases. You can determine a base pair location by multiplying 71 bases by the number of the last full line of the sequence and then count out to the site of interest. This Lambda sequence has 48,481 bases.
2. Given the specific series of bases needed for an enzyme to cleave allows you to search the sequence and
   i) determine (while your gel runs) how many times the enzyme cuts the DNA,
   ii) the resulting number of fragments and
   iii)how large they are (base pair length) expected to be.
   As you gather this information, record it in your manual, and then map the locations.

Searching the Sequence.

Enzyme information for Enzyme 1: Eco R1

Eco R1 cuts at 5'… g aattc… 3'

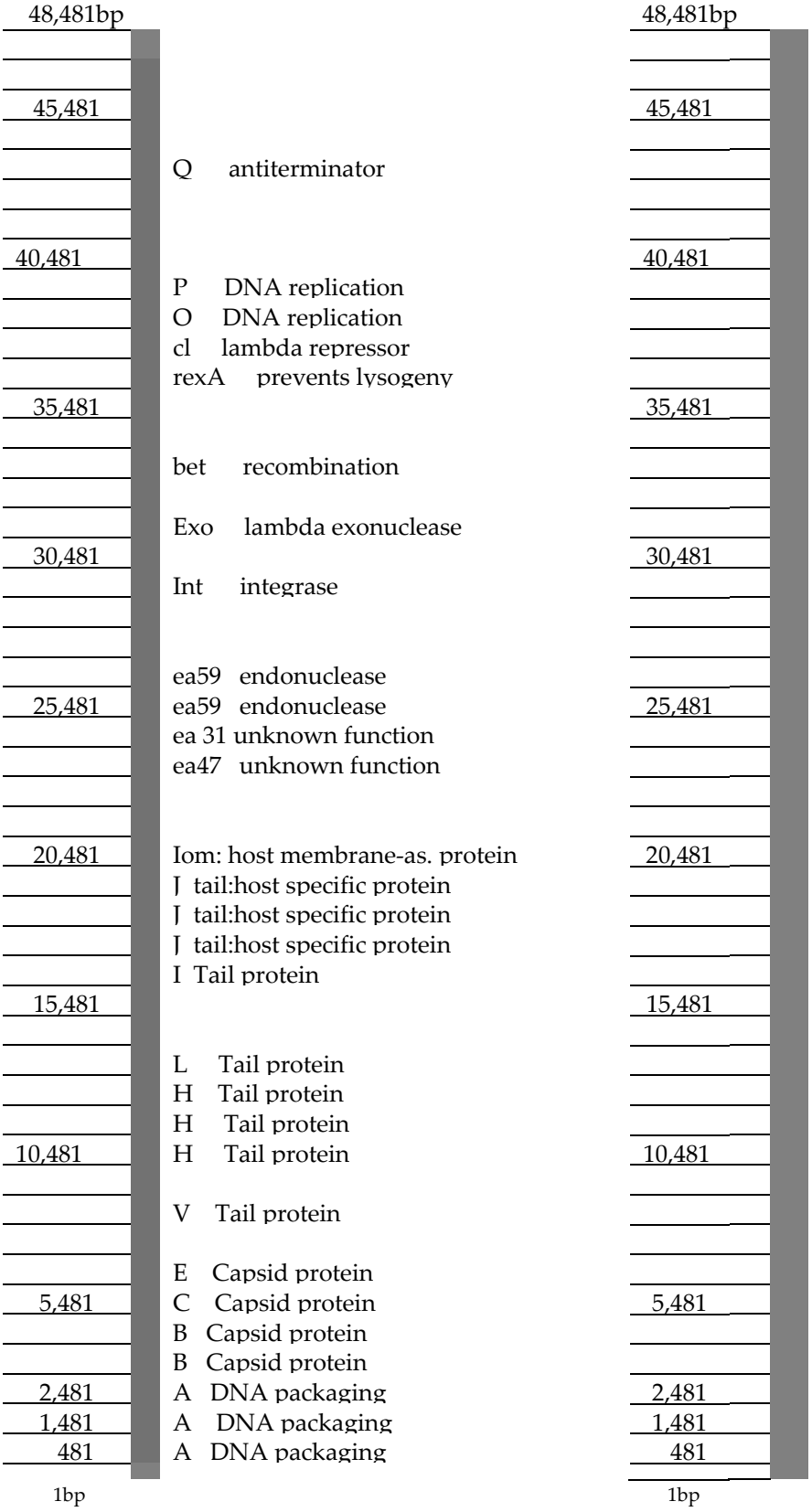
Search for locations by using the find command and type in this sequence of bases. Record site (base number) of all matches found.

3. Use the find command to search within the sequence for the location and number of times that the enzyme in your restriction digest, cleaves the Lambda DNA and mark each on the map.
4. For the find dialog box: Go to the toolbar at the top of the sequence screen, rest the curser on "edit" and click. A menu of choices will become visible. Rest and click the curser on **find** (or type Ctrl+F). A dialog box will open, in the open rectangle type the known sequence of bases that exist where ECOR I cleaves DNA. (**gaattc)** only type the letters, no spaces, no parentheses**.** Double check that it is correctly typed and press 'enter' on the keyboard.
5. It will find that portion of the sequence that matches what you asked for. Determine the base pair number by looking to the left of the row where the match was found to get the line number, but use the last full line of the sequence and multiply by 71. Then add what you count out to where it cuts on the row with the match. Record the location and indicate it on "Map 2. ".

6. Then click "find next" to find the next location that the enzyme cleaves the sequence. When you know that you have covered the entire sequence, mark on the map each expected cut.
7. **Mapping:** On the next page are 2 lambda genome maps. The wide dark vertical line represents the DNA sequence of the Lambda genome. Map 1. **Lambda** on the left is for reference. It numbers the genome sequence starting at the bottom with 1 base-pair (bp), the first line drawn above that is at 481 base-pairs (bp) and the distance between lines after that is 1000 base-pairs. This is there to help you to indicate the approximate base pair location on the neighboring map (#2). The reference map also includes gene information. Map 2 is your map.
8. On map 2, you are to indicate at the base-pair locations where you expect the enzyme ECOR I to cut.
9. After you indicate the cuts, draw a vertical line representing the fragment created between cuts. These are the expected number of fragments the enzyme creates and their size relative to each other. You should see the same number on your gel in the digest lane.
10. You know the size of each fragment as well, since you know the number where each fragment starts and ends. (subtract one from the other) record it next to the vertical line.
11. Once you have finished your digest Map, use it to fill in the expected length column in the results table below. **Start with the largest fragments** that you expect the enzyme to create.
12. You will fill in the millimeters moved and determine the estimate of the size of each when the gel results are visible, if not today then next week. You will be able to count the number of bands (DNA fragments) from each sample, measure how far each moved and estimate their sizes based on the standard curve you will generate. You may not need the entire table, there may be extra rows.

Maps Each line represents 1000 bp except the 481 line (2nd line up from the bottom)

1. Lambda Reference Map **2. Lambda & Enzyme (EcoRI) Digest Map**



Indicate here on Map 2 where the enzyme ECO RI cleaves Lambda's DNA.

Results Table λ = lambda

DNA Standard			Uncut λ DNA				λ DNA Digest with ECO RI			
Band #	mm	Known bp length	**Band #**	mm	**Expected** bp length	**Observed** bp estimate	**Band #**	mm	**Expected** bp length	**Observed** bp estimate
1		23,130	1				1			
2		9,416					2			
3		6,557					3			
4		4,361					4			
5		2,322					5			
6		2,027					6			

Additional comments regarding results:

III. Observed Results (Gel)

1. **Observe your gel:** look at your notebook how may lanes did you load things into? Are there bands of DNA where you would expect ?

2. **Observe the standard**

Observe the lane of the DNA standard, its location should be recorded in your notebook, but it is probably the first one on the left. Your TA loaded it and will be able to verify that.

a. The six bands and their sizes are listed in the results table above. Does your gel have the 6 bands in the standard lane? The one closest to the well is the largest (23,130bp). What is the size of the one closest to the bottom of the gel?

You can make a rough estimate of the sizes of other DNA bands on the gel by comparing them to this lane.

b. Visual estimate :Do your expectations of number and size appear to be met for the bands created by the digest ?

c. You can calculate a size estimate of unknown fragments by creating what is called a standard curve. Use a ruler to measure the distance that each known fragment of DNA moved in the standard lane relative to the well. Record it in millimeters in the second column of the chart below under the heading "mm". Start with the largest band and repeat with the rest.

d. **Nucleic acids migrate in agarose at a rate that is inversely proportional to their size. A linear relationship exists between the logarithm of their size (in Kilobase pairs) and mobility.** Plot this on the included sheet of semi-log graph paper. The x-axis will simply be the distance migrated. The y-axis will be the log of the size of the fragment expressed in kilobase-pair length. **This is your standard curve for this gel - it is unique to your gel.** Create a best fit line through the plotted data points, then use the line or equation to estimate the other unknown sizes on the gel based on how far they moved. It allows you to estimate the base-pair length of all other pieces of DNA on that gel. You will do this and compare your observed gel results with the expected results that you gathered on the computer and mapped previously.

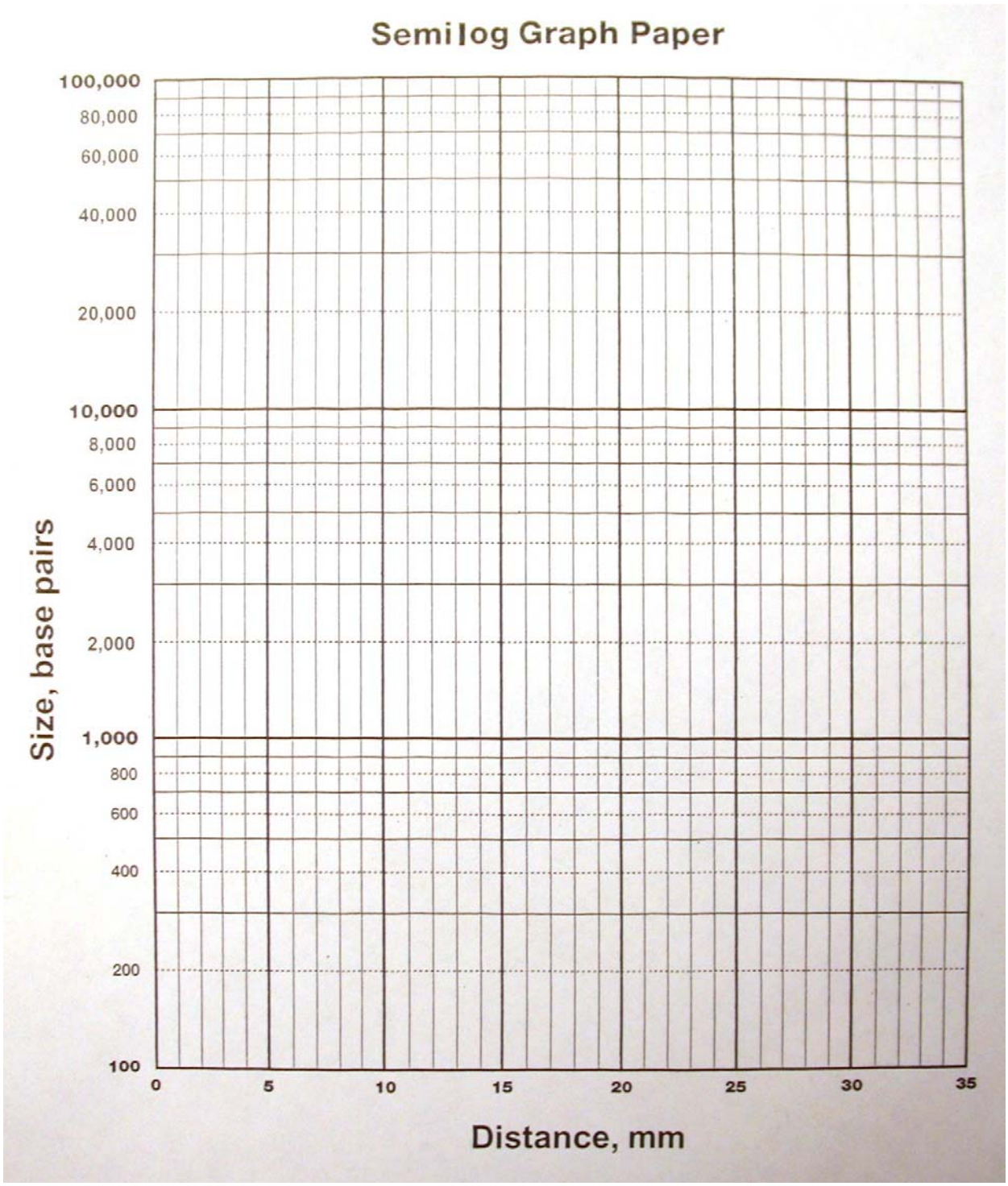
e. **If your gel does not yield useful results a backup gel or photo of one will be available to use in lab.**

