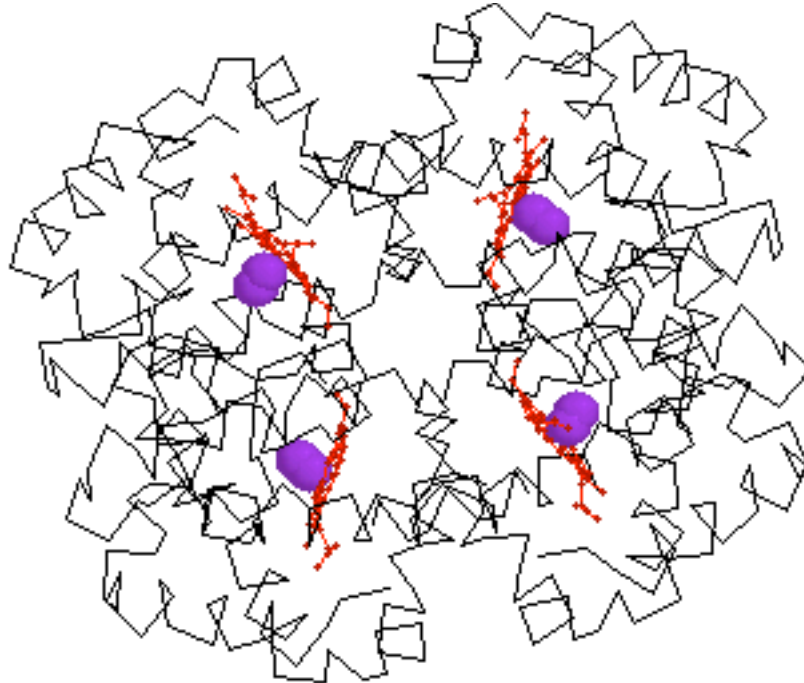


Biology 111



Lab Manual and Course Materials

Fall 2006

Name: _____

Lab Section #: _____

Lab Instructor's Name _____

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Exams from Fall 2000

(solutions included in the course handouts).

Exam	Page	Solutions can be found in this handout
Exam #1, part 1	141	Chemistry 2
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Bio 111 Pre-Lab for Lab #01: Name_____

Virtual Genetics Lab (VGL) I. TA & Sect._____

As a warm-up for this lab, you should do Genetics Problem V1 which can be found starting on page 9 of *A Problems Approach to Introductory Biology (APAIB)*. Your answers to this pre-lab will be based on this question.

1) In step (7a), you are asked to select two individual creatures, find their genotypes, and cross them. Do this and use the results to fill in the blanks below (do not use the example results presented in the book):

a) Genotype of male parent_____

b) Genotype of female parent_____

c) Give the expected results of cross.

<u>Genotypes</u>	<u>EE</u>	:	<u>Ee</u>	:	<u>ee</u>
(expected ratio)	_____	:	_____	:	_____
(for example 1:2:1)					

<u>Phenotypes</u>	<u>Extra</u>	:	<u>Crooked</u>
(expected ratio)	_____	:	_____
(for example 3:1)			

d) Results of cross:

Number of offspring with Extra Antennae_____

Number of offspring with Crooked Antennae_____

e) Do the actual results match your prediction? Explain why or why not.

Virtual Genetics Lab (VGL) I

Objective

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.

Description

VGL is a computer simulation of the genetics of an imaginary insect. The computer 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.

Warm-up

As a warm-up to this lab, you will work through Problem 1.1.2 from the Genetics chapter of *A Problems Approach to Introductory Biology (APAIB)*.

The task:

To solve two different problems generated by VGL. A solution is a genetic model that accounts for all your data. This will be your first introduction to hypothesis testing. Here, the hypotheses are your genetic models, the experiments are the crosses you do, the results are the offspring the crosses generate, and the conclusions are the model you finally choose as the correct one.

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 all of the computers in the lab. You can run the program on your home computer (Mac or PC); it can be found on the CD-ROM that came with the APAIB textbook.

3) To run the VGL program: double-click the VGL icon in the dock at the bottom of the screen. The icon looks like a little blue fly.

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 on the next page:

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 body

7 females with green body

2 males with blue body

1 female with blue body"

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:

<u>allele</u>	<u>contribution to phenotype</u>
---------------	----------------------------------

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 "Level2". 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 will need to solve one more problem for your lab report. This must also be a Level2 problem. For this problem, you should take careful notes about which flies were crossed and what the results were. You will need these results for your lab report (see later). You should read over the instructions for the lab report BEFORE starting this problem. You will need to choose a cross that 'proves your model'; that is, one that gives results that are consistent with one model and inconsistent with the alternative (where the other character is dominant).

14) 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 report:

- Must be typed; handwritten reports will not be accepted.
- Due next week at the start of the lab session you are currently in. This is a firm deadline.
- Although you will perform these experiments as a group, each member of the group must turn in an individual lab report.

Your lab report must include:

1) Your model of the inheritance of a particular trait. Note that, in these problems, there are only two alternatives:

For example, suppose that the shape of the thorax is controlled by one gene with two alleles: there are two possible models; only one can be correct:

Model 1: “Tetraltera is dominant”

<u>allele</u>	<u>contribution to phenotype</u>
T	tetraltera (dominant)
t	grooveless (recessive)

Model 2: “Grooveless is dominant”

<u>allele</u>	<u>contribution to phenotype</u>
G	grooveless (dominant)
g	tetraltera (recessive)

In your report, you should give **both models** and **indicate clearly** which you think is correct. If you thought that model 1 was correct, you’d say something like, “I feel that model 1 is correct”.

2) The results of one cross that ‘prove your model’; that is, results that are consistent with model 1 and inconsistent with model 2. 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)

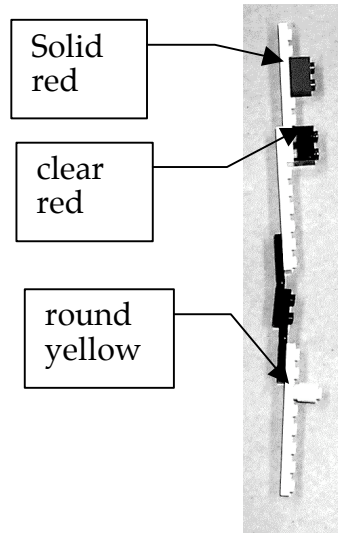
	G	g
g	Gg	gg
g	Gg	gg

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

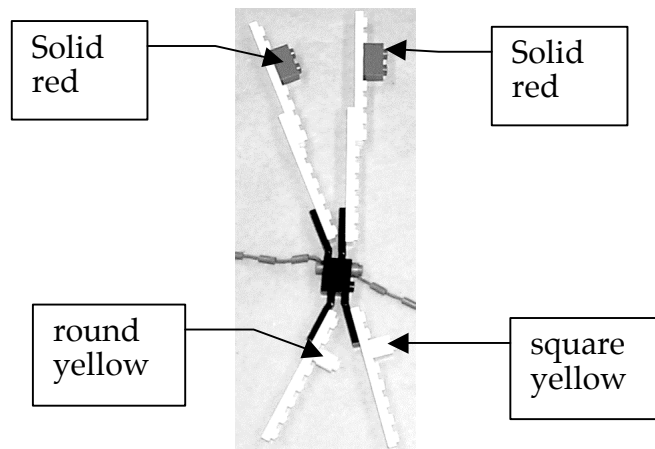
Bio 111 Pre-Lab for Lab #02: Name_____

Mitosis, Meiosis, etc. TA & Sect._____

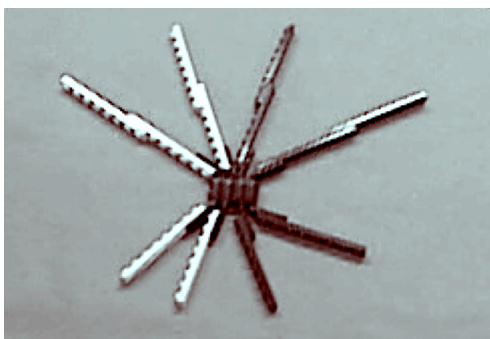
1) What is wrong with the genes on this chromosome? Why?



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



3) From the video of which process (mitosis, meiosis, or fertilization) was this picture taken? Relevant videos can be found on the course web site in the On-Line Lab Manual for this lab.



Mitosis - 1

Mitosis, Meiosis, & Fertilization

Objectives

- Follow genes, alleles, and chromosomes through mitosis, meiosis, and fertilization.
- Get a clearer picture of gene/allele and how they connect to chromosomes.
- See the connection between the symbols and the mechanism behind them.
- A 'behind the scenes' view of a Punnett square.

Description:

You will use Lego pieces to model the behavior of genes, alleles, chromosomes, and the mitotic spindle. You will construct demonstrations of these three processes; these demonstrations will be checked off by other students in the lab.

The Genetic System:

You will be modeling the genetics of Furbies - hypothetical diploid animals with only 2 chromosomes. We will consider three genes on these two chromosomes. The chromosomes from the nucleus of a diploid Furby cell are shown below:

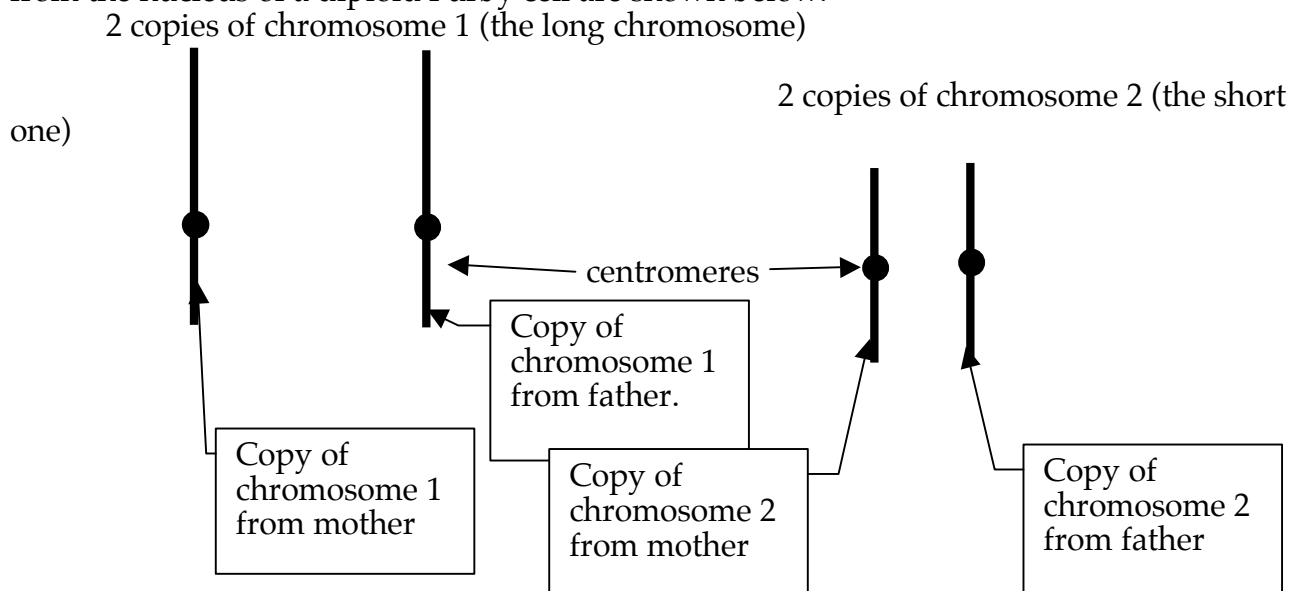


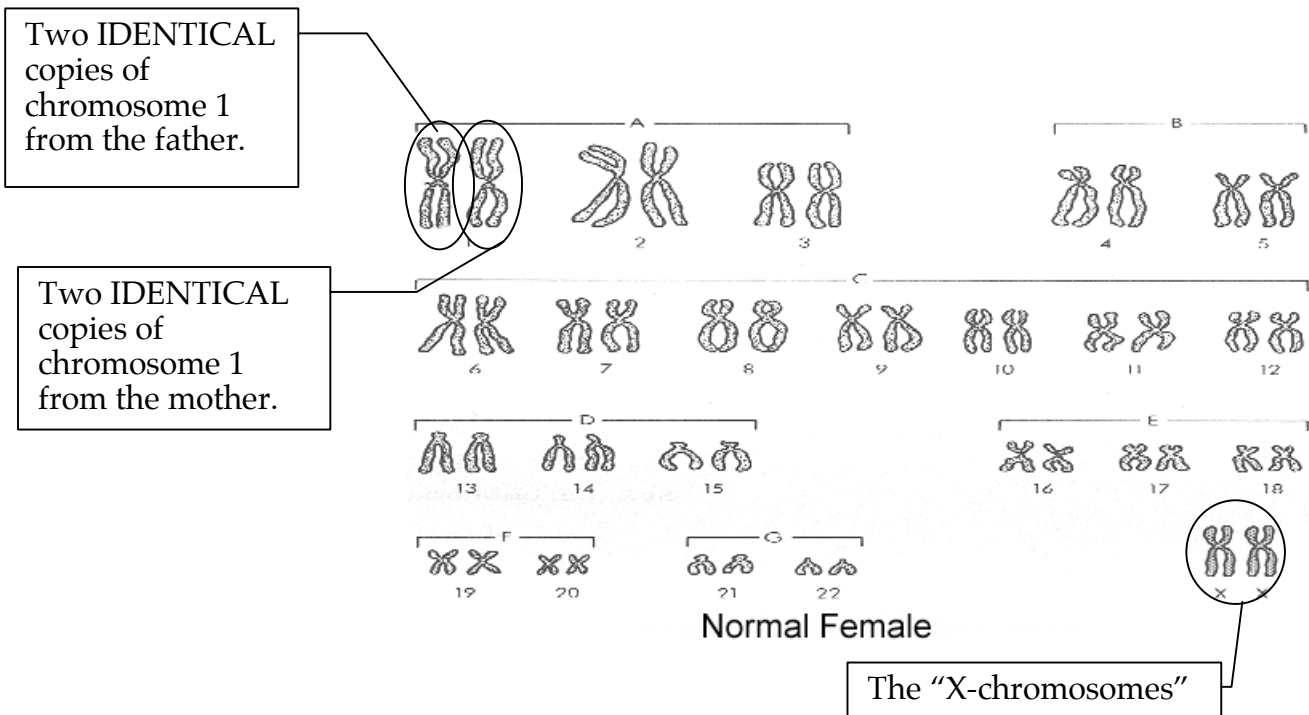
Figure 1

When we say "Furbies have two chromosomes", we mean "two different types of chromosomes" – in this case, the two different chromosomes are the long chromosome (#1) and the short chromosome (#2). Thus $N = 2$. Therefore, a diploid cell, which is $2N$, will have two copies of each different chromosome for a total of 4 chromosomes (if $N = 2$, then $2N = 4$). A haploid cell (N) would have one copy of each type of chromosome (one long & one short) for a total of 2 chromosomes.

The members of each pair of chromosomes are said to be homologous - that is, on both maternal and paternal copies of chromosome 1, all the genes are in the same locations, although the alleles may differ.

Using the same terminology, we'd say "humans have 23 different types of chromosomes" or $N=23$. Thus, a diploid cell from a human (most of the cells in your body) will have 2 copies of each chromosome ($2N = 46$). The picture on the following page shows

the chromosomes from a human cell after the chromosomes have replicated (prophase of mitosis), thus there are 4 copies of each chromosome ($4N=92$). Each "X" is two identical copies of one chromosome. The "X"s are arranged in homologous pairs - the "X" from the father and the "X" from the mother. **Note** although all of the chromosomes here look like "X"s, they are not all "X-chromosomes" (the chromosomes involved in sex-linked traits).



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

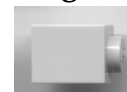
(1) The gene for Height

<u>allele</u>	<u>contribution to phenotype</u>
---------------	----------------------------------

H	tall (dominant)
---	-----------------

h	short (recessive)
---	-------------------

Lego Piece



square yellow "one"



round yellow "one"

(2) The gene for Eye color

<u>allele</u>	<u>contribution to phenotype</u>
---------------	----------------------------------

B	black (dominant)
---	------------------

b	blue (recessive)
---	------------------

Lego Piece



black flat "two"



blue flat "two"

(3) The gene for Blood-type

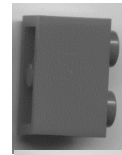
allele

contribution to phenotype

Lego Piece

Q^X

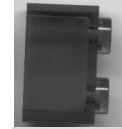
type X blood (co-dominant)



solid red "two"

Q^Y

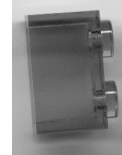
type Y blood (co-dominant)



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 matter which alleles are present, each gene is *always* in the same place on its particular chromosome.

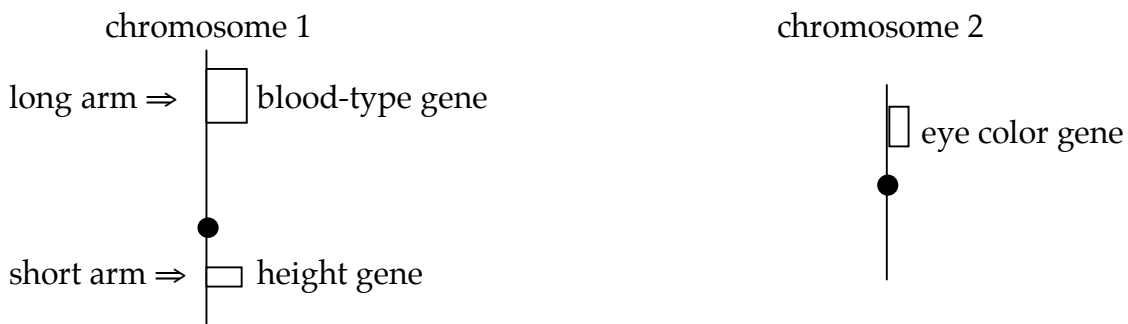


Figure 3

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

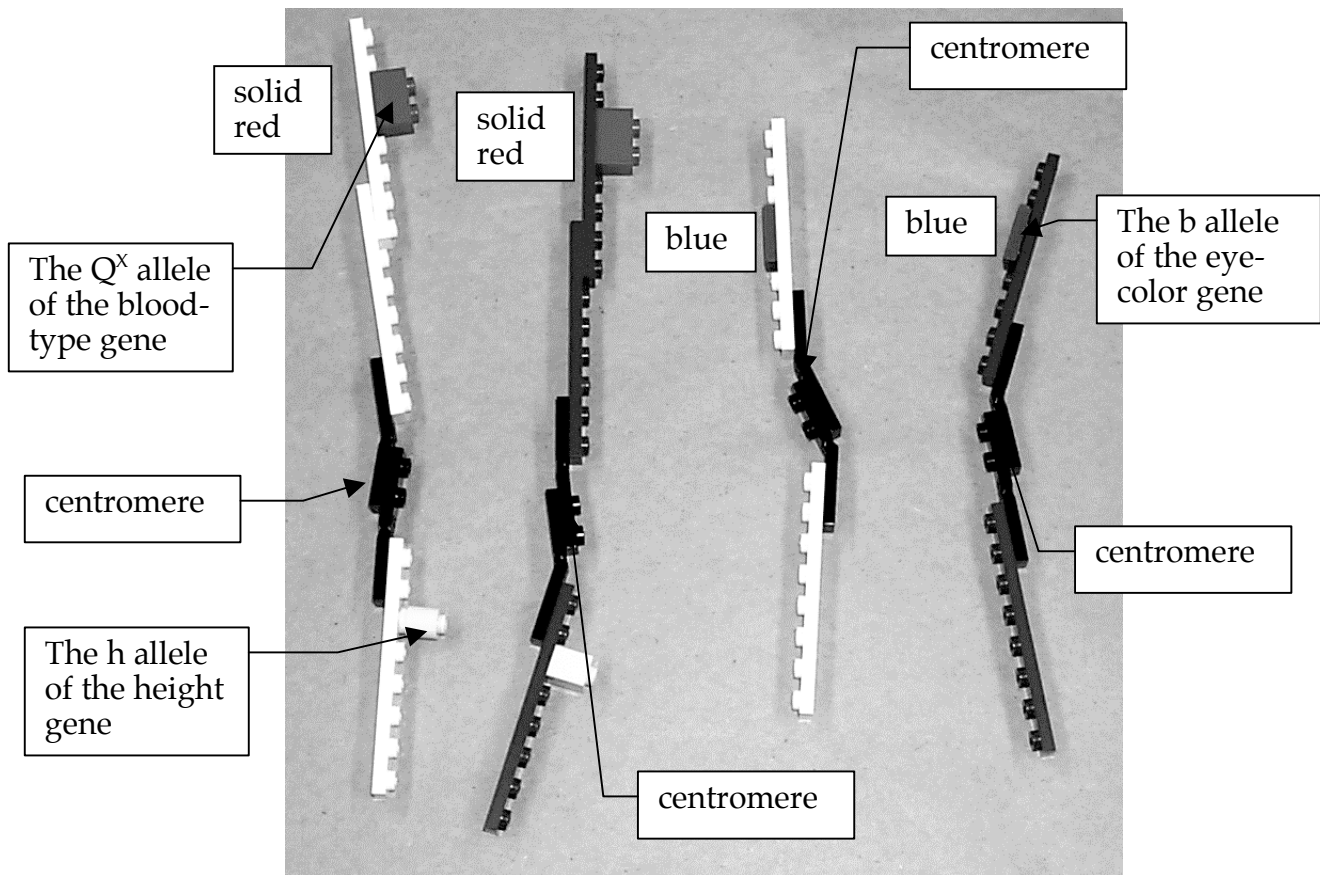


Figure 4

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 black. Therefore, the sperm had the genotype: $Q^x H b$.

Notes:

- 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.
- while the exact position of the alleles along the chromosomes is not critical, they should be in similar positions.

The complete kit of parts is shown in the following pages:

this page intentionally left blank

10 paternal chromosome parts
(purple)

10 maternal chromosome parts
(white)

4 b
alleles
(blue)

8 centromeres (black)

4 h
alleles

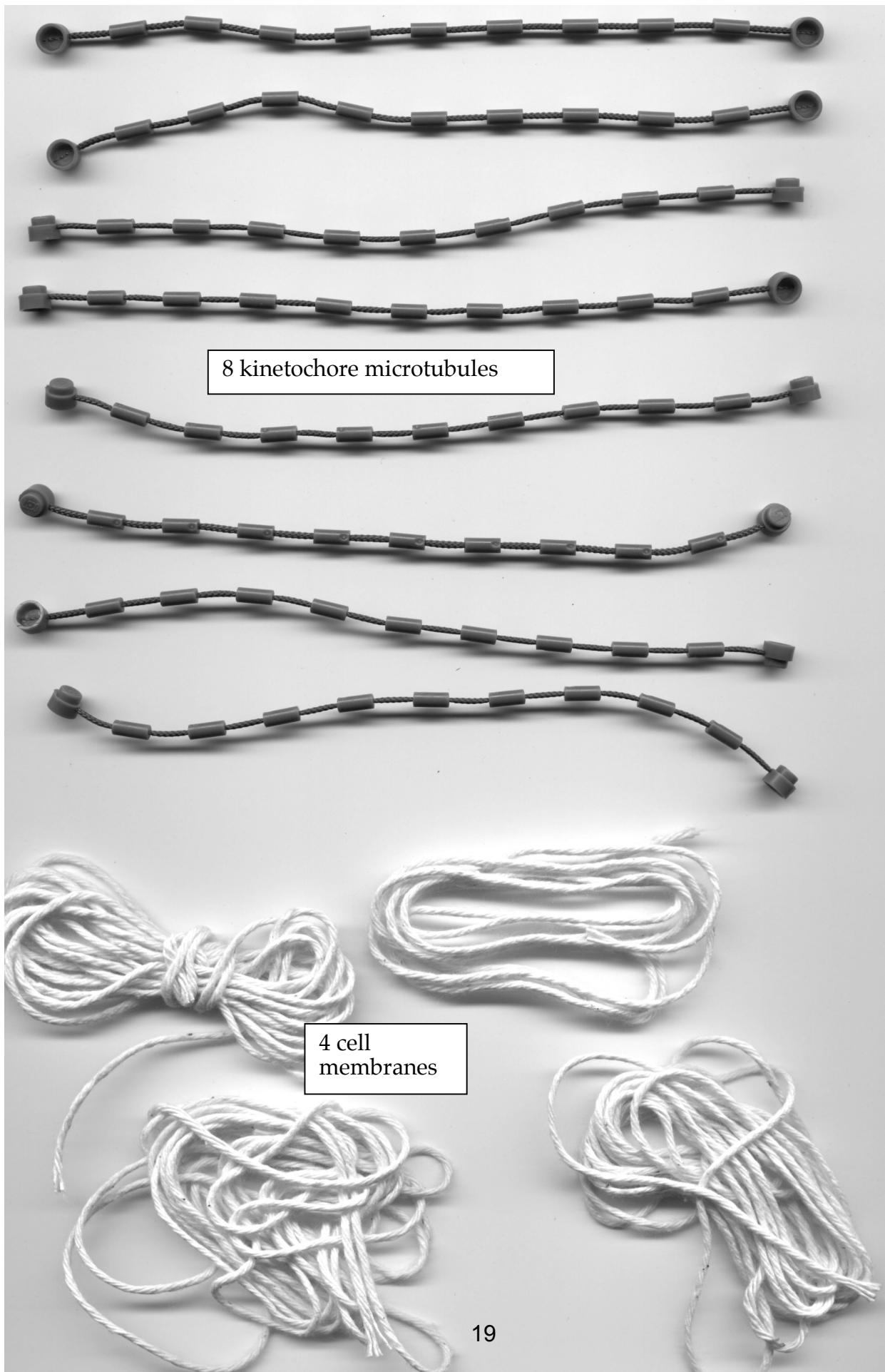
4 H
alleles

4 B
alleles
(black)

4 Q^Y
alleles
(clear
red)

4 Q^Z
alleles
(clear
pink)

4 Q^X
alleles
(solid
red)



8 kinetochore microtubules

4 cell
membranes

Procedure

(1) Open up the container of Lego and place each piece on its part of the photos on the previous pages to be sure that you have all the pieces.