In Conclusion, show your TA your gel, maps, result chart and standard curve.

1. How well did your observed results from your gel match up with the expected information? How many lanes have DNA ?

2. Are the number and sizes of bands reflective of what was expected?

3. If not, what explanation do you propose?


Semilog Graph Paper
100,000
80,000
60,000
40,000
20,000
10,000
8,000
6,000
4,000
2,000
1,000
800
600
400
200
100
Size, base pairs
0
5
10
15
20
25
30
35
Distance, mm

B. Genome Sequence Assembly

Determine Order of Fragments

1. Using BLAST you will align two fragments at a time. The output of the program will provide you with the lengths of the fragments and the regions that overlap.

http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi

a. record the length of each fragment

Fragment #	Length
1	
2	
3	
4	
5	

b. indicate whether there is an overlap between fragments (Y or N)

	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5
Fragment 1					
Fragment 2					
Fragment 3					
Fragment 4					
Fragment 5					

c. Record the regions of overlap (which bases) between the fragments.

d. Draw a diagram of how the fragments align with each other.

e. What is the order of the fragments? _____

f. What is the length of the full sequence? _____

>Fragment 1
AAAGCAAAACTTGGCAAGCAAACCTTCGATTGATCTCTAAGTTTGATACTGTTGAAGACTTTT
GGGCTCTATACAACCATATCCAGTTGTCTAGTAATTTAATGCCTGGCTGTGACTACTCACTTTTTAAGGA
CGGGATTGAGCCTATGTGGGAAGATGAGAAAAACAAACGAGGAGGACGGTGGCTGATCACACTGAACAAG
CAGCAGAGACGGAGTGACCTCGATCGCTTCTGGCTAGAGACACTGCTGTGCCTTATTGGAGAATCTTTCG
ATGACTACAGTGATGATGTGTGTGGAGCTGTTGTTAATGTTAGAGCTAAAGGTGATAAGATAGCAATATG
GACTACTGAGTGTGAAAACAGAGATGCAGTCACACACATAGGGAGGGTATACAAGGAAAGGTTAGGACTT
CCTCCGAAGATAGTGATTGGTTATCAGTCCCACGCAGACACAGCTACAAAGAGCGGCTCCACCACTAAAA
ATAGGTTTGTTGTTTAAGAAGACACCTTCTGAGTATTCTCACAGGAGACTGCGTCACGCAATCGAGATTG
GGAGCTGAACCAAAGCCT

>Fragment 2
GGAGGCGGAGGGAGCTGGTCCTTAAGGAAGGCACGCGCTTGCTTCTAGATTCCGAAGCGTTTTCAAAGCT
GGTTACAGTCCTTACCACAGCACACCCTTGTGAGGAGCGGTTGTGCGATCAGATCGATCTAAGATGGCGA
C

>Fragment 3
GGAACCGGAAACCACCCCTACCACTAATCCCCCACCTGCAGAAGAGGAAAAAACAGAGTCTAATCA
GGAGGTTGCTAACCCAGAGCACTATATTAAACACCCTCTACAGAACAGGTGGGCACTCTGGTTTTTTAAA
AATGATAAAAGCAAAACTTGGCAAGCAAACCTTCGATTGATCTCTAAGTTTGATACTGTTGAAGACTTTT
GGGCTCTATACAACCATATCCAGTTGTCTAGTAATTTAATGCCTGGCTGTGACTACTCACTTTTTAAGGA
CGGGAT

>Fragment 4

GAGTGACCTCGATCGCTTCTGGCTAGAGACACTGCTGTGCCTTATTGGAGAATCTTTCG
ATGACTACAGTGATGATGTGTGTGGAGCTGTTGTTAATGTTAGAGCTAAAGGTGATAAGATAGCAATATG
GACTACTGAGTGTGAAAACAGAGATGCAGTCACACACATAGGGAGGGTATACAAGGAAAGGTTAGGACTT
CCTCCGAAGATAGTGATTGGTTATCAGTCCCACGCAGACACAGCTACAAAGAGCGGCTCCACCACTAAAA
ATAGGTTTGTTGTTTAAGAAGACACCTTCTGAGTATTCTCACAGGAGACTGCGTCACGCAATCGAGATTG
GGAGCTGAACCAAAGCCTCATCAAAGCAGAGTGGACTGCACTGAAGTTGATTCCATCCAAGTGTTGCTAA
GATATAAGAGAAGTCTCATTCGCCTTTGTCTTGTACTTCTGTGTTCATTCTCCTCCCCCACCCCCAATTT
TTGCTAGTGTGTCCACTATCCCAATCAAAGAATTACAGTATACGTCACCCCAGAACCCGCAGATGTGTTC
CTGGCCCGCTCTGTAACAGCCGGTTAGAATTACCATGACACACACATTTGCCTTTCCACAGTATTCGAAA

>Fragment 5
GCGGTTGTGCGATCAGATCGATCTAAGATGGCGA
CTGTGGAACCGGAAACCACCCCTACCACTAATCCCCCACCTGCAGAAGAGGAAAAAACAGAGTCTAATCA
GGAGGTTGCTAACCCAGAGCACTATATTAAACACCCTCTACAGAACAGGTGGGCACTCTGGTTTTTTAAA
AATGATAAAAGCAAAACTTGGCAAGCAAACCTTCGATTGATCTCTAAGTTTGATACTGTTGAAGACTTTT
GGGCTCTATACAACCATATCCAGTTGTCTAGTAATTT

Appendix

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Due week of 2/8/10

1) What do the four elements most abundant in life - carbon, oxygen, hydrogen, and nitrogen - have in common?
A) They all have unpaired electrons in their valence shells.
B) Each element exists in only one isotopic form.
C) They are elements produced only by living cells.
D) They all have the same number of valence electrons.
E) They are equal in electronegativity.

2) Atoms whose outer electron shells contain eight electrons tend to
A) be stable and chemically nonreactive, or inert.
B) form ionic bonds in aqueous solutions.
C) be isotopes and very radioactive.
D) form covalent bonds in aqueous solutions.
E) be unstable and chemically very reactive.

3) One of the buffers that contribute to pH stability in human blood is carbonic acid (H_2CO_3). Carbonic acid is a weak acid that when placed in an aqueous solution dissociates into a bicarbonate ion (HCO_3^-) and a hydrogen ion (H^+). Thus,

$$H_2CO_3 \Leftrightarrow HCO_3^- + H^+$$

If the pH of the blood increases, one would expect

A) an increase in the concentration of HCO_3^- and a decrease in the concentration of H_2O.
B) a decrease in the concentration of HCO_3^- and an increase in the concentration of H_2O.
C) an increase in the concentration of H_2CO_3 and a decrease in the concentration of H_2O.
D) a decrease in the concentration of H_2CO_3 and an increase in the concentration of H_2O.
E) a decrease in the concentration of HCO_3^- and an increase in the concentration of both H_2CO_3 and H_2O.

4) Which of the following statements is (are) true about the phosphate ion?
A) It is negatively charged.
B) It has acid properties.
C) It is hydrophobic.
D) Only A and B are true.
E) A, B, and C are true.

5) Which of the following contains nitrogen in addition to carbon, oxygen, and hydrogen? A) a hydrocarbon such as benzene
B) an amino acid such as glycine
C) a monosaccharide such as glucose
D) an alcohol such as ethanol
E) a steroid such as testosterone

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Due week of 2/15/10

6) A molecule with the formula C with subscript((18))H with subscript((36))O with subscript((2)) is probably a
A) hydrocarbon.
B) carbohydrate.
C) lipid.
D) nucleic acid.
E) protein.

7) Upon chemical analysis, a particular protein was found to contain 556 amino acids. How many peptide bonds are present in this protein?
A) 556 B) 554 C) 558 D) 139 E) 555

8) Sucrose is a disaccharide, composed of the monosaccharides glucose and fructose. The hydrolysis of sucrose by the enzyme sucrase results in
A) the release of water from sucrose as the bond between glucose and fructose is broken.
B) utilization of water as a covalent bond is formed between glucose and fructose to form sucrase.
C) breaking the bond between glucose and fructose and forming new bonds from the atoms of water.
D) production of water from the sugar as bonds are broken between the glucose monomers.
E) bringing glucose and fructose together to form sucrose.

9) Consider the following: Succinate dehydrogenase catalyzes the conversion of succinate to fumarate. The reaction is inhibited by malonic acid, which resembles succinate but cannot be acted upon by succinate dehydrogenase. Increasing the ratio of succinate to malonic acid reduces the inhibitory effect of malonic acid. Which of the following is correct?
A) Succinate dehydrogenase is the enzyme, and malonic acid is the substrate.
B) Succinate is the substrate, and fumarate is the product.
C) Succinate dehydrogenase is the enzyme, and fumarate is the substrate.
D) Malonic acid is the product, and fumarate is a competitive inhibitor.
E) Fumarate is the product, and malonic acid is a noncompetitive inhibitor.

10) An enzyme catalyzes a reaction by
A) lowering the energy of activation of a reaction.
B) increasing the amount of free energy of a reaction.
C) lowering the DeltaG of a reaction.
D) supplying the energy to speed up a reaction.
E) changing the equilibrium of a spontaneous reaction.

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Due week of 2/22/10

11) Where does glycolysis takes place?
A) mitochondrial matrix
B) mitochondrial outer membrane
C) cytosol
D) mitochondrial intermembrane space
E) mitochondrial inner membrane

12) In the presence of oxygen, the three-carbon compound pyruvate can be catabolized in the citric acid cycle. First, however, the pyruvate 1) loses a carbon, which is given off as a molecule of CO_2, 2) is oxidized to form a two-carbon compound called acetate, and 3) is bonded to coenzyme A. These three steps result in the formation of
A) acetyl CoA, FAD, H_2, and CO_2
B) acetyl CoA, NADH, H^+, and CO_2
C) acetyl CoA, $FADH_2$, and CO_2
D) acetyl CoA, NAD^+, ATP, and CO_2.
E) acetyl CoA, O_2, and ATP.

13) In the absence of oxygen, yeast cells can obtain energy by fermentation, resulting in the production of
A) ATP, pyruvate, and acetyl CoA.
B) ATP, CO_2, and lactate.
C) ATP, CO_2, and ethanol (ethyl alcohol).
D) ATP, NADH, and pyruvate.
E) ATP, pyruvate, and oxygen.

14) In alcohol fermentation, NAD^+ is regenerated from NADH during the
A) oxidation of pyruvate to acetyl CoA.
B) reduction of acetaldehyde to ethanol (ethyl alcohol).
C) reduction of pyruvate to form lactate.
D) phosphorylation of ADP to form ATP.
E) oxidation of NAD^+ in the citric acid cycle.

15) You have a friend who lost 7 kg (about 15 pounds) of fat on a "low carb" diet. How did the fat leave her body?
A) Chemical energy was converted to heat and then released.
B) It was released as CO with subscript((2)) and H with subscript((2))O.
C) It was converted to ATP, which weighs much less than fat.
D) It was converted to urine and eliminated from the body.
E) It was broken down to amino acids and eliminated from the body.

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Due week of 3/1/10

16) A plant has a unique photosynthetic pigment. The leaves of this plant appear to be blue and purple. What wavelengths of visible light are not being absorbed by this pigment?
A) green and yellow
B) red and yellow
C) green, blue, and violet
D) blue and purple
E) blue, green, and red

17) All of the following are directly associated with photosystem II except
A) release of oxygen.
B) harvesting of light energy by chlorophyll.
C) P680 reaction-center chlorophyll.
D) extraction of hydrogen electrons from the splitting of water.
E) NADP+ reductase.

18) In a plant cell, where are the ATP synthase complexes located?
A) thylakoid membrane
B) plasma membrane
C) inner mitochondrial membrane
D) A and C
E) A, B, and C

19) What are the products of the light reactions that are subsequently used by the Calvin cycle? A) water and carbon
B) electrons and photons
C) carbon dioxide and RuBP
D) oxygen and carbon dioxide
E) ATP and NADPH

20) All of the events listed below occur in the light reactions of photosynthesis except
A) carbon dioxide is incorporated by Rubisco.
B) light is absorbed and funneled to reaction-center chlorophyll a.
C) ADP is phosphorylated to yield ATP.
D) NADP+ is reduced to NADPH.
E) oxygen is produced.

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Due week of 3/8/10

21) The process of transduction usually begins
A) when the signal molecule changes the receptor protein in some way.
B) after the target cell divides.
C) when the hormone is released from the gland into the blood.
D) after the third stage of cell signaling is completed.
E) when the chemical signal is released from the alpha cell.

22) In general, a signal transmitted via phosphorylation of a series of proteins
A) allows target cells to change their shape and therefore their activity.
B) brings a conformational change to each protein.
C) cannot occur in yeasts because they lack protein phosphatases.
D) requires phosphorylase activity.
E) requires binding of a hormone to a cytosol receptor.

23) Which of the following is s widely used second messenger in signal transduction pathways?
A) inositol trisphosphate
B) cyclic AMP
C) calcium ions
D) A and B only
E) A, B, and C

24) Which of the following would be inhibited by a drug that specifically blocks the addition of phosphate groups to proteins?
A) G-protein-linked receptor signaling
B) phosphatase activity
C) ligand-gated ion channel signaling
D) receptor tyrosine kinase activity
E) adenylyl cyclase activity

25) The response of a particular cell to a signal depends on
A) its particular collection of signal receptor proteins.
B) the proteins needed to carry out the response.
C) its relay proteins.
D) A and B only
E) A, B, and C

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Due week of 3/22/10

26) What is a chromatid?
A) another name for the chromosomes found in genetics
B) a special region that holds two centromeres together
C) a chromosome found outside the nucleus
D) a replicated chromosome
E) a chromosome in G1 of the cell cycle

27) What is the name for the special region on a duplicated chromosome that holds the sister chromatids together?
A) kinetochore
B) centromere
C) microtubule organizer region
D) desmosome
E) centrosome

28) All of the following occur during mitosis except the
A) disappearance of the nucleolus.
B) uncoupling of chromatids at the centromere.
C) synthesis of DNA.
D) condensing of chromosomes.
E) formation of a spindle.

29) Cytokinesis usually, but not always, follows mitosis. If a cell completed mitosis but not cytokinesis, the result would be a cell with
A) high concentrations of actin and myosin.
B) two abnormally small nuclei.
C) two nuclei.
D) a single large nucleus.
E) two nuclei but with half the amount of DNA.

30) Which of the following organisms does not reproduce cells by mitosis and cytokinesis?
A) cow
B) mushroom
C) bacterium
D) banana tree
E) cockroach

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Due week of 3/29/10

31) What is a genome?
A) an ordered display of chromosomes arranged from largest to smallest
B) the complete complement of an organism's genes
C) a specialized polymer of four different kinds of monomers
D) a specific segment of DNA that is found within a prokaryotic chromosome
E) a specific sequence of polypeptides within each cell

32) How do the two members of a pair of homologous chromosomes differ from each other?
A) their length
B) the identity and relative position of the genes present on each of the chromosomes
C) their staining patterns
D) the precise sequence of the DNA within each of the chromosomes
E) the position of the centromere within each of the chromosomes

33) Which of the following terms belongs with the words synapsis, tetrads, and chiasmata?
A) crossing over
B) autosomes
C) prophase II
D) fertilization
E) haploid

34) Independent assortment of chromosomes is a result of
A) the relatively small degree of homology shared by the X and Y chromosomes.
B) the random nature of the fertilization of ova by sperm.
C) the random distribution of the sister chromatids to the two daughter cells during anaphase II.
D) the random and independent way in which each pair of homologous chromosomes lines up at the metaphase plate during meiosis I.
E) all of the above

35) Which of the following is the term for a human cell that contains 22 pairs of autosomes and two X chromosomes?
A) a sperm cell
B) a female somatic cell
C) a male somatic cell
D) an unfertilized egg cell
E) both A and D

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Due week of 4/5/10

36) What is the difference between a monohybrid cross and a dihybrid cross?
A) A monohybrid cross produces a single progeny, whereas a dihybrid cross produces two progeny.
B) A monohybrid cross results in a 9:3:3:1 ratio whereas a dihybrid cross gives a 3:1 ratio.
C) A monohybrid cross involves a single parent, whereas a dihybrid cross involves two parents.
D) A monohybrid cross is performed only once, whereas a dihybrid cross is performed twice.
E) A monohybrid cross involves organisms that are heterozygous for a single character, whereas a dihybrid cross involves organisms that are heterozygous for two characters.

37) What is genetic cross between an individual showing a dominant phenotype (but of unknown genotype) and a homozygous recessive individual called?
A) a dihybrid cross
B) a self-cross
C) a testcross
D) a hybrid cross
E) an F1 cross

38) A 9:3:3:1 phenotypic ratio is characteristic of which of the following?
A) a dihybrid cross
B) a trihybrid cross
C) a monohybrid cross
D) linked genes
E) both A and D

39) P = purple, pp = white. The offspring of a cross between two heterozygous purple-flowering plants (Pp multiply Pp) results in
A) all white-flowered plants.
B) all purple-flowered plants.
C) all pink-flowered plants.
D) purple-flowered plants and white-flowered plants.
E) two types of white-flowered plants: PP and Pp.

40) Hydrangea plants of the same genotype are planted in a large flower garden. Some of the plants produce blue flowers and others pink flowers. This can be best explained by
A) the allele for blue hydrangea being completely dominant.
B) the alleles being codominant.
C) environmental factors such as soil pH.
D) the fact that a mutation has occurred.
E) acknowledging that multiple alleles are involved.

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Due week of 4/12/10

41) In trying to determine whether DNA or protein is the genetic material, Hershey and Chase made use of which of the following facts?
A) DNA contains phosphorus, but protein does not.
B) DNA does not contain sulfur, whereas protein does.
C) DNA contains nitrogen, whereas protein does not.
D) A and B only
E) A, B, and C

42) What kind of chemical bond is found between paired bases of the DNA double helix?
A) sulfhydryl
B) ionic
C) hydrogen
D) covalent
E) phosphate

43) A new DNA strand elongates only in the 5' to 3' direction because
A) Okazaki fragments prevent elongation in the 3' to 5' direction.
B) DNA polymerase can only add nucleotides to the free 3' end.
C) DNA polymerase begins adding nucleotides at the 5' end of the template.
D) the polarity of the DNA molecule prevents addition of nucleotides at the 3' end.
E) replication must progress toward the replication fork.

44) Which of these mechanisms ensures that the DNA sequence in the genome remains accurate?
A) complementary base pairing during DNA replication
B) mismatch repair
C) proofreading during DNA replication
D) excision repair
E) all of the above

45) A eukaryotic cell lacking telomerase would
A) be highly sensitive to sunlight.
B) undergo a reduction in chromosome length.
C) produce Okazaki fragments.
D) have a high probability of becoming cancerous.
E) be unable to repair thymine dimers.

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Due week of 4/19/10

46) Which of the following statements best describes the termination of transcription in prokaryotes?
A) RNA polymerase transcribes through an intron, and the snRNPs cause the polymerase to let go of the transcript.
B) RNA polymerase transcribes through a stop codon, causing the polymerase to stop advancing through the gene and release the mRNA.
C) RNA polymerase transcribes through the polyadenylation signal, causing proteins to associate with the transcript and cut it free from the polymerase.
D) RNA polymerase transcribes through the terminator sequence, causing the polymerase to fall off the DNA and release the transcript.
E) Once transcription has initiated, RNA polymerase transcribes until it reaches the end of the chromosome.

47) What is a ribozyme?
A) An enzyme that synthesizes RNA as part of the transcription process
B) An enzyme made up of RNA
C) An enzyme that uses RNA as a substrate
D) An enzyme that catalyzes the association between the large and small ribosomal subunits
E) An enzyme that synthesizes RNA primers during DNA replication

48) All of the following are directly involved in translation except
A) tRNA.
B) DNA.
C) ribosomes.
D) aminoacyl-tRNA synthetase enzymes.
E) mRNA.

49) What is an anticodon part of?
A) mRNA
B) tRNA
C) DNA
D) a ribosome
E) an activating enzyme

50) Sickle-cell disease is probably the result of which kind of mutation?
A) nonsense
B) point
C) nondisjunction
D) frameshift
E) both B and D

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Due week of 4/26/10

51) If you discovered a bacterial cell that contained no restriction enzymes, which of the following would you expect to happen?
A) The cell would be unable to replicate its DNA.
B) The cell would be easily infected and lysed by bacteriophages.
C) The cell would become an obligate parasite.
D) The cell would create incomplete plasmids.
E) Both A and D would occur.