Part I: Mitosis

(2) Choose a genotype for your starting cell. Write this genotype here_____
_____ (note that your genotype must include all three genes)

(3) Build a set of diploid chromosomes that models the genotype you picked in step (2). Use figure 4 as a model. Have a member of another lab group sign off in the box below.

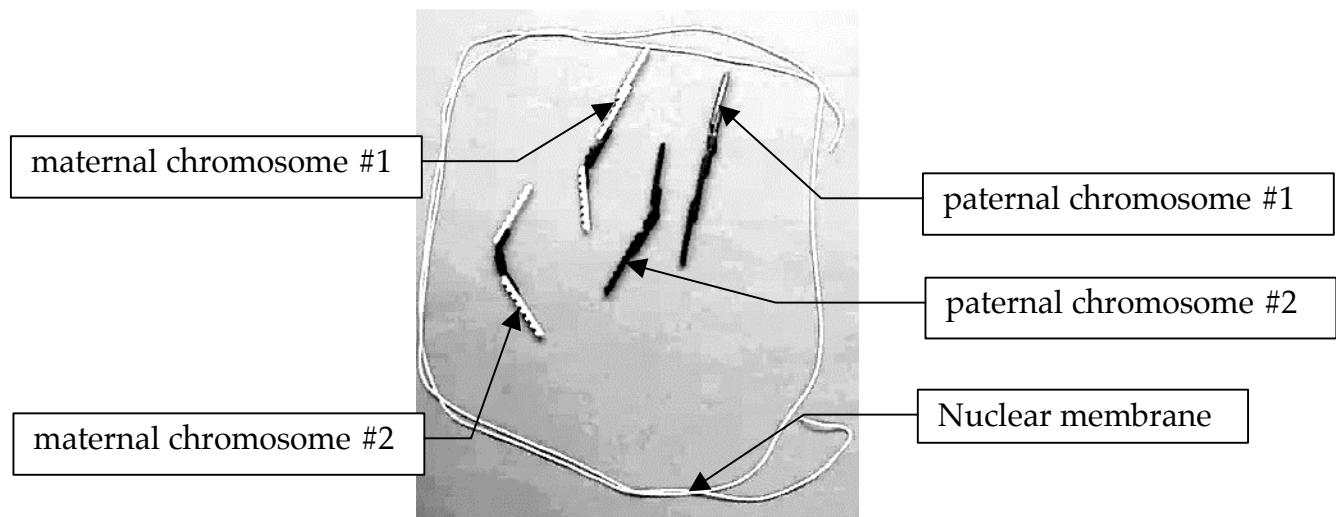
I certify that this lab group has built and demonstrated this part correctly.

Name (print)_____ sign_____

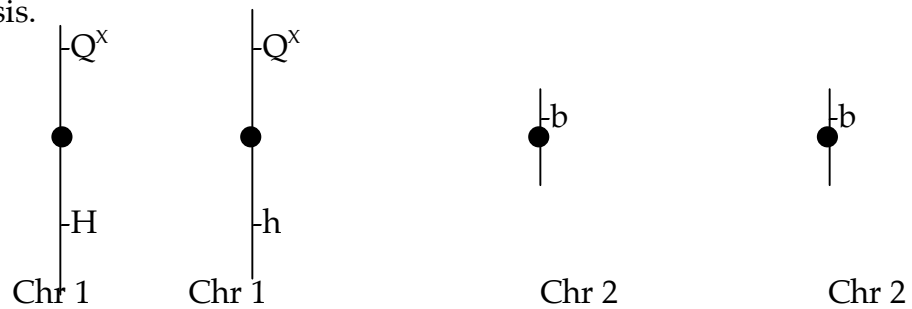
(4) Consulting Figure 12.6 in Campbell, use the Lego to model the mitosis of a Furby cell with the genotype you picked in step (2). You should use the figures that follow as a guide. Note that these figures do not show the genes and alleles; your models must include the genes and alleles. In order to get checked off, you must demonstrate all the stages shown below to a member of another lab group.

a) A resting cell (before the chromosomes have been replicated)

(**Note** the chromosomes are not visible in a real cell at this stage; we are showing them for the purposes of illustration only)

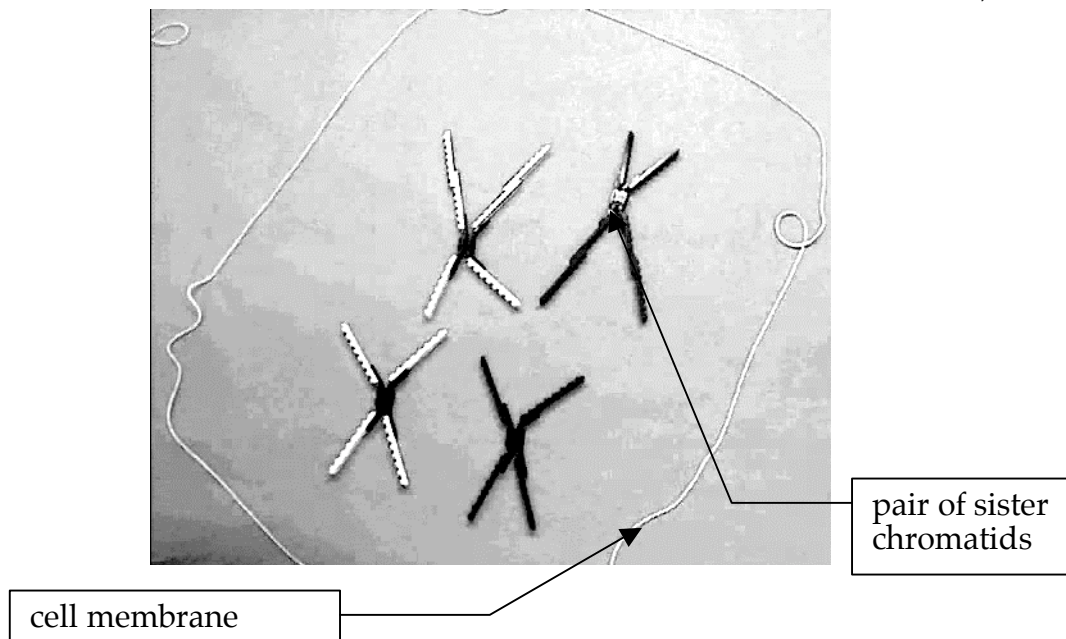


The drawing below shows a schematic of the chromosomes at this stage assuming a genotype of Q^xQ^xHhbb . Use this as a model for the drawings you will make of the later stages of mitosis and the stages of meiosis.

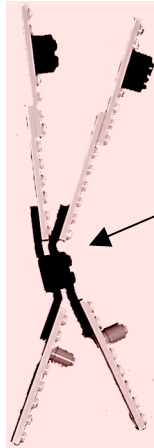


Draw a similar diagram for the genotype you chose in part (2) as they would appear in a resting cell:

b) Prophase (chromosomes have duplicated; 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)



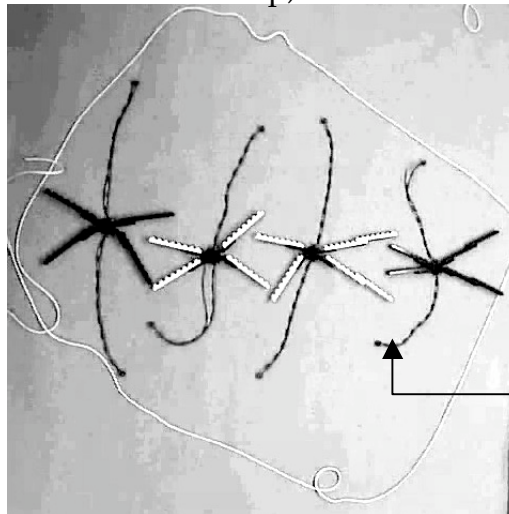
Note that the sister chromatids are exact duplicates. Therefore the alleles should be identical for all genes in a given pair. This is shown below:



Note that the sister chromatids are joined at the centromere.

Draw a schematic diagram of the chromosomes for the genotype you chose in part (2) as they would appear in a cell at prophase:

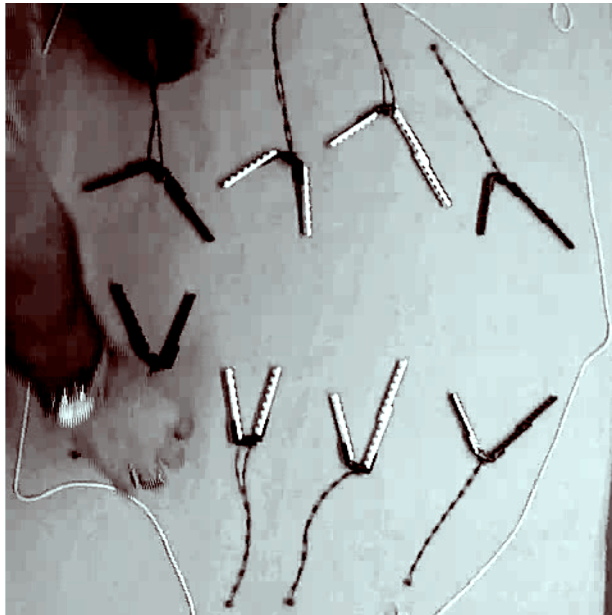
c) Metaphase (chromosomes have lined up)



kinetochore
microtubule

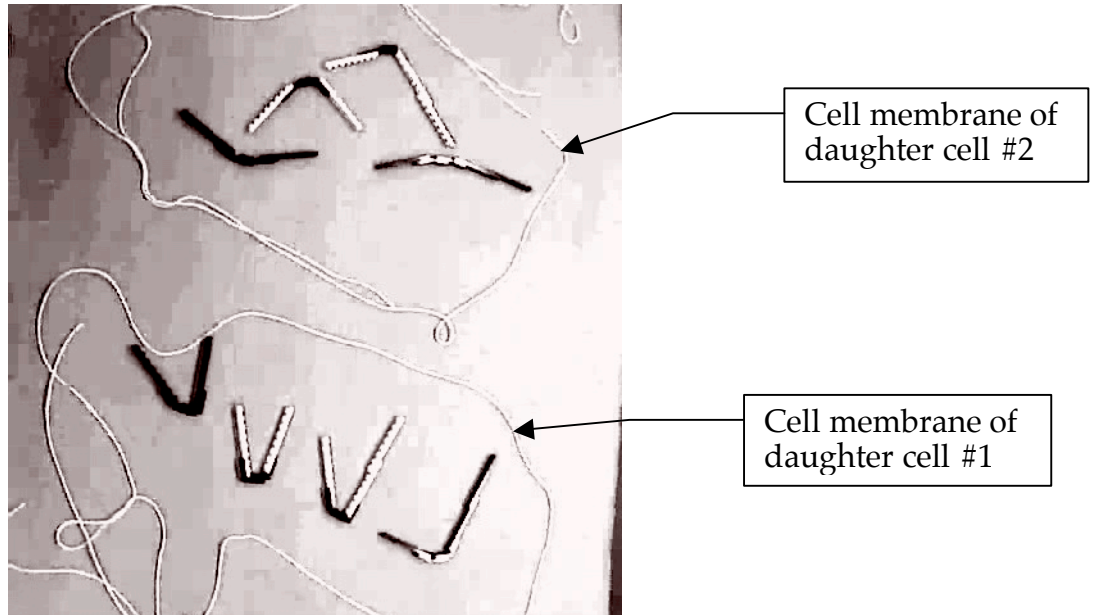
Draw a schematic diagram of the chromosomes for the genotype you chose in part (2) as they would appear in a cell at metaphase:

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 a schematic diagram of the chromosomes for the genotype you chose in part (2) as they would appear in a cell at the end of cytokinesis:

I certify that this lab group has built and demonstrated this part correctly.

Name (print)_____ sign_____

Part II: Meiosis

(5) Construct a model of the diploid chromosomes of a Furby cell with the genotype $Q^xQ^x Hh Bb$.