52) Assume that you are trying to insert a gene into a plasmid. Someone gives you a preparation of genomic DNA that has been cut with restriction enzyme X. The gene you wish to insert has sites on both ends for cutting by restriction enzyme Y. You have a plasmid with a single site for Y, but not for X. Your strategy should be to
A) cut the DNA again with restriction enzyme Y and insert these fragments into the plasmid cut with the same enzyme.
B) insert the fragments cut with X directly into the plasmid without cutting the plasmid.
C) cut the plasmid with enzyme X and then insert the gene into the plasmid.
D) cut the plasmid with restriction enzyme X and insert the fragments cut with Y into the plasmid.
E) cut the plasmid twice with restriction enzyme Y and ligate the two fragments onto the ends of the DNA fragments cut with restriction enzyme X.

53) What is the enzymatic function of restriction enzymes?
A) to add new nucleotides to the growing strand of DNA
B) to cleave nucleic acids at specific sites
C) to repair breaks in sugar-phosphate backbones
D) to join nucleotides during replication
E) to join nucleotides during transcription

54) How does a bacterial cell protect its own DNA from restriction enzymes?
A) by adding methyl groups to adenines and cytosines
B) using DNA ligase to seal the bacterial DNA into a closed circle
C) adding histones to protect the double-stranded DNA
D) by reinforcing the bacterial DNA structure with covalent phosphodiester bonds
E) by forming "sticky ends" of bacterial DNA to prevent the enzyme from attaching

55) What is a cloning vector?
A) an agent, such as a plasmid, used to transfer DNA from an in vitro solution into a living cell
B) the sticky end of a DNA fragment
C) a DNA probe used to locate a particular gene in the genome
D) an enzyme that cuts DNA into restriction fragments
E) the laboratory apparatus used to clone genes

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Due week of 5/3/10

56) Which of the following modifications is least likely to alter the rate at which a DNA fragment moves through a gel during electrophoresis?
A) methylating the cytosine bases within the DNA fragment
B) decreasing the length of the DNA fragment
C) neutralizing the negative charges within the DNA fragment
D) increasing the length of the DNA fragment
E) altering the nucleotide sequence of the DNA fragment

57) The polymerase chain reaction (PCR) has been used to amplify DNA from which of the following?
A) fossils
B) viruses
C) bacteria
D) fetal cells
E) all of the above

58) DNA microarrays have made a huge impact on genomic studies because they
A) can be used to introduce entire genomes into bacterial cells.
B) allow physical maps of the genome to be assembled in a very short time.
C) dramatically enhance the efficiency of restriction enzymes.
D) can be used to eliminate the function of any gene in the genome.
E) allow the expression of many or even all of the genes in the genome to be compared at once.

59) Plasmids are important in biotechnology because they are
A) surfaces for respiratory processes in bacteria.
B) proviruses incorporated into the host DNA.
C) recognition sites on recombinant DNA strands.
D) surfaces for protein synthesis in eukaryotic recombinants.
E) a vehicle for the insertion of foreign genes into bacteria.

60) Which of the following best describes the complete sequence of steps occurring during every cycle of PCR?
1. The primers hybridize to the target DNA.
2. The mixture is heated to a high temperature to denature the double stranded target DNA.
3. Fresh DNA polymerase is added.
4. DNA polymerase extends the primers to make a copy of the target DNA.

A) 3, 4, 1, 2 B) 3, 4, 2 C) 2, 1, 4 D) 1, 3, 2, 4 E) 2, 3, 4

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THE MICROPIPETTOR

Learning Goal: To familiarize the student with correct use of a micropipettor.
Introduction: The micropipettor is a tool used in the lab to measure and transfer small volumes of liquid. There are many different brands of micropipettors, the most common being the Pippetman produced by the company Gilson. Though different models exist, see **Table** 1, the basic components of micropipettor are the same and are depicted in **Figure 1** below. This is meant to give you a general model of how to use a micropipettor, keep in mind that each brand may vary a bit, so be prepared to look at each that you encounter and ask yourself how it works. This foundation should help you to develop that ability.

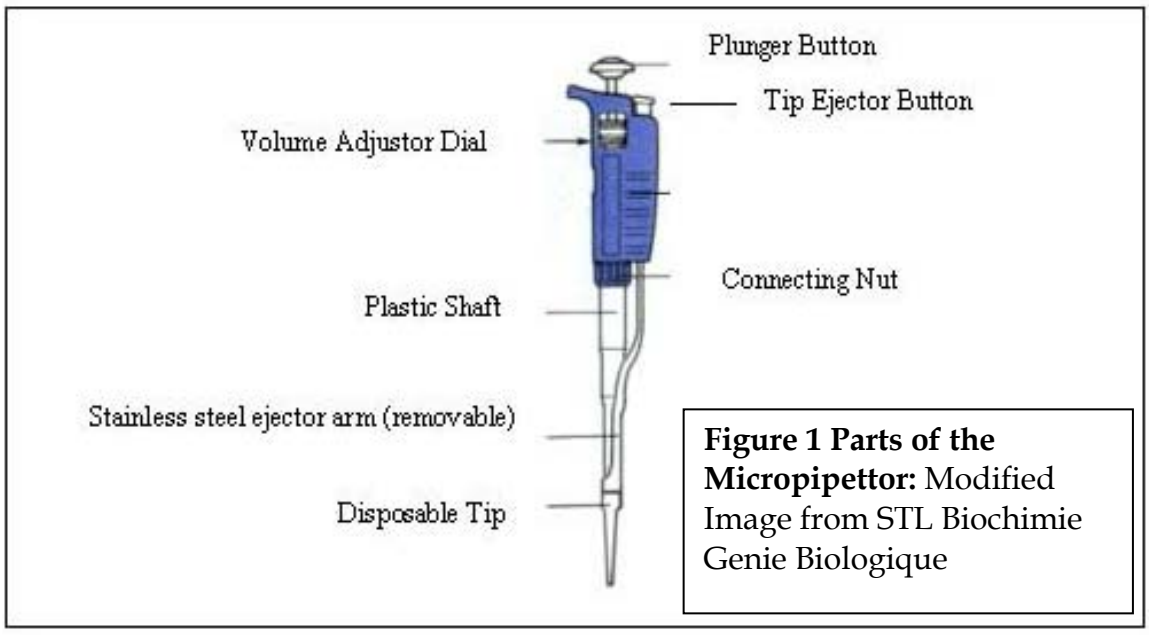


Figure 1 Parts of the Micropipettor: Modified Image from STL Biochimie Genie Biologique

	Model of Micropipettor	**Recommended Range in µL**
	P-2	0.1 to 2
	P-10	0.5 to 10
*	P-20	2 to 20
	P-100	10 to 100
*	P-200	20 to 200
*	P-1000	200 to 1000
	P-5000	500 to 5000
*	L-20	2 to 20
*	L-200	20 to 200
*	L-1000	100 to 1000
	Eppendorf - 20	1 to 20
*	Eppendorf - 200	20 to 200
*	Eppendorf - 1000	100 to 1000

Table 1 Overview of Different Available Micropipettor Models: An asterisk indicates the model is used in the laboratory. Many more models are available than shown.

Figure 2 Top images of the P20, P200, and P1000: Micropipettors usually contain a label at the top of the device indicating the model. Modified image from University of Michigan-Dearborn.

Prior to using a micropipettor it is crucial to know what model you are using, how to set the volume, how to read the volume, and how to attach a tip. The type of micropipettor is represented on the micropipettor as shown in **Figure 2**.

HOW TO READ THE VOLUME:

Figure 3 Volume Indicator Dials: The volume indicators are shown for a p20, p200, and p1000. Modified image from University of Michigan-Dearborn.

In Figure 3 above, the p20, which has a range of 2 to 20 µL is set to measure 6.86 µL. The dial contains 3 slots for numbers, with the bottom slot in red. The first slot, which is set to zero in **Figure 3**, sets the ten's place. The second slot, which is set to 6 in **Figure 3**, sets the one's place. The red slot, indicates the tenth's (whole number) and hundredth's place (notches). Each red notch measures 0.02 µL.

In **Figure 3** above, the p200, which has a range of 20 to 200 µL is set to measure 132.4 µL. The dial contains 3 slots for numbers. The first slot, which is set to 1 in **Figure 3**, sets the hundred's place. The second slot, which is set to 3 in **Figure 3**, sets the ten's place. The third slot, sets the one's (whole numbers) and tenth's place (notches). Each notch measures 0.2 µL.

In **Figure 3** above, the p1000, which has a range of 200 to 1000 µL is set to measure 262 µL. The dial contains 3 slots for numbers, with the top slot in red. The red slot, which is set to zero in **Figure 3**, sets the thousand's place. This slot should never be set to a number other than 0 or 1! The second slot, which is set to 2 in Figure 3, sets the hundred's place. The third slot sets the ten's place (whole number) and the one's place (notches). Each notch measures 2 µL.

HOW TO ATTACH A TIP PROPERLY:

i. Find the correct tips. Different models of the micropipettor require different sizes and/or types of tips. If you use the wrong size tip you may not measure the volume accurately.
ii. Leave the tips in their tip boxes.
iii. Firmly attach the tip to the shaft as depicted in **Figure 4a**.


Figure 4a

Figure 4a. Attaching and removing tips: Above, the proper method for attaching a tip from a tip box. Right, the proper method for using the tip ejector button. Images acquired from University of Michigan-Dearborn.

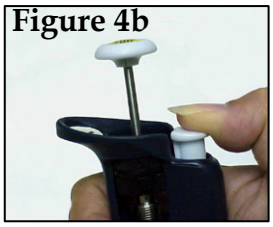


Figure 4b

Figure 4b. Attaching and removing tips: To remove a tip, use the tip ejector button, as shown in **Figure 4b**. Always use fresh clean tips for each sample in order to prevent contamination.

When using a micropipettor, there are a few guiding principles to keep in mind:

- Be consistent with speed and smoothness when using the plunger
- Hold consistent pressure on the plunger at the first stop
- Maintain vertical positioning of the micropipettor
- Avoid air bubbles
- Change tips in order to prevent contamination.
- **NEVER** drop a micropipettor.
- **NEVER** rotate the volume adjuster either below or above the range of the instrument.
- **NEVER** lay a filled micropipettor on its side. (This will contaminate the shaft.)
- **NEVER** immerse the barrel of a micropipettor in a liquid above the tip.
- **NEVER** allow the plunger to snap up when liquid is being drawn into the tip.

Procedure

Step 1. Push down plunger to first stop. Use your thumb!!

Step 2. Insert pipette tip into solution. (Make sure the tip is fully submerged in the solution.)

Step 3. Slowly release the plunger with your thumb in a controlled fashion. As you do this, you will see the solution rise up the pipette tip.

Step 4. Remove the pipette tip from the solution. (Make sure you do this before step 5!)

Step 5. Dispense the fluid inside the pipette tip by pushing down on the plunger all the way (to the second stop).

Figure 5. Using the Plunger: Left, the plunger is not depressed. Middle, the plunger is at the first stop. Right, the plunger is at the second stop.