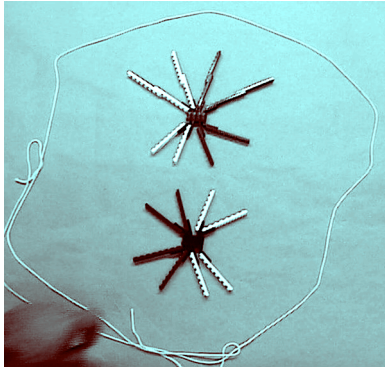
(6) Consulting Figure 13.8 in Campbell, use the Lego to model the meiosis of a Furby cell with the genotype from step (5). You should use the figures that follow as a guide. Note that these figures do not show the genes and alleles; your models must include the genes and alleles. In order to get checked off, you must demonstrate all the stages shown below to a member of another lab group.

a) resting cell (before the chromosomes have duplicated)



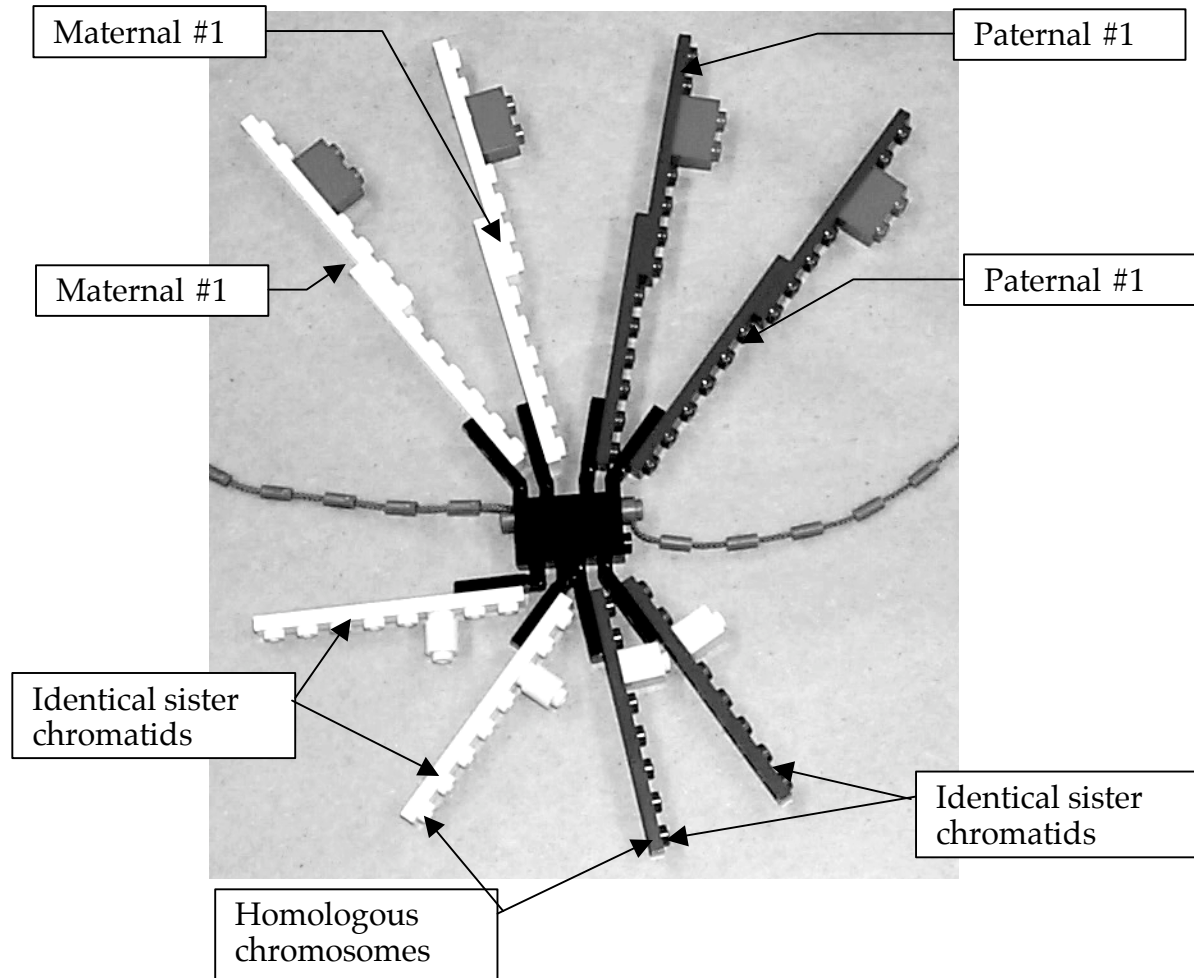
Draw a schematic diagram of the chromosomes for the genotype $Q^xQ^x Hh Bb$ as they would appear in a resting cell:

b) Prophase I (chromosomes have duplicated and homologs have paired to form tetrads) (note that we will not worry about chiasmata, recombination, or crossing over)

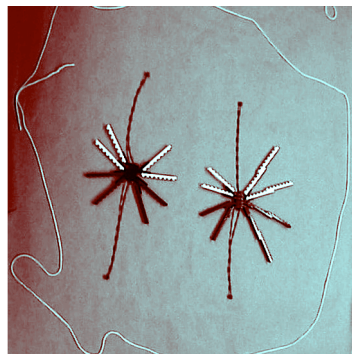


Draw a schematic diagram of the chromosomes for the genotype $Q^xQ^x Hh Bb$ as they would appear in a cell at prophase I of meiosis (see photo on next page for hints):

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. Note that, although crossing over (recombination) happens here, we won't worry about that.

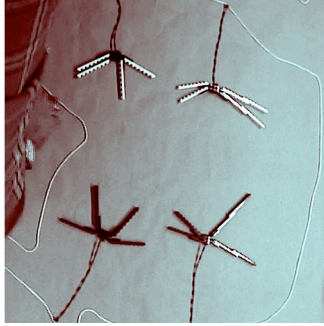


Draw a schematic diagram of the chromosomes for the genotype $Q^xQ^x Hh Bb$ as they would appear in a cell at Metaphase I of meiosis:

configuration 1:

configuration 2:

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

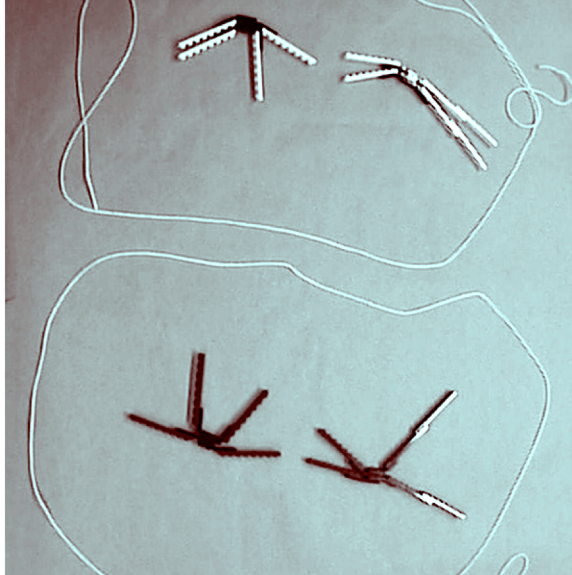


Draw a schematic diagram of the chromosomes for the genotype $Q^xQ^x Hh Bb$ as they would appear in a cell at Anaphase I of meiosis:

configuration 1:

configuration 2:

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

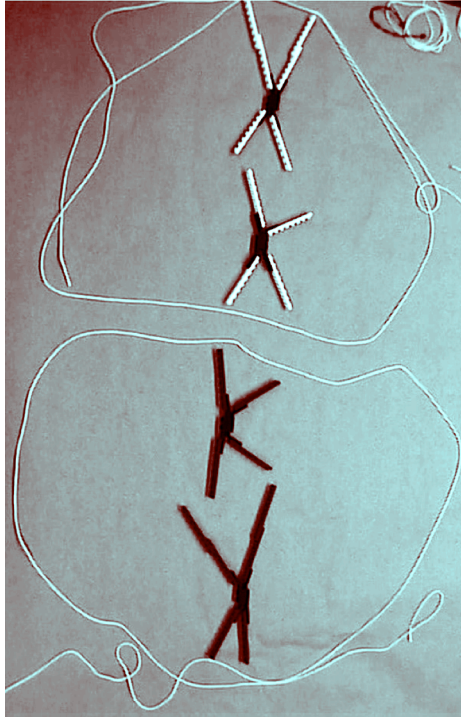


Draw a schematic diagram of the chromosomes for the genotype $Q^xQ^x Hh Bb$ as they would appear in a cell at the end of Cytokinesis I of meiosis:

configuration 1:

configuration 2:

f) Metaphase II (chromosomes have lined up)

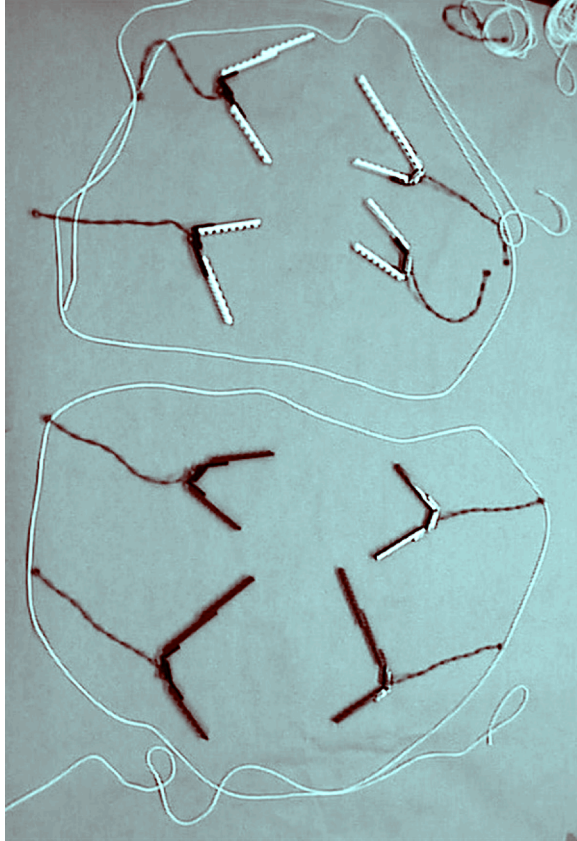


Draw a schematic diagram of the chromosomes for the genotype $Q^X Q^X Hh Bb$ as they would appear in a cell at Metaphase II of meiosis:

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 in order to be checked off. (See Campbell figure 13.8)

I certify that this lab group has built and demonstrated this part correctly.

Name (print)_____ sign_____

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?

Finishing up

(7) Have your TA note that all the parts have been signed off by members of another group.

(8) Place all your Lego pieces on the photos at the beginning of this section and have your TA check off that you are returning all of them.

Lab Report:

- Due next week at the start of the lab session you are currently in. This is a firm deadline.
- Although the lab work was done as a group, each member of the group must turn in an individual lab report.
- Your lab report MUST include the following:

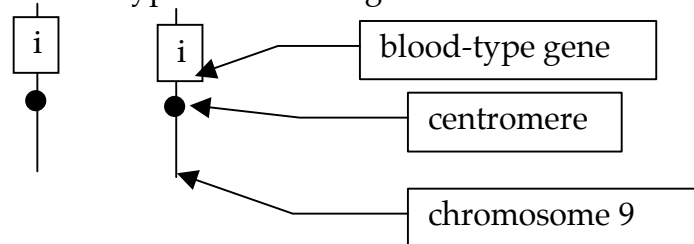
Note that this lab report is based on what you did in lab, but it is **not** a description of what you did in lab.

Your lab report will be based on the following scenario:

A **human** mother, Katie, has blood-type AB. Her husband, Joe, has blood-type A. Their daughter, Anne, has blood-type B. The gene for blood-type in humans is located on chromosome 9.

and should consist of the following :

(1) A diagram of Katie's copies of chromosome 9 as they would appear (if they were visible) in a resting cell labeled as shown below. Here is a sample diagram of the copies of chromosome 9 carried by an individual with blood-type O. Your diagram should be labeled like this:



(2) A diagram of Joe's copies of chromosome 9 as they would appear (if they were visible) in a resting cell labeled as shown in part (1). Use symbols similar to those you used in question (1).

(3) A diagram of Katie's copies of chromosome 9 as they would appear as they went through the stages of meiosis you modeled in step (6) of the lab. You must have a drawing for each of the stages shown in the lab manual (6b through 6h). Note that there is only one possible configuration here.

(4) A diagram showing the copies of chromosome 9 contained in the egg and sperm that gave rise to Anne. Draw egg and sperm as separate cells and label as 'egg' or 'sperm' as appropriate.

(5) A diagram of Anne's copies of chromosome 9 as they would appear as they went through the stages of mitosis you modeled in step (4) of the lab. You must have a drawing for each of the stages shown in the lab manual (4a through 4e).

Pitfalls to avoid

- Use the right symbols. Blood types are A,B, AB, and O. The alleles are I^A , I^B , and i . People have blood types; alleles go on chromosomes.
- Humans are diploid so a human genotype **MUST** contain two alleles. The only exceptions are eggs and sperm which are haploid (only one allele).
- Each chromosome carries only **ONE** allele for a given gene.
- Label your diagrams **THOROUGHLY** - be sure you've marked all that the question calls for.
- Chromosomes duplicate only **ONCE** before the cell divides. Thus, in a resting cell there are 2 copies of each chromosome (homologs) and at metaphase there are 4 copies (2 copies of each homolog).

Bio 111 Pre-Lab for Lab #03: Name_____

Genetics Problems TA & Sect. _____

Consider the following scenario:

George has a genetic disease we'll call Q-osis. Paula does not have the disease. Paula and George have 4 daughters. One of the 4 daughters has Q-osis, the other 3 do not. Paula's father has Q-osis; Paula's mother does not. Both of George's parents do not have Q-osis.

1) Draw a pedigree for this family. Shade in the symbols of the individuals with Q-osis.

2) Is it possible that Q-osis is inherited in an autosomal dominant manner? Explain your reasoning.

Genetics Practice Problems

We will work through the following problems during this lab period.

The problems can be found in the Genetics chapter of *A Problems Approach to Introductory Biology (APAIB)*. They are:

- 1.3.4
- 1.3.5
- 1.3.6
- 1.3.7
- 1.5.1
- 1.2.5

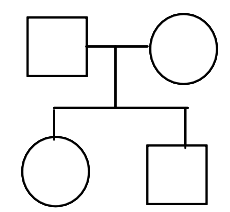
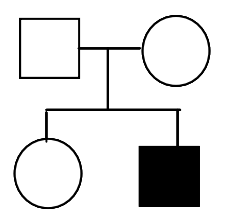
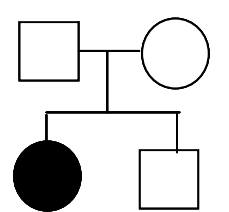
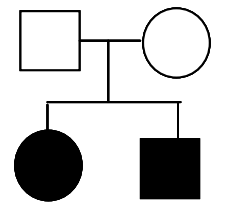
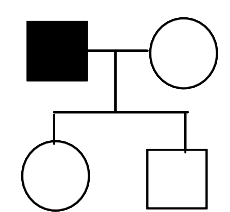
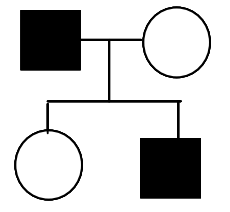
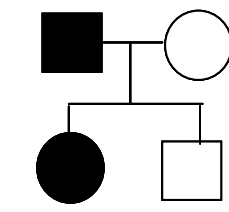
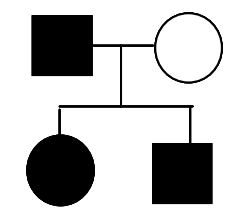
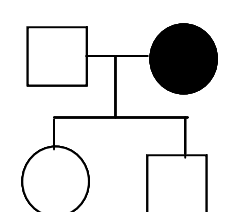
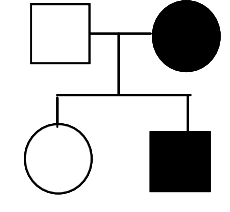
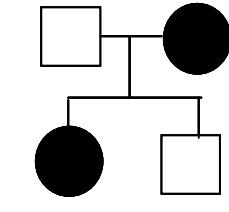
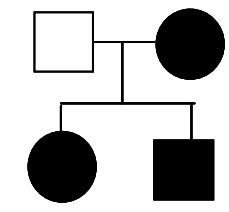
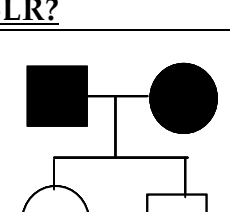
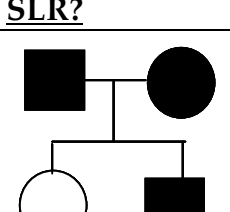
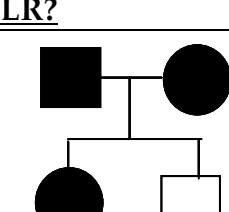
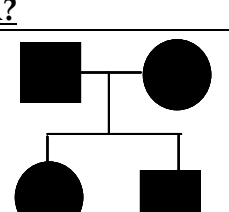
In addition, if there is time, you should work through this problem in lab.

“Sixteen Pedigrees”

The table on the next page shows the 16 possible pedigrees that could occur in a family of four (mom, dad, brother, sister). For each pedigree:

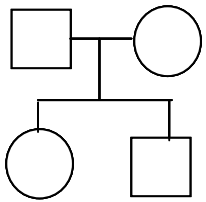
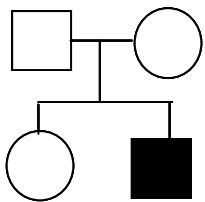
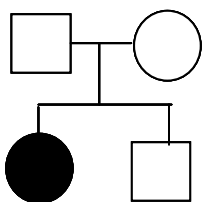
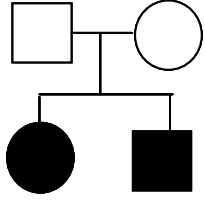
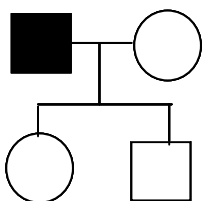
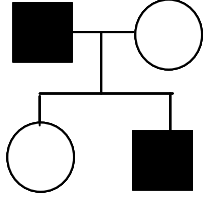
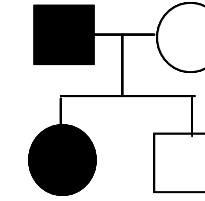
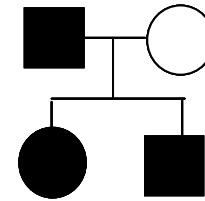
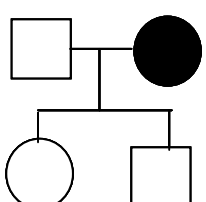
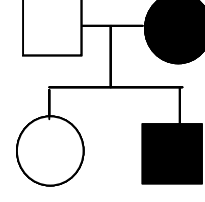
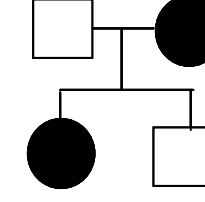
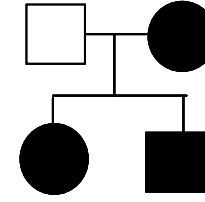
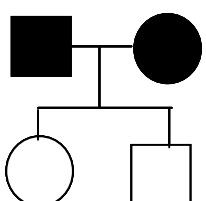
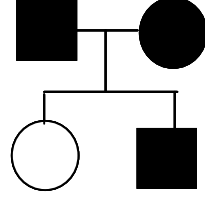
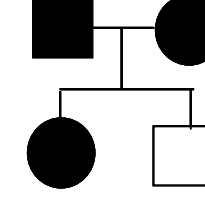
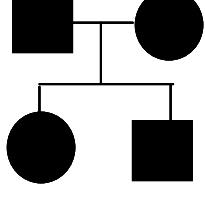
- is it consistent with an autosomal recessive mode of inheritance?
 - if yes: write “consistent” after AR? and write in the number of unrelated individuals who bring in at least 1 disease allele that are required to complete the pedigree.
 - if no: write “inconsistent” after the AR?
- is it consistent with an autosomal dominant mode of inheritance?
 - if yes: write “consistent” after AD? and write in the number of unrelated individuals who bring in at least 1 disease allele that are required to complete the pedigree.
 - if no: write “inconsistent” after the AD?
- is it consistent with an sex-linked recessive mode of inheritance?
 - if yes: write “consistent” after SLR? and write in the number of unrelated individuals who bring in at least 1 disease allele that are required to complete the pedigree.
 - if no: write “inconsistent” after the SLR?

Solutions to this problem can be found at the end of this section of the lab manual.

 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>
 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>
 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>
 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>	 <p><u>AR?</u> <u>AD?</u> <u>SLR?</u></p>

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Solutions to "Sixteen Pedigrees"
(number of "unrelated carriers" in parentheses)

 <p><u>AR?</u> consistent <u>AD?</u> consistent <u>SLR?</u> consistent</p>	 <p><u>AR?</u> consistent (2) <u>AD?</u> inconsistent <u>SLR?</u> consistent (1)</p>	 <p><u>AR?</u> consistent (2) <u>AD?</u> inconsistent <u>SLR?</u> inconsistent</p>	 <p><u>AR?</u> consistent (2) <u>AD?</u> inconsistent <u>SLR?</u> inconsistent</p>
 <p><u>AR?</u> consistent (1) <u>AD?</u> consistent (1) <u>SLR?</u> consistent (1)</p>	 <p><u>AR?</u> consistent (2) <u>AD?</u> consistent (1) <u>SLR?</u> consistent (2)</p>	 <p><u>AR?</u> consistent (2) <u>AD?</u> consistent (1) <u>SLR?</u> consistent (2)</p>	 <p><u>AR?</u> consistent (2) <u>AD?</u> consistent (1) <u>SLR?</u> consistent (2)</p>
 <p><u>AR?</u> consistent (1) <u>AD?</u> consistent (1) <u>SLR?</u> inconsistent</p>	 <p><u>AR?</u> consistent (2) <u>AD?</u> consistent (1) <u>SLR?</u> consistent (2)</p>	 <p><u>AR?</u> consistent (2) <u>AD?</u> consistent (1) <u>SLR?</u> inconsistent</p>	 <p><u>AR?</u> consistent (2) <u>AD?</u> consistent (1) <u>SLR?</u> inconsistent</p>
 <p><u>AR?</u> inconsistent <u>AD?</u> consistent (2) <u>SLR?</u> inconsistent</p>	 <p><u>AR?</u> inconsistent <u>AD?</u> consistent (2) <u>SLR?</u> inconsistent</p>	 <p><u>AR?</u> inconsistent <u>AD?</u> consistent (2) <u>SLR?</u> inconsistent</p>	 <p><u>AR?</u> consistent (2) <u>AD?</u> consistent (2) <u>SLR?</u> consistent (2)</p>

Bio 111 Pre-Lab for Lab #04: Name_____

Virtual Genetics Lab II TA & Sect._____

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

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

Genotype	Phenotype
$X^W X^W$	
$X^R X^R$	
$X^g X^g$	
$X^W X^R$	
$X^W X^g$	
$X^R X^g$	
$X^R Y$	
$X^W Y$	
$X^g Y$	

2) Which phenotype(s) are present only in females?

Virtual Genetics Lab (VGL) II

Objective

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. You will also be asked to write up a lab report in the approximate format of a scientific paper; this will help you to understand hypothesis testing and the process of science.

Description

VGL is a computer simulation of genetics. The computer 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 creatures with random genotypes called the Field Population.

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

The task:

To solve two different problems generated by VGL. A solution is a genetic model that accounts for all your data. These are the most challenging VGL problems - there are 12 possible genetic models. All the problems in VGL at this level involve genetic models with one gene that has two or three alleles. Based on this, there are several features that can vary:

- The number of alleles; this can be either:
 - Two alleles (Models 1, 2, 3, 4, 5, and 6). Given this, there are two possible **Interactions between the alleles**:
 - Simple Dominance: (Models 1, 3, and 5) The heterozygote has the same phenotype as the dominant homozygote. That is, with two alleles A and a:
 - Incomplete Dominance: (Models 2, 4, and 6) The heterozygote has a different phenotype than either homozygote. In nature, this is usually intermediate; in VGL it need not be.
 - Three Alleles (Models 7, 8, 9, 10, 11, and 12). Given this, there are two possible **Interactions between alleles**:
 - Hierarchical Dominance: (Models 7, 9, and 11). A is dominant to all; A' is dominant to A'' and recessive to A; A'' is recessive to all ($A > A' > A''$).
 - Circular Dominance: (Models 8, 10, 12). B is dominant to B'; B' is dominant to B''; B'' is dominant to B.
- Whether the trait is sex-linked or not; this can be either:
 - Not sex-linked (Models 1, 2, 7, and 8) - the gene for the character is carried on an autosome so it is inherited identically in both sexes.
 - Sex-linked - the gene for the trait is located on a sex-chromosome so it is inherited differently in different sexes. **This can be either**:
 - XX/XY (Models 3, 4, 9, and 10) Females are XX; males are XY. Here, Y carries no genes except those needed to make the organism male.
 - ZZ/ZW (Models 5, 6, 11, and 12). Females are ZW; males are ZZ. Here, W carries no genes except those needed to make the organism female.

This leads to twelve possible genetic models.

Model 1: 2-alleles; Simple Dominance; Autosomal.

Model 2: 2-alleles; Incomplete Dominance; Autosomal.

Model 3: 2-alleles; Simple Dominance; XX/XY Sex-linked.

Model 4: 2-alleles; Incomplete Dominance; XX/XY Sex-linked.

Model 5: 2-alleles; Simple Dominance; ZZ/ZW Sex-linked.

Model 6: 2-alleles; Incomplete Dominance; ZZ/ZW Sex-linked.