References:

University of Michigan-Dearborn:
http://www.umd.umich.edu/casl/natsci/slc/slconline/MICRPIP/index.html

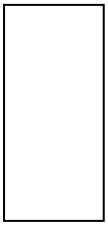
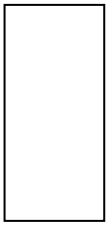
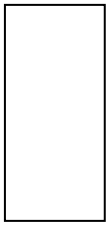
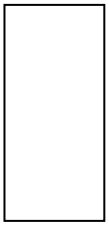
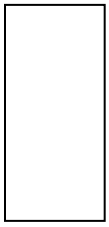
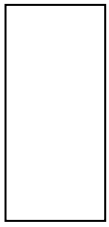
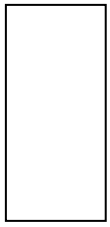
STL Biochimie Genie Biologique:
http://stlbgb.apinc.org/spip.php?article8

Practice PROBLEMS

1. For each of the diagrams of a volume indicator below, indicate the volume the device is set for.

p20	p200	p1000	p20	p200	p1000	p1000
1 2 0	0 4 3	1 0 0	2 0 0	2 0 0	0 2 0	0 5 8
_____	_____	_____	_____	_____	_____	_____

2. Draw a diagram of a volume indicator, including the notches, for each of the following volumes and micropipettors.

p20	p200	p1000	p20	p200	p1000	p1000
						
12.4 µL	182.6 µL	330 µL	5.7 µL	24.5 µL	839 µL	200 µL

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How to Use a Microscope

Part I: The Microscope

Learning Goals: After completing this laboratory exercise you will be able to:

1. Name and give the function of the parts of a light microscope.
2. Explain magnification, depth of field, and resolution in terms of microscopy.
3. Calculate the diameter of field and magnification of each lens setting.
4. Prepare biological material for analysis using microscopy.
5. Correctly use a microscope in the analysis of biological material.

Introduction: If a biologist has a universal tool, it is certainly the light microscope. Most biologists use microscopes in their work as a means of investigating small objects. A certain amount of skill with a microscope acquired at an early stage in one's biological training will be of use in this course and in further biology courses. Always treat the microscope with great care. Make certain that you do not touch any part of the lens system with anything abrasive (such as a slide or dirty water) or greasy (such as even the cleanest fingers). Never clean a lens with anything except clean lens paper! If the view gets foggy (as it probably will sometime during the semester), and lens paper will not clean it, call your laboratory instructor.

I. Structure of the Compound Microscope

It is very important that you familiarize yourself with the parts of the microscope and their function. Your first task is to locate all of the parts named in the diagram on the next page. Place the microscope so that it is at right angles to you. In addition to the stand (arm & base) and a movable stage by which the object can be positioned and focused for viewing, the microscope consists of the following two sub-units:

1. Object illuminating system: light, diaphragm and condenser.
2. Lens system (magnifies the object): ocular, body tube and objective lens

A. The System of Illumination.

Keep the microscope in the same position: plug it in, turn on the light, move the diaphragm lever as far to the left as possible.

1. Place a clean slide on the stage over the condenser and put a piece of white paper about 25 mm square on top of the slide.
2. Now slide the condenser knob and move the condenser up and down while observing the light on the piece of paper (do not look through the microscope but continue to look at the paper with your naked eye).

3. Note that you see a fairly intense small circle of light when the condenser is at its uppermost position and that this circle gets larger and more diffuse as one lowers the condenser. For most work with the l0X and 40X objectives it is best to have the condenser near the top of its travel.

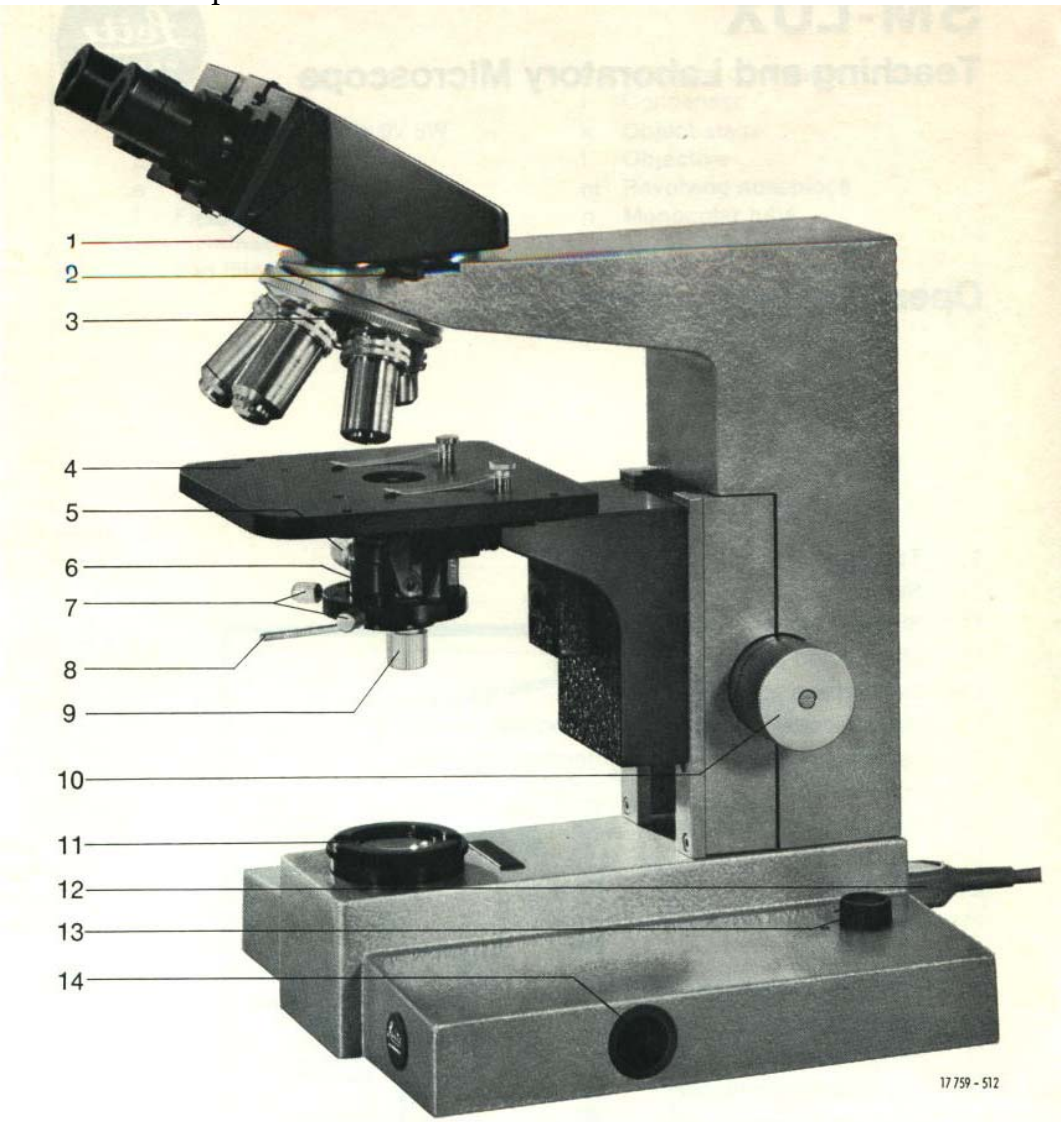

Fig. 1:
SM-LUX with binocular tube S and object stage No. 24a, with permanently mounted condenser

1 Binocular tube S
2 Tube changing lever
3 Revolving nosepiece with objectives
4 Object stage No. 24a with permanently mounted condenser
5 Lever for swing-out lens of condenser
6 Condenser
7 Centring screws
8 Aperture-stop lever
9 Control knob for vertical adjustment of condenser
10 Single control knob for coarse and fine focusing
11 Protective glass with field diaphragm
12 Mains cable
13 Control knob for transformer
14 Lamp socket

Put your eye at table level and look up at the bottom of the condenser. Now move the diaphragm lever and observe what happens. This is an iris diaphragm. Why do you suppose it is called this?

Look at the piece of paper again while opening and closing the diaphragm. The **diaphragm** serves to regulate the amount of light passing through the condenser. It also serves to cut down stray light. Later when you look through the microscope you will see that the diaphragm can be kept partly closed without cutting down on the light passing through the lens (i.e., only light beyond the field of the lens is being blocked). Further closing of the diaphragm will cause less light to enter the lens and decrease the resolving power of the lens while increasing contrast in the viewed object. **Resolving power** is how well specimen detail is preserved. **Contrast** is the ability to see particular detail against its background. Control of the light entering the microscope is very important.

B. The Lens System. Light passes through the condenser, through the object which is placed on the slide and into the lens system. The lens system consists of: (l) an objective lens - the revolving nosepiece of your microscope has at least two of these, (2) a body tube - in your microscope the body tube has prisms in it to allow the tube to be inclined and (3) the ocular lens. Basically, the objective lens magnifies the object and forms an image in the tube which is further magnified by the ocular lens. (If you are skeptical about this, ask your instructor to demonstrate the image in the tube.) The objective lens is the most important (and most expensive) part of the microscope and the quality of a microscope is largely a question of the quality of its objective lens. The ones in your microscope are very good indeed and deserve care. The l0X objective (low power) has a working distance (the distance from lens to object when the object is in focus) of about 4 mm. The 40X objective (high power) has a working distance of about l mm.

While still looking at the microscope from the side, move the stage down well clear of the objective lenses by turning the coarse adjustment knob. Now rotate the nosepiece and notice that each lens clicks into the proper position. Move the l0X objective into position. Next move the stage up until the lens is about 4 mm from the slide. Notice while doing so that the knob you are turning is both a coarse and fine adjustment (most microscopes have separate knobs for these) and that extreme movement of the knob moves the stage rapidly, but immediately after you reverse the direction of movement, the stage moves almost imperceptibly for a short distance. This fine adjustment allows precise focusing.

II. Principles of Microscopy

A. Magnification is roughly the product of the magnification of each lens (objective x ocular). With l0X objective and l0X ocular the magnification should be l00X. A microscope with a straight body tube is designed to project the image to the level of the bench surface. Your microscope, since it has an inclined tube, projects the image at

an angle to the bench. The magnification refers to the increase in size of this apparent image over the object on the slide. Put the microscope on low power (l0X objective).

B. Depth of Field is very limited when using a compound microscope. It is necessary to continually focus up and down to get an impression of depth. Make a slide using colored threads which cross over each other, i.e., put a red thread on the slide and a blue cross over it to form an X. Add water and a cover slip and observe. Notice that you can focus clearly on only one of the threads at the point where they cross and must focus up or down to get the other clearly in view. This is especially noticeable under high power. To get an impression of depth with the microscope, one continually focuses up and down and takes optical sections through the object.

C. Resolution refers to the ability to discriminate between two objects which are very close together. Draw two heavy lines on a piece of paper less than l mm apart. Put the paper on the wall on the other side of the room. If the paper is far enough away, you will see the two lines as one, but as you walk toward the paper you will be able at some distance to resolve those lines as two distinct lines. At its best, the naked eye can resolve lines which are about l00 microns apart (very close to the eye), but has difficulties with objects closer together than that. What limits the resolving power of your eye? What limits the resolution of a camera?

Resolution in a microscope can be limited by many things. The most important as far as the quality of the microscope is concerned is the quality of the objective lens. It is fairly easy to make a condenser and light system of good quality and an ocular lens of good quality, but good objective lenses, properly corrected, are difficult to make. Magnification is of secondary importance in a microscope. Your microscope will magnify a well resolved object 400X, a Mickey Mouse microscope will magnify a blur 400X.

The ultimate limit on the resolving power of the light microscope is the wavelength of light itself. The shorter the wave length, the better the resolution. A good light microscope will resolve objects about l/2 micrometer (micron) apart.
Now turn the microscope around so that you can look through the ocular. We are now ready to look at something.

III. Preparing a Slide and Making Observations.

Your lab instructor will show you how to make a slide. The great art here is to avoid air bubbles when you lower the coverslip! Make a water mount of a piece of paper with a small letter "e" on it. Put the slide on the stage, check to be sure that the "e" is in the circle of light coming through the condenser and that the l0X objective is in place and about 4 mm from the surface of the slide. Look through the ocular and by moving the focusing knob get the paper in focus (having the objective slightly closer than 4 mm and focusing upward by moving stage downward until the image appears is

the best way to do this). When the paper is in focus, move the slide back and forth and up and down until the "e" comes into view. Is the image of the letter oriented in the same way as the actual letter on the slide? When you move the slide to the left does the image move to the left? As you move the slide about it is necessary to constantly keep adjusting the focus. A good microscopist will always keep his or her hand on the focus adjustment and continue to make the fine adjustments in focus as he or she looks at an object.

Assuming that the microscope is in focus with the l0X objective, switch to the 40X objective by rotating the nosepiece. It is a good idea to look at the nosepiece as you rotate it to be sure that the high power objective does not strike the coverslip. The lens should come into position about l mm above the coverslip and be nearly in focus when you look through the ocular. Lenses which come into focus in this manner are referred to as being parfocal. Continued refocusing with the fine adjustment is more important here than with the low power lens. This is because the depth of field is less. When you switched from low to high power, did the object get brighter or dimmer? Why? You may have to readjust the light (open or close the diaphragm). Note that though the object is larger, the field of vision is smaller. Under high power (40X) the diameter of the field of vision is 0.4 mm, under low power (l0X) the diameter is l.35 mm.

Points to Remember: The following are worth remembering as you use the microscope.

1. Be sure that you are using the condenser and diaphragm correctly.
2. Do all preliminary focusing under low power.
3. Do not focus downward when first getting the object in focus (i.e. beware of smashing the slide and lens together).
4. Try to use the microscope with both eyes open - it will seem hard at first, but is easier in the long run.
5. Use the fine adjustment constantly to keep things in focus.
6. Use lens paper to clean the lenses occasionally, you will find that the microscope works best when clean.

IV. Preparation and Analysis of Biological Samples.

Peel a piece of onion (*Allium cepa*) epidermis from an inner leaf (your instructor will show you how) and make a wet mount of it as you did with the letter "e". After careful inspection of several cells, make a drawing of a "typical" cell indicating the cell wall, cytoplasm, nucleus and vacuole. Your instructor will show you how the drawing should be made and labeled. Assume that a typical onion cell is 10 micrometers in height. Measure the height of the cell in your drawing and calculate the "magnification" -- how much did you magnify the cell when you drew it? (There are 1000 micrometers in one millimeter.)

Ideally the light should be fairly bright, but you will find it difficult to distinguish cell parts. Cutting down on the light will make more visible. Cutting down on the light increases the contrast, but reduces the resolution. You will find that you will often have to sacrifice resolution to gain contrast in order to see the various parts of something which looks to be various shades of gray. There are two solutions to this problem:

l) to use a special microscope called a phase contrast microscope or

2) to stain the object and gain contrast by differential uptake of stain by different parts of the object.

V. The Dissecting Microscope

If time permits, and you did not learn this in an earlier lab, learn to use the dissecting microscope. Your instructor will give you pointers here. A coin or dollar bill are good objects to practice on. Note:

l. That the magnification is much less than with the compound microscope.

2. That the working distance is much greater.

3. That the depth of field is much greater and one has a stereoscopic view of the object.

4. That the image is not inverted.

5. That one does not necessarily have to use transmitted light for viewing.

Adjustment of the binocular microscope ocular

If you have normal binocular vision, you will want to adjust your binocular dissecting microscope so that you see in three dimensions:

1. Move the two oculars outward or inwards until they are in the correct position for your eyes. People vary in the width of the face, and your eyes may be farther apart, or closer together, then the previous user of your microscope.

2. Note that the left ocular has a knurled ring which can be turned to change focus, while the right ocular is fixed in focus. Put something on the stage of the microscope which you can focus on, close your left eye, and focus on the object, using only your right eye. Use the main focusing knob on the arm of the microscope to achieve a sharp focus.

3. Now close your right eye, and use the knurled ring on the left ocular to achieve the same sharp focus for your left eye.

4. Now both eyes should be in focus for any object, and you need only to use the main focusing knob to focus on new objects. Since people vary in the adjustments they make, you may have to repeat this entire procedure every time you use a dissecting microscope which has been used by others.

Organizing and Displaying Data: Tables and Graphs

**Give as assignment to students who are struggling as "extra"

Tables and graphs are frequently used in scientific writing, to organize data and to visually display relationships between two or more variables. In this exercise, you will consider some of the principles involved, and practice graphing using some supplied data sets. Use this information when writing your lab reports.

Tables

For every table

- There must be a clear overall title.
- Columns or rows must be clearly titled.
- It must be clear what the numbers in the table represent. (Are they counts or measurements? What are the units?)

Graphs

For every graph

- There must be a clear overall title.
- Each axis must be clearly labeled, and scaled in such a way as to accurately display any relationship between variables. For a linear-scaled axis, each increment of distance along the axis must represent an equal increment of change in the represented variable.
- It must be clear what the data points shown in the graph represent, including units of measurement.

Use the type of graph that is appropriate for the data. The two most basic types are the line graph and the bar graph.

In the first data set below (Table 1; Figure 1), ocean water temperature is what is referred to as the dependent variable—that is, the water temperature *depends* upon the time of year, the independent variable, and not vice versa. By convention, the dependent variable is displayed on the y axis, and the independent variable on the x axis.

Line graphs are appropriate for data sets in which the independent variable is scalar—its values can be represented by points on a scale. Time (in this case,

month of the year) is one example of a scalar independent variable. Other frequently encountered examples are temperature and concentration.

Temperature of ocean water at 1m depth, Savin Hill Cove, Dorchester Bay (2002)

Month	Temperature, Monthly Average (Degrees Celsius)
Jan	36
Feb	35
Mar	39
Apr	47
May	56
Jun	65
Jul	70
Aug	71
Sep	66
Oct	58
Nov	49
Dec	39

Table 1.

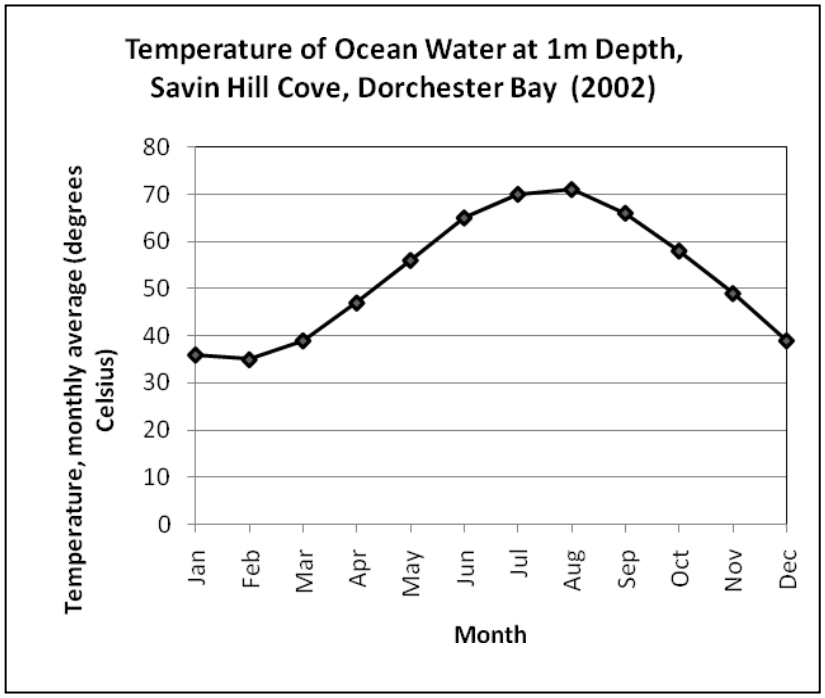

Figure 1.

Bar graphs are used when the independent variable is *qualitative* (categories, as opposed to time points, for example), as shown below. Here, the independent variable is location.

Pollutant X in Ocean Water Samples

Location	Pollutant X Concentration (ppm)
Ogunquit, ME	10
Boston Harbor	150
Hyannis, MA	15
Block Island, RI	19
New London, CT	230

Table 2.

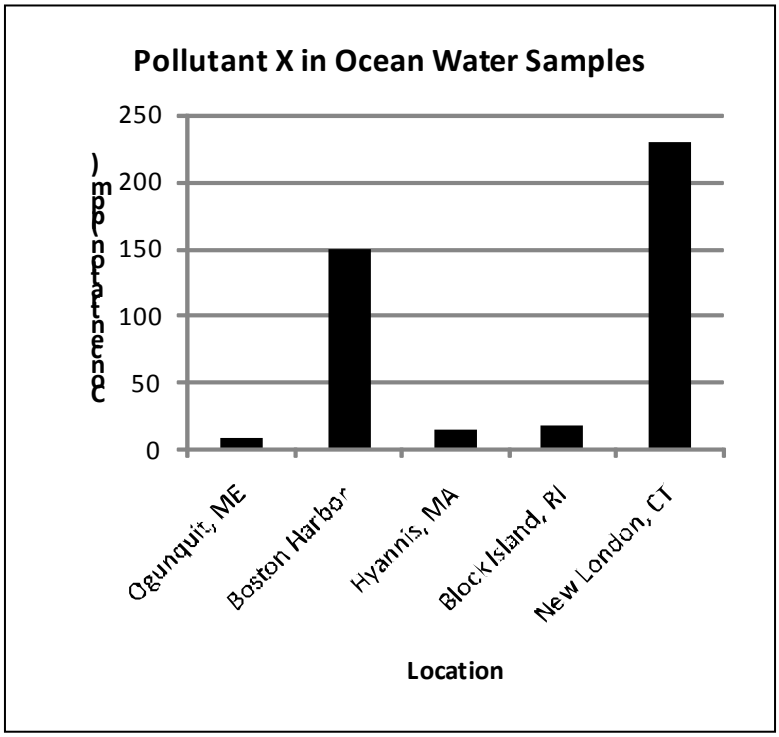

Figure 2.

Your instructor will provide some practice data sets for you to graph. Graph them using the appropriate style of graph, and keep in mind the conventions and guidelines as explained above. It is usually best to master the principles of graphing by hand first, before learning to use computer software to produce graphs.

1. Produce one line graph and one bar graph by hand, using the graph paper and rulers provided, to be checked by your TA before leaving. Consult with your TA as needed. You may also find the link below useful for information on graphing basics:

   http://www.ncsu.edu/labwrite/res/res-homepage.htm

Figure 2.

2. If time permits, see the tutorial at the same link on using the program Excel to produce simple graphs. Your instructor may ask you to try to reproduce one or more of your hand-done graphs using this software program.

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How to Write a Laboratory Report.

Objectives

1. Write a laboratory report in a scientific manner.
2. Rewrite a laboratory report in a scientific manner.

Introduction :

Being able to write a coherent account of an event is important for people working in any field. Biology lab reports should be considered as much an exercise in this art as are English themes or history term papers. While great eloquence in style is not crucial, clarity and conciseness are.