Model 7: 3-alleles; Hierarchical Dominance; Autosomal

Model 8: 3-alleles; Circular Dominance; Autosomal

Model 9: 3-alleles; Hierarchical Dominance; XX/XY Sex-linked

Model 10: 3-alleles; Circular Dominance; XX/XY Sex-linked

Model 11: 3-alleles; Hierarchical Dominance; ZZ/ZW Sex-linked

Model 12: 3-alleles; Circular Dominance; ZZ/ZW Sex-linked

Your task is to find the genetic model that best fits your data.

You should know that, since VGL selects traits randomly, the particular traits do not necessarily indicate the dominance relationships. That is, although you might expect otherwise, having no antennae may be dominant to having antennae. Similarly, having 4 legs may not be the heterozygote of 2-legs and 6-legs.

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 all of the Macintosh computers in the lab. You can also download the program and run it from your home computer; go to <http://intro.bio.umb.edu/VGL/index.htm>

3) To run the VGL program, click on the VGL icon in the Dock. It looks like a small fly-like insect.

4) If you want to read the VGL manual, click on the Help button at the top of the VGL window.

5) You should choose Level 10 problems for this lab session. For practice, you can choose Level 9, 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). The problem you demonstrate to your TA and the problem you solve for your lab report must be Level 10.

6) 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"

7) 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.

8) Finally, you must solve one more Level 10 problem for your Lab Report. We *strongly* advise you to work through this problem in lab, rather than at home. In lab, your TA can help you to make sure that you have worked it through completely. In addition, although you can run VGL on your home computer, there is no guarantee that this will work as well as it does in lab.

The problem you solve for your lab report must be more complex than a "two-allele, simple dominance, autosomal" problem. That is, it must include at least one of the following: sex-linkage, three-alleles, or incomplete dominance. Lab reports describing simpler problems, or problems worked in practice mode, will receive no credit.

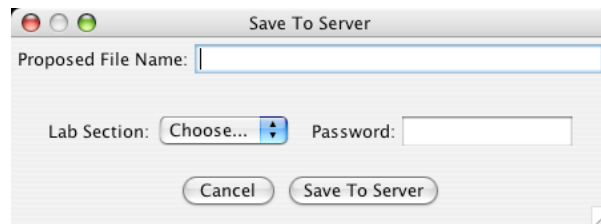
9) Once you have solved this problem to your satisfaction, you **must** save this problem on the Introductory Biology Web Server. This is for several reasons:

- So that you can go back and look at all of your data after you leave lab.
- So that you can do more crosses in your problem after you've left lab.
- So that your TA can check to be sure that you analyzed your data correctly.

For those reasons, saving the problem to the server is worth 5 points on the Lab Report.

Procedure

a) From the "File" menu, choose "Save to Server...". You should see this:



b) Enter a descriptive (but not too long) name for your problem file.

c) Select your lab section number from the list.

d) Enter the password; your TA will provide it.

e) Click "Save To Server".

f) The program will respond with a slightly-modified name for your file. This is the name that it will be listed by on the web page. **You should be sure to write this name down** (that's why the program will tell it to you twice!)

File name _____

g) You can check to see if your file is there by launching Safari and following the links to the "VGL Problem Archive" from the OLLM for this lab. It will be filed under your lab section number in alphabetical order. You can either view your data or run the problem itself (if you want to do more crosses).

10) You should also take careful notes of your results or print your data (Choose "Print Work" from the "File" menu) in case the server malfunctions.

Lab report:

It is very important that you follow **all** of the directions for preparing your lab report in order to receive full credit. Feel free to contact your TA in advance of the due date if you have any questions.

Overall notes

- Your lab report is due at the start of lab on the date listed in the syllabus. It will not be accepted late.
- Although you and your group members worked on the same problem, your lab report must be in your own words.
- Your lab report must be typed; handwritten lab reports will not be accepted.

Special notes for this lab

- (5 points) You must bring in two copies of your lab report. This lab is under development and we need to have a copy of your report that we can analyze to help develop the lab.
- (5 points) You must give the name of the file that corresponds to the problem described in your lab report.
- The lab report **must be in the format described below** including titles for each section (Abstract, etc.). Lab reports that are not in this format will receive an automatic deduction of 10 points.

Parts of the Lab Report

This will be structured like a scientific paper to introduce you to this important format as well as the aspects of the nature of science that it illustrates.

The most important thing to keep in mind is that a scientific paper is not the story of what you did. It is a logical argument in a prescribed format designed to convince your reader that your conclusions are correct. The most crucial issue is making a clear and logical argument that is supported by data. This means that, in most cases, you will not present your data in the order that you got it; you should present it in a way that makes the clearest case for your interpretation.

These are the remaining parts of the lab report. *Descriptions in italics indicate their role in the argument you are developing.*

- (5 points) **Abstract** A 1-3 sentence description of the genetic model that explains the inheritance of your trait. *This introduces your reader to the model you are going to be arguing for.*
- (5 points) **Introduction** A short paragraph that explains the research question being addressed (the inheritance of your trait) and why the methodology being used (crossing individual organisms and looking at the offspring) is believed to be able to answer that question. *This introduces the background behind your studies and why they are worth reading about.*
- (5 points) **Materials and Methods** A short paragraph that explains what you did. **Do not** give the details of **all** the crosses you did. All you need here is a few sentence summary of: how many crosses you did; roughly how many of those were necessary to figure out the model; and any strategies you used in choosing what to cross (just describe these in general **do not** talk about each and every cross in detail). *This tells the reader the details of the techniques you used.*
- (25 points) **Results and Discussion** *This is the most important part of the paper. It is where you make the argument for your interpretation of the data. Start by introducing the genetic*

model you have found and defining appropriate symbols (5 points). Then go point-by-point to show the evidence for your model. This is not a narrative or a history, it is a logical argument supported by data. Do not present all your crosses in the order you did them; present them in the order that makes the clearest argument for the model you described in the Abstract.

It is probably best to break this down into parts. For each part of your model, give the evidence for that part. For example, if you want to argue that red eyes are XX/XY sex-linked dominant, you would have separate arguments to show that it is (1) XX/XY sex-linked and (2) dominant.

In addition to the 5 points for defining the symbols, we will grade this part using the following rubric:

- *All parts of model are supported* (0 - 8 points). All parts of the model you presented are specifically supported by one or more pieces of data. Less than full credit will be given for answers that do not support all parts of your model. That is, if you say it is “ZZ/ZW sex-linked incomplete dominant”, you have to show data that it is ZZ/ZW, sex-linked, and incomplete dominant.
- *Data rule out all possible alternative models* (0 - 6 points). You present data that specifically exclude all alternative models. Less than full credit if other interpretations are possible. That is, if you choose the above model, you would have to show that it cannot be autosomal, XX/XY sex-linked, circular dominance, etc.
- *Only relevant data are presented* (0 - 6 points). All pieces of data presented are part of an explicit argument that supports the selected model or rules out other models. Less than full credit for data presented that are not part of your argument.

Live Long and Prosper

Purpose

- To explore hypothesis testing in a more open-ended and less clearly-defined situation
- To extend your knowledge of genetics with a more complex exercise
- To introduce the genetics of more than one character (or gene) at a time.

Introduction

The core of the scientific process is the testing of hypotheses with empirical data. This process has given rise to all the knowledge that is taught in this course. In the 'real world' of science, this process can be very complex; as Jim Ryder put it, "conclusions do not often arise un-problematically from data". Understanding the basics of hypothesis testing as well as its subtleties is an important part of being a scientist as well as a citizen.

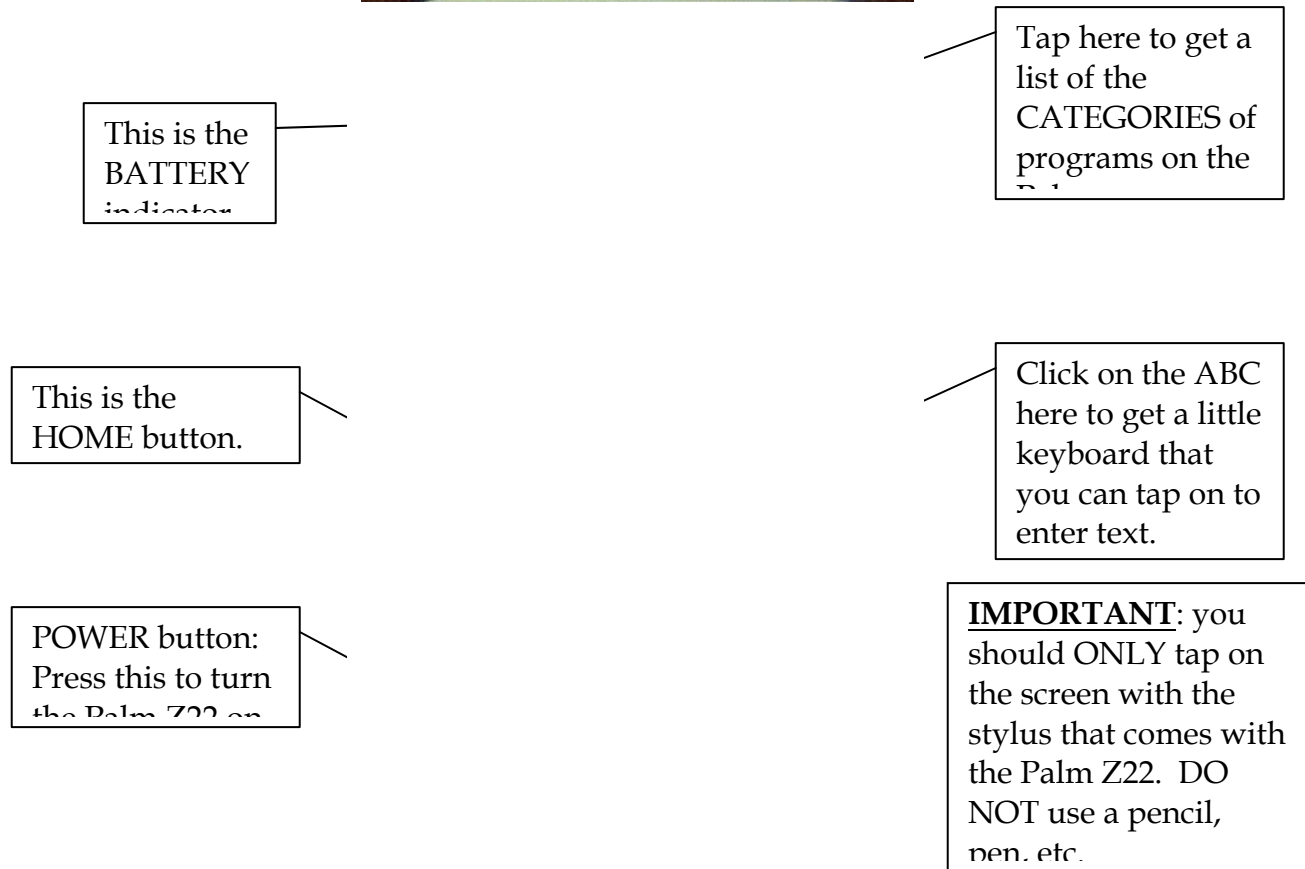
In the Virtual Genetics Labs, you were testing hypotheses. We called them "genetic models" but they are hypotheses that attempt to explain the patterns of inheritance you observed. You tested those hypotheses by crossing particular flies and observing the results. In this lab, you will use a similar strategy to explore some more complex traits. In VGL, the traits were clearly defined; for example, the flies were either red or green and the program sorted offspring by color cleanly - there were no reddish-green or greenish-red flies. In the real world, it would likely be more complex (like in the Marfan's syndrome problem, where it is not clear who is affected and who is not). Furthermore, in previous problems, we have dealt with only one character (or gene) at a time; in this lab, you will deal with several characters and genes simultaneously. This will help to prepare you for more advanced courses in genetics.

In this lab, you will need to *define* the traits in addition to determining how they are inherited. As a result, the discussion will be more complex and more like it would be in a research lab.

In this lab, each student will play the role of a creature with several characters. This will be simulated by a program that runs on the Palm Pilot called "Live Long and Prosper" which was developed at MIT. In this program, each Palm Pilot simulates a simple diploid organism with several characters.

Each organism lives for a particular amount of time and then dies. While you are alive, you can mate with some other creatures (your fellow classmates) by having your Palm Pilots communicate with each other using their Infra Red communication ports. Once you have mated, your creature is replaced by one of the possible offspring of you and your mate. In this way, you can perform crosses to determine how the various characters are inherited. You will need to coordinate with your classmates to devise and carry out experiments and to pool the class data to draw final conclusions.

Each student will sign out and use a Palm Z22; shown below:



Procedure

Part I: Starting the Palm Z22 and running the Genetics program

1) Press the POWER button. You should see something like the screen shown above. If you don't see the "Genetics" icon:

- tap the HOME button
- tap on the "CATEGORIES" menu at the top right of the screen
- select "Bio" from the menu that appears. You should see the screen above.