A lab report indicates several things: how well you can carry out an experiment, how well you understood what you did in lab, how methodically and logically you can present your results and conclusions, and whether you have thought carefully about your results and consulted references which will help you to interpret them.

Aim for clarity and thoroughness as well as good grammar, correct spelling, and proper sentence structure when you write lab reports.

Format of a Lab Report:

Introduction: This statement should indicate clearly what ideas are being investigated and state specifically what the purpose of your experiment is. If you do several experiments in one lab, the purpose of each should be given. Do not merely copy what is in the lab manual: present the purpose in your own words. For many experimental labs, you should frame a hypothesis that you intend to test.

Materials and Methods: Ask your lab instructor whether you should write out the entire methods and materials section or whether you should merely state "as in lab manual". If written out, the materials you used and the method followed should be written clearly and with enough detail so that another person could perform the experiment based on what you have written. Don't just copy the lab manual methods section: use your own words and perhaps improve on the way the manual explains techniques! No results should be included here.

Results: Here you should include all tables, graphs, diagrams, or whatever else helps to illustrate what happened in the experiment. Descriptive observations may supplement the data. All drawings should show accurately what you saw: they should not be copied from other sources. Do not try to interpret your data in this section (see discussion), but do state in words what each graph, table or whatever shows, and what are the main patterns present in the data.

Discussion: This section includes an interpretation of your results and a discussion of what these results mean. If something did not turn out the way you would have expected, discuss that here. If you think your experiment could have been designed differently for better results, tell how you could have improved on it. You can also discuss your results in comparison to others in the lab or in books or published articles (see below for how to do that).
References: In scientific reports, footnotes are not usual (as they are in many history papers, for instance). Instead, we refer to a book or article from which we gained information as follows: "Typical RQ values which have been obtained in different germinating seeds range from 0.5 to l.0 (Street & Opik. l975, p. l5). This statement might appear in the discussion of an experiment such as the one that you will perform when you study respiration. At the end of your lab report, you would give the full reference to the book or article, and if you used several articles, you would give them in alphabetical order by the first author's last name. Suppose you had used the book above (by H.E. Street and Helgi Opik) or a textbook. At the end of your lab report you would list (under the subheading References:):

Campbell, Neil A. 1996. Biology (4th ed.) Benjamin/Cummings Publ. Co., Redwood City, CA.

Street, H.E. & Helgi Opik. l975. The Physiology of Flowering Plants. American Elsevier Publishing Co., New York.

If you have used an article from a journal, the proper format would be similar to the above when you refer to it in the text, and in the references at the end you would state, for instance:

Bjorkman, Olle & Joseph Berry. 1973. High-Efficiency Photosynthesis. Scientific American 229: 80-93.

(note: journal titles can be abbreviated if certain conventions are followed: Scientific American becomes Sci. Am.)

Plagiarism: Plagiarism refers to the copying of statements (whether exact words or paraphrased) or ideas of others without acknowledging where you obtained them. It is a very serious crime in the world of academia and, no matter what the customs were in your former schools, you need to learn how to properly acknowledge where you got your information. Often, you will simply acknowledge information as we did above, showing where you found the details of seed RQ values. If you use the exact words of the author you must make that clear by using quotes: for instance, 'Street & Opik (1975) state on p.17 that "The RQ is therefore clearly not a value determined by one single process, but the resultant of a number of biological reactions involving oxygen and carbon dioxide." For the most part, though, you should not directly quote others -- put the idea into your own words. You will show how well you understand a concept if you do that!

Plagiarism may also refer to the copying of papers or lab reports (etc.) and that is an equally serious crime to that of not acknowledging an idea or fact which you may have obtained from a book. Lab partners should **NEVER**, copy each others' lab reports. You may do an

experiment together, and accumulate data; however, the analysis and presentation of results done individually, and lab reports should clearly reflect your own attempt to make sense out of what you did in lab. Of course students may consult with each other when they are trying to analyze data - that is very different form copying lab reports! Usually, your own judgment will tell you how to (and how often to) acknowledge a source of information, but if in doubt, ask your instructors.

Sample lab report:

Introduction to the Spectrophotometer:

Wavelength, Absorbance, and Concentration

In Methylene Blue

Kevin Donnelly

2 March 2006

Cell Biology

TA: Alex Trachtenberg

Lab partners: Tamara Jette and Wayne Thornton

INTRODUCTION

The spectrophotometer is an essential tool for biologists and chemists in analyzing chemical and biological samples. Gaining familiarity with its operating protocols and understanding what its outputs mean are very important in the development of lab technique for students of cell biology. This experiment will help laboratory students gain experience in using the spectrophotometer.

This instrument takes advantage of the regular light absorption and scattering patterns of chemical structures (Lab Manual, p.19). Specifically, it detects compounds absorbing light at selected wavelengths and produces a number corresponding to its absorption (Alberts, 2004). Each compound absorbs and scatters this light more than others at specific wavelengths. Years of research have yielded information on a myriad of compounds, permitting one to use spectrophotometry as a way of identifying unknown compounds and determining the concentration of a substance.

Specifically, the spectrophotometer measures quantitatively the amount of light passing through a compound in solution as a fraction of the light emitted by the machine ("Spectrophotometry"). A monochromator is used to produce light in very small ranges of wavelength. A photodetector detects how much of the light emitted by the machine actually transmits through the solution. A specific wavelength is input, and a display indicating percent transmittance (and its inverse, absorption or optical density) communicates to the user the absorption of a compound at this wavelength.

This experiment includes two pieces related to the spectrophotometer. First, a determination of the wavelength at which a compound (in this case, methylene blue) absorbs light best is made. Second, using this wavelength, solutions of varied concentrations are measured for their absorptions; this measurement relies on the idea that a greater number of molecules in a given volume will absorb more light than one that has fewer (Lab Manual, p. 23).

Two hypotheses may be made prior to performing this experiment. First, the absorption spectrum obtained from methylene blue should peak at an intermediate range approximately equal to 668 nm ("Optical Absorption of Methylene Blue"). In addition, the absorptions of serial dilutions of methylene blue should yield a linear relationship, as the absorbance of a substance should be proportional to its concentration.

METHODS

This experiment consisted of three parts. First, an absorption spectra was created using the Spectronic-20 spectrophotometer. Using information gleaned from the absorption spectra and serial dilutions of the substance subsequently measured for absorption, a standard curve of absorption against concentration was created. Finally, a sample of unknown concentration was tested for its absorption level and, using the standard curve, its concentration was determined.

To begin the experiment, the spec-20 spectrophotometer was turned on to allow time for it warm up. This was done before all else, and left on for approximately 15 minutes prior to any use of the machine. To prepare for the measurement of the absorbance spectrum, two test tubes were prepared. A test tube containing 5 ml of the stock solution of methylene blue, stated to be

a 3.5×10^{-5} M concentration, was prepared with the label "stock." Also, a test tube containing 5 ml water was made, labeled "blank." Separate pipettes were used for each test tube filling, to protect from contamination of the blank or dilution of the stock. With the machine properly warmed and ready for experimentation, the wavelength was set to 400 nm. Next, the machine was set to zero. With no tube in the machine, the zero control was turned to adjust the needle to a 0% transmittance/infinite optical density (OD). The blank tube was placed in the sample chamber, the cover to the chamber was closed, and the transmittance/absorbance control knob was adjusted so the needle read 100% transmittance/0 OD. The blank tube was then removed.

With the machine warmed and properly zeroed, data for the absorbance spectrum was collected. With the wavelength set to 400 nm, the stock solution tube was placed in the sample chamber, and its OD was recorded. The tube was removed, the wavelength control set to 425 nm, and the machine was zeroed again. Though the dial for 0% transmittance was not observed to be adjusted when the machine was empty and the wavelength adjusted, it was necessary to place the blank tube in the sample chamber and the dial adjusted to reestablish a reading of 100% transmittance. The blank tube was replaced by the stock tube, and a second OD reading was recorded. This same set of steps (adjust the wavelength control by 25 nm, zero the machine, measure the OD of the stock solution at the new wavelength) was repeated 21 times, until readings for the same stock solution across a wide spectrum of wavelengths was recorded. The only extra adjustment needed during this process occurred at the 600 nm reading, when the machine's filter lever needed be flipped to its second position; this was forgotten at first, and resulted in initial readings at 600nm, 625nm, and 650nm inconsistent with the hypothetical

trend. The final readings recorded, however, reflect the proper adjustment of the lever as required by the spec-20. The results of this part of the experiment were recorded in a table, and then plotted using Microsoft Excel. A peak wavelength was recorded for use in the next step in the experiment.

To determine the standard curve of absorbance versus concentration, several steps were necessary. First, serial dilutions of the stock solution were made. Using the pipettes from step 1, 5 ml of stock solution was placed in one vial without any water. A second vial containing 4.5 ml and 0.5 ml of stock solution and water, respectively, was made. This process was continued for a total of 9 vials (each totaling 5 ml, with remaining ratios of stock:water::4.0:1.0, 3.5:1.5, 3.0:2.0, 2.5:2.5, 2.0:3.0, 1.5:3.5, 1.0:4.0). The wavelength was held constant through this experiment. The wavelength used was the peak wavelength from the previous portion of this experiment: 625nm.[1] The machine was zeroed for this wavelength using the steps outlined earlier. Each dilution tube was placed individually in the sample chamber and the resulting OD was recorded. Alongside this data, the molarity of each sample was calculated and their corresponding g/L concentrations were, as well. This data was plotted using Microsoft Excel in an X-Y scatter plot. A best fit line (standard curve) was calculated and plotted using Excel, and the equation for this line included.

A tube of the stock solution with unknown concentration labeled "B" was obtained from the instructor for evaluation. This tube was placed in the sample chamber of the spec-20

[1] 625 nm is not the actual peak wavelength recorded for the stock solution used. Two higher results were excluded because there was instruction to exclude wavelengths with correlated OD values greater than 0.8. See Table 1 in the results section for these values.

without adjustment from the previous step in the experiment; its OD was recorded at a wavelength of 625 nm and its OD was recorded. Having established the standard curve for this wavelength, the sample of unknown concentration was plotted on the best fit line using OD. Its correlating concentration was then found easily.

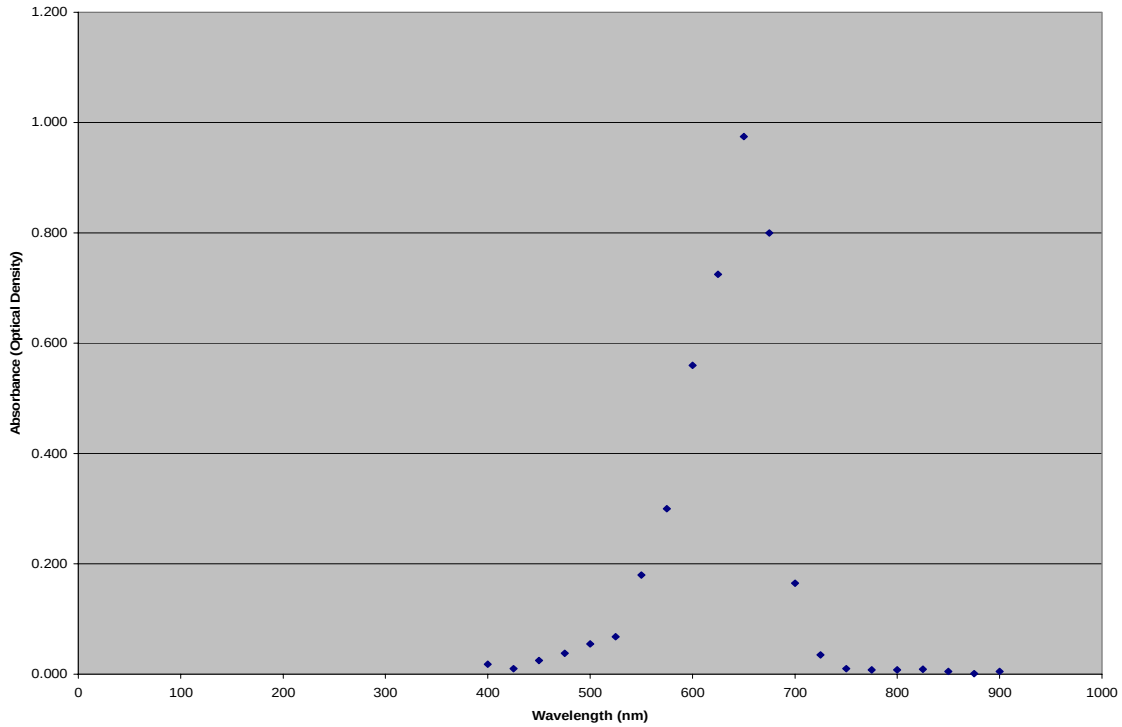
RESULTS

The first experiment yielded wavelength and absorbance (OD) readings as shown in Table 1. These results were plotted in Figure 1. Figure 1 shows the absorbance increasing as the wavelength is increased until approximately 650 nm is reached; from this point, the absorbance pattern decreases rapidly. Therefore the peak wavelength for Methylene Blue is approximately 650 nm.

Table 1

Wavelength (nm)	Absorbance (OD)	Wavelength (nm)	Absorbance (OD)
400	0.018	675	0.800
425	0.010	700	0.165
450	0.025	725	0.035
475	0.038	750	0.010
500	0.055	775	0.008
525	0.068	800	0.008
550	0.180	825	0.009
575	0.300	850	0.005
600	0.560	875	0.001
625	0.725	900	0.005
650	0.975		

Figure 1:
Absorbance vs. Wavelength

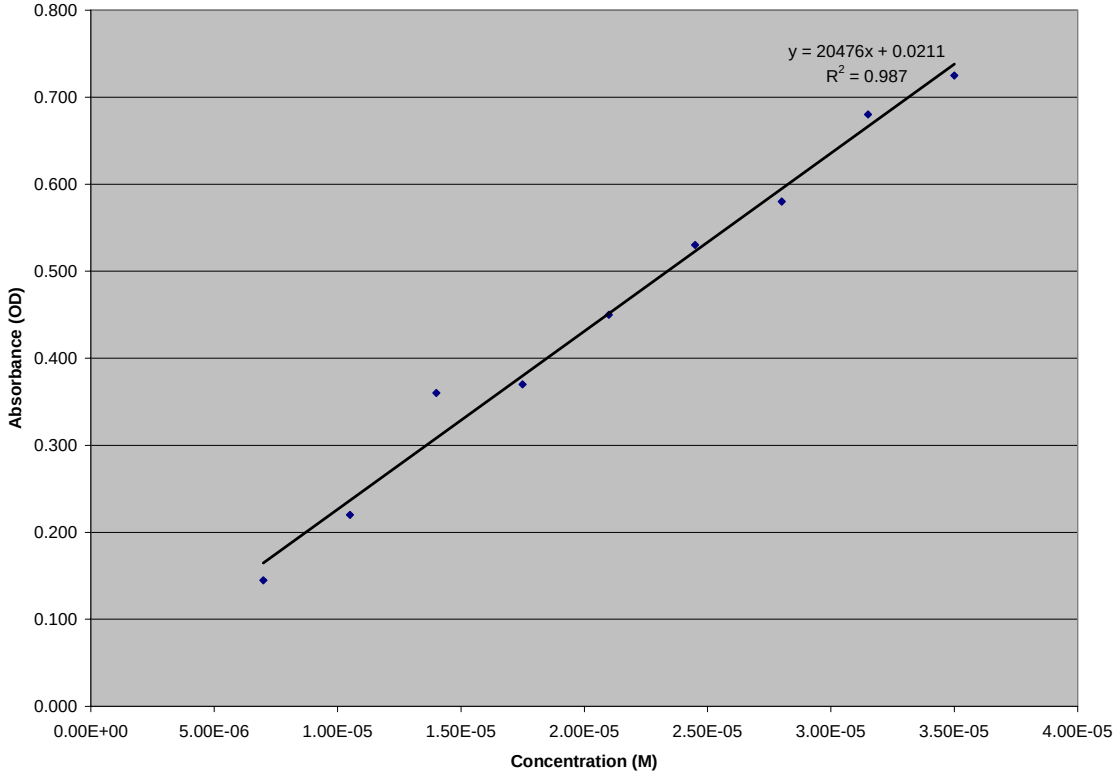


Using the peak wavelength (below 0.8 OD), Table 2 and its corresponding Figure 2 were created. The equation for the best line is: $Y = 20476X + 0.0211$. Also, the value $R2 = 0.987$ for this line was determined by Excel. Finally, the value for the unknown concentration B shown in Table 2 was calculated based on the equation for the best fit line. This value was measured on the graph using a ruler to be approximately 1.00×10^{-6} M; using the formula it was determined to be 9.76×10^{-6} M. In addition, concentration in g/L were calculated, and included in Table 2. The unknown concentration B in these terms was found to be 3.11×10^{-6} g/L.

Table 2

Tube	ml of stock	ml of water	Concentration (mol/L)	Absorbance (OD)	grams/L
1	5.0	0.0	3.50E-05	0.725	1.12E-02
2	4.5	2.5	3.15E-05	0.680	1.01E-02
3	4.0	1.0	2.80E-05	0.580	8.95E-03
4	3.5	1.5	2.45E-05	0.530	7.84E-03
5	3.0	2.0	2.10E-05	0.450	6.72E-03
6	2.5	2.5	1.75E-05	0.370	5.60E-03
7	2.0	3.0	1.40E-05	0.360	4.48E-03
8	1.5	3.5	1.05E-05	0.220	3.36E-03
9	1.0	4.0	7.00E-06	0.145	2.40E-04
Unknown (B):			**9.76E-06**	0.220	**3.11E-03**

Figure 2:
Absorbance vs. Concentration



DISCUSSION

Two hypotheses were evaluated in the course of this experiment. The first hypothesis, that methylene blue would absorb light at 668 nm, was not able to be falsified. The peak wavelength occurred at 650 nm. The next highest occurred at 675 nm, and the third at 625 nm. Given that the OD measured at 675 nm was slightly higher than that at 625 nm, it is likely that the peak wavelength actually exists somewhere between the measures evaluated at 650 nm and 675 nm. The second hypothesis, that a linear relationship between absorbance and concentration would be found, was also not falsified based on the experiment. The R^2 value for the best fit line was 0.987; in statistics, the R^2 value can be anywhere from 0 to 1, with 0 being

least likelihood of correlation, and 1 indicating a perfect correlation between events. As the R^2 value was very close to 1, a high degree of correlation was found.

Given the results, little error appears likely in the experimental procedures. However, any doubts regarding the results may be traced to a few elements of the experiment that lend themselves to possible error. The need to zero the machine between each of the readings in obtaining the absorption spectrum, and resulting peak wavelength, leaves room for error between each reset procedure. Though the student performing the zeroing was supported in his procedure by two lab partners, the precision with which a person can accurately adjust the needle on the spectrophotometer to zero is limited. Another place where error is likely is in the serial dilution of methylene blue. Though there is a presumption that the pipetting was done accurately, similar room for human error in measurement is possible as in the repeated zeroing of the instrument. Finally, reading the OD outputs became less precise the higher the OD reads; the machine measures more precisely smaller increments, with larger increments closer together on the dial.

A lack of precision in all three of the stated procedures above is possible. However, error appears to have been minimized in the experiment, assuming the hypotheses made were not misguided. One way of determining the accuracy of these results would be to repeat this experiment several times, finding a mean between the data obtained.

REFERENCES

Alberts, Bruce. Dennis Bray, et al. Essential Cell Biology. New York: Garland Science, 2004. p. 103

Lab Manual, Cell Biology. Atrium Graphics, 2006.

"Monochromator." http://en.wikipedia.org/wiki/Monochromator

Prahl, Scott. "Optical Absorption of Methylene Blue,"
http://omlc.ogi.edu/spectra/mb/index.html.

"Spectrophotometry." http://en.wikipedia.org/wiki/Spectrophotometry

Wikipedia is not considered an acceptable reference. Do not use it!

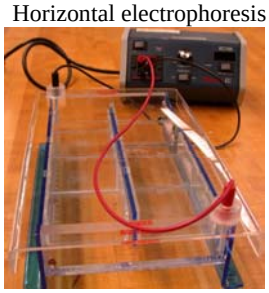
Freshman Biology (Bio 111) Lab Equipment Picture Glossary

Compound Microscope

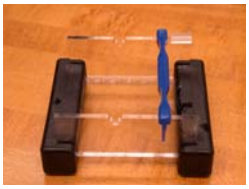
Dissection Microscope

Oxygen Electrode

Horizontal electrophoresis gel rig



Gel tray with dams and comb

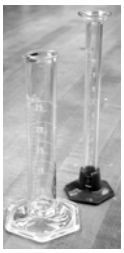


Glassware & tubes

Beakers

Erlenmeyer Flask

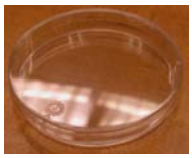
Graduated Cylinder



Funnels

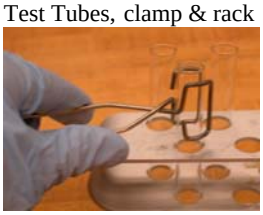
Mortar & Pestle

petri dish

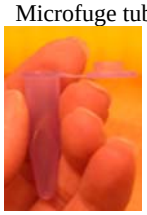


smith fermentation tubes

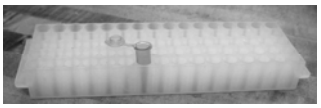
Test Tubes, clamp & rack



Microfuge tube



Microfuge tube rack



Microfuge tube floating rack

Slide drying rack & coplin jar

Weigh Boats

Balance

Power pack

Microcentifuge

Clinical centrifuge

Pipet pumps

2ml 10ml 25ml

Pasteur Pipet bulb

Pump next to serological glass pipet

Pipets are used to transfer exact amounts of liquid, the pump size should match the pipet size. (use a 10ml pipet with a 10ml pump)
Micropipetters and removable tips are used to transfer 1ml and less: very tiny amounts in units of microliters.
Use the micropipet or pipet <u>with the volume range closest to that which you wish to transfer</u> for accuracy. (if you are transferring 1.5ml use a 2ml pipet and pump not a 10ml)

Pipets Serological (10ml)

Connected for use

Bulb & glass Pasteur pipet

Pasteur pipet

Transfer pipet

plastic

Micropipet & tips (example: up to 200µl)

Total volume range display

Adjusted volume setting (quantity to transfer)

Pipet tips