2) Tap on the "Genetics" icon. You will see this:

10/10/2020

2) Enter your name on the dotted line. You can do this by tapping on ABC in the lower left part of your Palm Z22, which brings up a keyboard. Tap in your name **but don't tap Start yet!!**

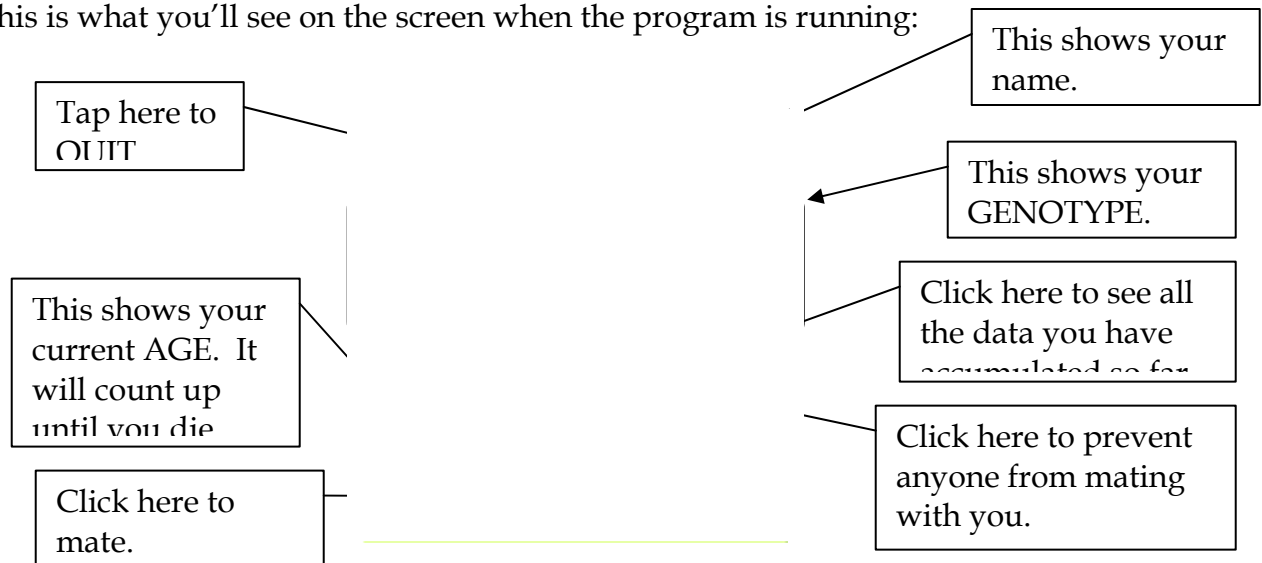
3) When everyone is ready, you should all tap "Start" at the same time. You will see this:

This part of the display shows your genotype.

- Each of the twisted things is a different gene.
- The order of the genes matters; that is, the left-most gene will code for the same character in all organisms in the class.
- Some genes code for characters, some don't. You will have to figure out which is which.
- The shading pattern shows the genotype of that gene. There are three possibilities:
 - Dotted
 - Striped
 - Blank

One of these corresponds to homozygous dominant, one to heterozygous, and the

This is what you'll see on the screen when the program is running:



Your goal is to figure out as much as you can about how the traits you've found are inherited.

Using the Genetics Program

Mating.

You can mate with other creatures by doing the following:

- be sure that both Palm Z22's are ready to mate
 - both should not be dead.
 - both should show the screen shown at the bottom of page 3.
 - the "Ready" button (above the "Mate" button) should say "Ready" not "Locked". If it is locked, tap the "Locked" button to make it ready.
 - be sure that there are no warning messages, etc. on either screen. If needed, tap the OK button on them to clear.
- lining up your Palm Z22's head to head (as shown below) and having ONE person tap the Mate button (one person is the sender and the other is the receiver).
- you will then get a "Finding Someone to Meet" message that tells you not to move your Palm Z22's while the mating is in process. This message will then disappear and one of the following will happen:
 - **No message appears:** this means that the two Palm's were unable to communicate. It may be possible for them to mate, but one or both of them are not ready to mate. See above for what to do to make them ready.
 - **A "Sorry" message appears** telling you that you and your partner are unable to mate. That means that the Palms did communicate and both organisms were ready, but they were genetically unable to mate. You will need to find out how this is inherited.
 - **A "Congratulations" message appears** telling you that you have mated, died, and been replaced by one of your offspring. When you reproduce successfully and become one of the offspring, your age will go back to zero, your generation will increase by 1 and your score will increase by whatever your age was at the time plus a bonus (age 21-40 = 5 points, age 41-60 = 10 points, age 60+ = 20 points).

Death. When you die, you will see something like this on your screen:

If you click “Start new game”, you will become a newborn creature with a randomly-assigned genotype; the underlying genetic model remains the same. If you click “View data”, you can look at the data you’ve collected so far (the matings and resulting offspring).

Part II: Warm-up

You should start off just trying to maximize your score so you can get a feel for how it’s working.

Part III: Hypothesis testing

As a class, you should discuss and implement strategies for figuring out what the characters are and how they are inherited.

Quitting the Program

You should tap on the “Genetics” menu at the upper left of the screen. Select “Exit Game” from the resulting menu. You should then turn the Palm Z22 off by pressing the POWER button.

Lab Report

This is the first year that we are doing this lab, so there is no lab report for this lab.

You will be given a grade based on your participation in the lab. This score will range from 0 (did not attend); 5 (attended but did not participate); to 20 (participated extensively). This score will be subjective and your TA has the final say on this score.

Bio 111 Pre-Lab for Lab #06: Name _____

Chemical Structures TA & Sect. _____

Note: You may find the Molecular Formula Calculator useful when answering the questions on this pre-lab. You can find it by going to the Bio 111 Home Page (<http://intro.bio.umb.edu/111-112>), clicking on the On-Line Lab Manual link, and going to the links for this lab.

1) Draw a molecule with the formula CH_2O . That is, a molecule that follows all the bonding rules and is made of exactly one atom of carbon, one atom of oxygen, and two atoms of hydrogen. Show all appropriate lone pairs and charges (if present).

2) Draw two different molecules, all having the same formula: $\text{C}_3\text{H}_5\text{NO}$. That is, each molecule should follow all the bonding rules and consist of 3 carbons, 5 hydrogens, one nitrogen, and one oxygen. Show all appropriate lone pairs and charges (if present).

a)

b)

3) This question deals with amino acid structure, which has yet to be covered in lecture. It is intended as a warm-up for the lab and the lectures. You should consult Campbell figure 5.17 for this problem. Pick any one of the 20 amino acids from Campbell figure 5.17 and draw it below. Circle the side chain with a solid line and draw a dotted line around the backbone.

Chemical Structures

Objectives

1. To practice with the different atoms used in Bio 111: to know the number of bonds made by each kind of atom, the structures that they form, and the charges they have.
2. To build molecular models of various bio-molecules.
3. To understand and be able to work with the different representations of molecules used in Bio 111.

Introduction

Matter is made up of approximately 100 elements. Of these, only carbon, hydrogen, oxygen, nitrogen, sulfur and about a dozen others are found in living organisms. Atoms of these elements can attach to one another by chemical bonds. There are three types of bonds important to us in Bio 111:

1. covalent bonds
2. hydrogen bonds
3. ionic bonds
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 ($-\text{C}-\text{C}-\text{C}-\text{C}-$) thus making possible very elaborate molecules with carbon "backbones".

Part I: Structures on paper and in 3-d

Using the model kits

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 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 electronegativities 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 bond are closer together than in a C-C, but not so close as in a C≡C bond.

Working in groups of three, build these molecules using the stick models and have your TA check them off.

1) Simple hydrocarbons.

methane CH₄

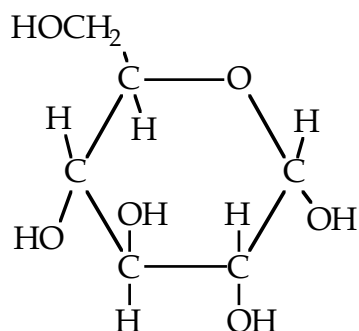
2) Alcohols

butanol C₄H₉-O-H

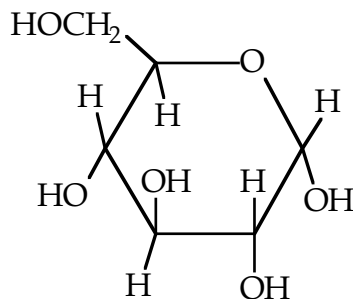
Note: there are 5 isomers of butanol. Three are structural isomers. Two are enantiomers - that is, they are mirror-image isomers (see Purves fig 2.21, page 32). Draw the three structural isomers and build models of the two enantiomers.

3) Sugars:

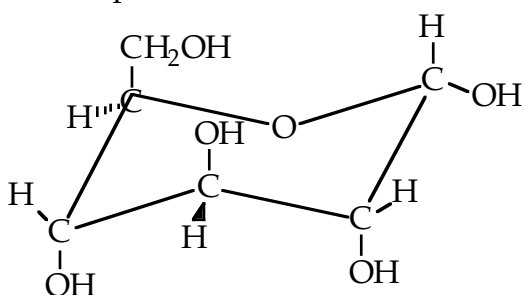
glucose



or:

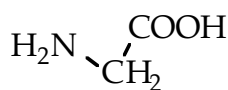


Note that all of the carbons in glucose are chiral – that is, it matters which groups point up from the ring and which point down. A more correct representation would be:

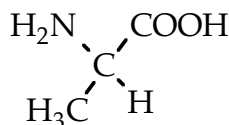


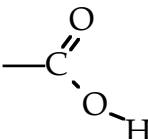
4) Amino acids

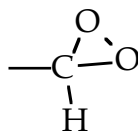
glycine



alanine

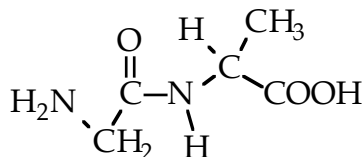


note that -COOH is  not

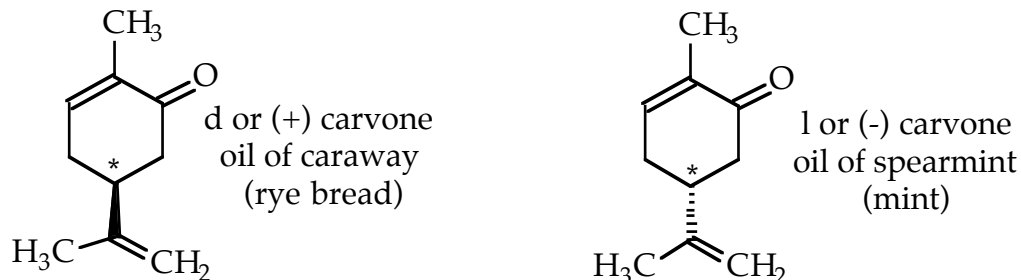


Take two amino acids and join them to make a dipeptide, such as the one below: what did you have to remove to make this molecule? You have made a peptide bond.

a dipeptide (glycine-alanine)



5) 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:



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.

Make one of the forms of carvone and have another group make the other. Convince yourself that they are not the same structure. They are mirror-image isomers, or enantiomers.

Your TA will give you samples of the two carvones to smell. Notice that their similar structures lead to similar smells, but there is a difference due to the slight difference in shape.

Part II: Molecules on the computer

Objectives

- To look at the structures of some important biological molecules and get a feel for their three-dimensional structure.
- To familiarize yourself with the representations of molecules used by the Jmol program, which we will use extensively in future labs.

Background

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

You will follow the exercise on the course web site for this lab and fill in the worksheet described below. You should work in groups of three; your worksheet will be a group effort for a group grade.

- 1) Go back to the dock at the bottom of the screen.
- 2) Click on the "Safari" icon.
- 3) Safari will start up and go to the Biology 111/112 home page.
- 5) Click on the link to the "OLLM" ("On-Line Lab Manual")
- 6) Click on the link to "Chemical Structures Exercises" for this lab.
- 7) Follow the exercise there and fill in the worksheet.

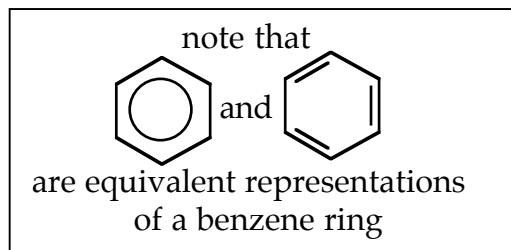
Briefly, you will:

(1) Based on the image on the computer, draw the structures of two sugars, glucose and fructose as well as an amino acid. These images have hydrogen atoms included as a warm-up. You will be asked to find the differences between the sugar structures and identify the amino acid.

(2) Your TA will assign you two randomly-chosen amino acids. You will look at its image in RasMol. You will draw the complete structure of each and identify which amino acid each is.

Lab report

There is no report for this part of the lab. You will turn in the worksheet to your TA at the end of lab to be graded. A copy of this worksheet is at the end of this section.



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Bio 111 Small Molecules Worksheet

Name _____ TA & Sect. _____
Name _____
Name _____ Score _____ / 20

This is a
group effort
for a group
grade.

This is due at the end of lab today.

The numbers of these questions correspond to the numbers on the web site.

1) Using the image from the web site, draw the structure of the linear form of glucose. You need not indicate the chiral parts of the molecule. (2 pts)

2) Using the image from the web site, draw the structure of the linear form of fructose. You need not indicate the chiral parts of the molecule. (2 pts)

3) On the structure in part (2), indicate the differences between glucose and fructose. (1 pt)

4) Using the image from the web site, draw the structure of the circular form of glucose. You need not indicate the chiral parts of the molecule. (2 pts)

5) On the structure in part (1), indicate which parts of the molecule have been linked to form ring structure. (1 pt)

6) Using the image from the web site, draw the structure of the amino acid for part (6). You need not indicate the chiral parts of the molecule. (1 pt)

7) Using the chart of amino acid structures in the lab manual, identify the amino acid you drew in part (6). (1 pt)

8) 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 hydrogens 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 hydrogens. Using the chart in the lab manual, identify the amino acid you have been assigned.

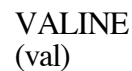
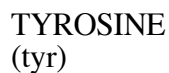
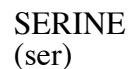
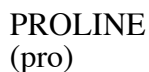
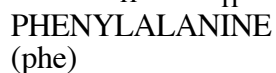
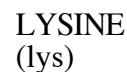
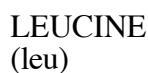
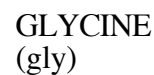
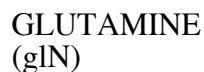
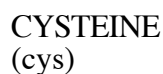
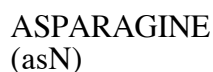
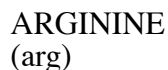
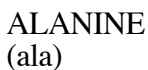
a) Number given by TA _____ Identity of amino acid _____ (2 pts)

Structure: (3 pts)

b) Number given by TA _____ Identity of amino acid _____ (2 pts)

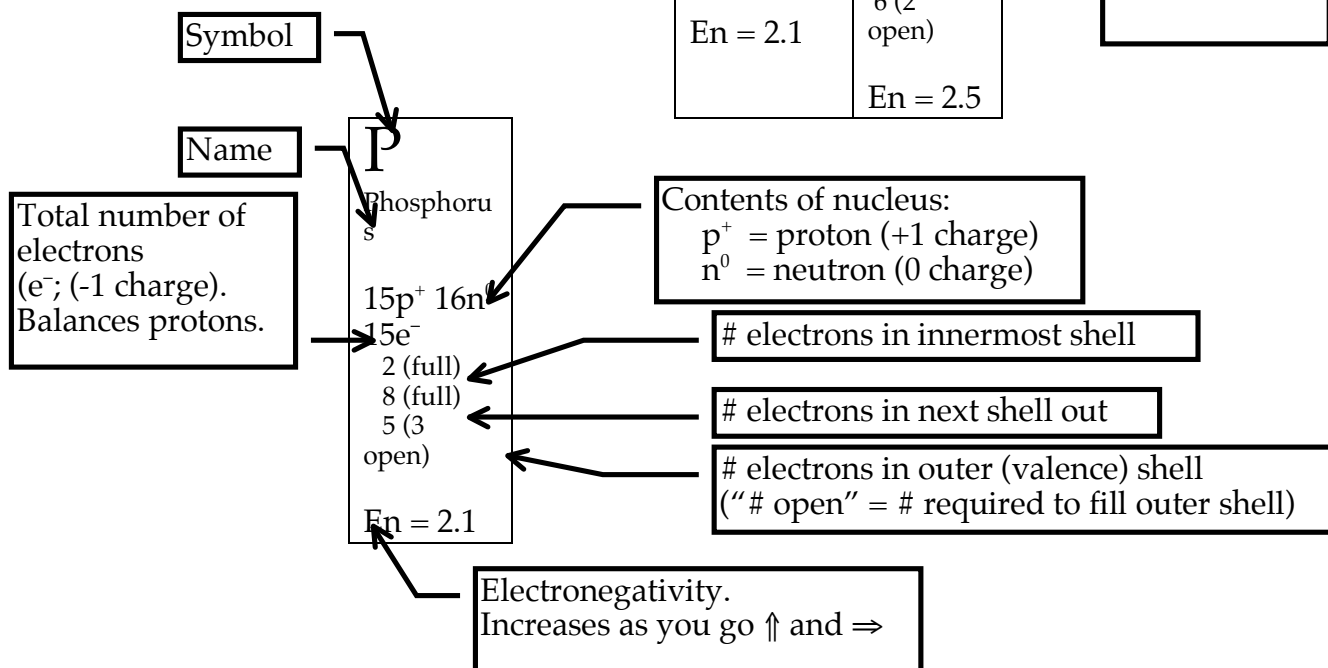
Structure: (3 pts)

STRUCTURES OF AMINO ACIDS



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



Bio 111 Pre-Lab for Lab #07: Name_____

Chemical Properties TA & Sect. _____

Choose any two molecules from Campbell, draw their structures below, indicate which one is more hydrophobic, and explain your reasoning briefly. You can use any two molecules from Campbell, but you must give the figure(s) where you found them.

Molecule 1

Campbell figure_____

Structure: (draw below)

Molecule 2

Campbell figure_____

Structure: (draw below)

Which molecule is more hydrophobic? (circle)

Molecule 1

Molecule 2

Explain your reasoning:

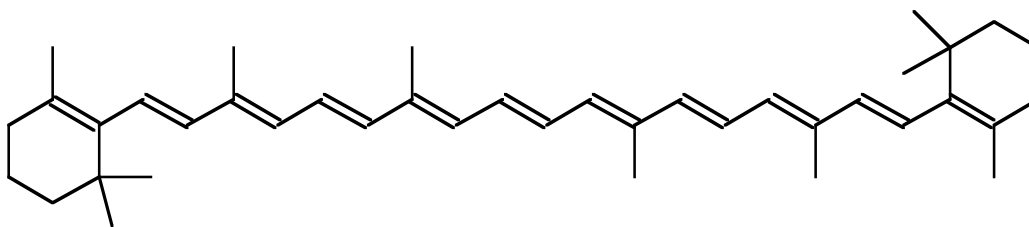
Chemical Properties

Objectives:

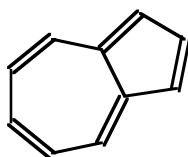
- to get a clearer understanding of hydrophobic/hydrophilic molecules
 - how to 'read' structures
 - the effects of various groups of atoms on the properties of a molecule
- to learn how to use a software program that you can use at home to strengthen your understanding of hydrophobic/hydrophilic molecules

Part I: Questions for Discussion:

1) Consider the four dye molecules:

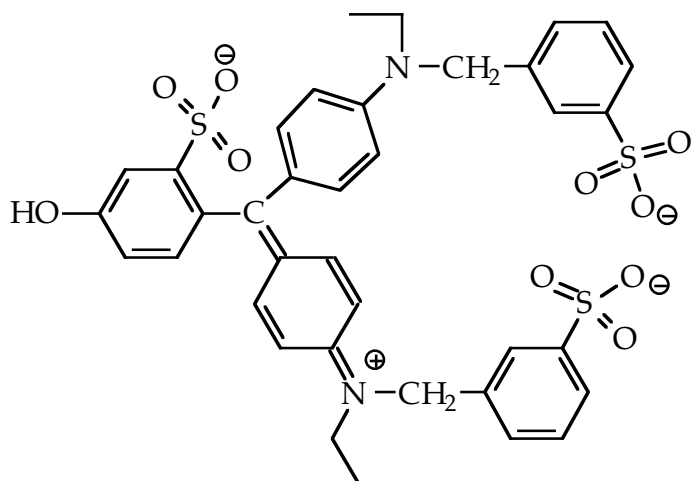


β -carotene (orange-red; present in carrots, etc.)

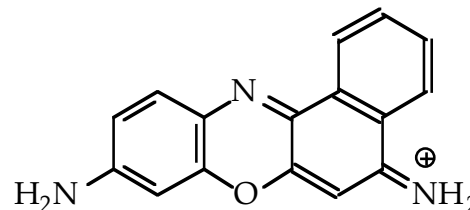


azulene
(deep purple)

fast green FCF
(blue-green)



cresyl violet
(red-purple)



For each of the molecules above, indicate the parts that are hydrophobic and those that are hydrophilic.

2) Two of these dyes are soluble in water and two are not. Predict which is which in terms of the bonds that each molecule can make with water and their hydrophobicity/hydrophilicity.

3) Your TA will demonstrate this solubility as follows. She will have prepared tubes with water on the bottom layer and hexane ($\text{H}_3\text{CCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ – very hydrophobic) on the top layer (remember that oils like hexane float on water). She will then add a small amount of a solution of each dye to the test tubes.

- Hydrophilic dyes will make favorable interactions with water, leaving the water colored and the hexane colorless.
- Hydrophobic dyes will not make favorable interactions with water and the hydrophobic effect will drive them to be in the hexane. Thus they will color the hexane and not the water.

Based on your predictions in part (2), predict which dyes will be found in water or hexane.

4) Work through problem 1.2.5 from Chapter 2 of APAIB.

Part II: Exercises on the Computer

The exercises in this lab can be found in *A Problems Approach to Introductory Biology* chapter 2, problem C4 (page 105). You may find it useful to review the use of the computer software using problem C1 (page 86). You can use the software on the APAIB CD-ROM or from the course website; go to the page for this lab in the On-Line Lab Manual.

You should do part (1) in your book; part (2) should be done on the attached worksheet.

Bio 111 Chemical Properties Worksheet

Name _____

Name _____

Name _____

TA & Sect. _____

Score _____ / 30

This is due at the end of lab this week.

Use the computer program to answer the questions below.

1) In organic chemistry "R" is shorthand used to represent "the rest of the molecule". To answer this question, you can use the "R group" of your choice; just be sure that you use the same "R group" for all four molecules.

Consider the following 4 molecules:



For a given R-group, two of these have high logP values and two have low logP values.

a) Choose an R group of your own design, draw the four variations of this molecule (R-CH_3 , R-OH , R-SH , and R-NH_2), and give their logP values. (4 pts)

b) In terms of the polarity of the bonds involved, explain why the two molecules with high logP are more hydrophobic and why the two with low logP are more hydrophilic. (6 pts)

2) Ethanol ($\text{H}_3\text{CCH}_2\text{OH}$) and di-methyl-ether (H_3COCH_3) have the same number of carbons, hydrogens, and oxygens ($\text{C}_2\text{H}_6\text{O}$) but differ in the following important way: in ethanol, the O is bonded to a carbon and a hydrogen, but in di-methyl-ether, the O is bonded to two carbons.

Create a similar pair of molecules.

- Both members of this pair should have the same number of carbons and hydrogens (check this using the formula that jlogp calculates).
- Both members should have only one oxygen (check this using the formula)
- One member should have the oxygen bonded to a carbon and a hydrogen; the other should have the oxygen bonded to two different carbon atoms.

a) Draw the two molecules. (3 pts)

b) In terms of their capability of forming bonds with water, predict which will be more hydrophobic and explain your reasoning. (5 pts)

c) Give the logP values for your two molecules. Do they agree with your prediction? (2 pts)

3) Adding an -OH (hydroxyl) group makes a molecule more hydrophilic; adding a -CH₃ (methyl) makes a molecule more hydrophobic. Approximately how many -CH₃'s are required to counterbalance the effect of an -OH? Note that this will depend on many factors and will not be the same for all molecules. (3 pts)

a) Start with a molecule of your choosing. Draw it below & calculate its logP: _____

b) Add an -OH to the molecule from part (3a). Draw it below & calculate its logP: _____

c) Keep adding -CH₃'s to the molecule from part (3b) until it has approximately the same logP as the original molecule (3a). Draw the molecule below, fill in the number of -CH₃'s you had to add, and give the logP. Discuss the results with your classmates.

of -CH₃'s required _____

logP _____

4) Adding a charged group -O⁻ or -NH₃⁺ group makes a molecule much more hydrophilic; adding a -CH₃ (methyl) makes a molecule more hydrophobic. Approximately how many -CH₃'s are required to counterbalance the effect of a charged group? Note that this will depend on many factors and will not be the same for all molecules. (3 pts)

a) Start with a molecule of your choosing. Draw it below & calculate its logP: _____

b) Add a charged group to the molecule from part (4a).

Draw it below & calculate its logP: _____

c) Keep adding -CH₃'s to the molecule from part (4b) until it has approximately the same logP as the original molecule (4a). Draw the molecule below, fill in the number of -CH₃'s you had to add, and give the logP. Discuss the results with your classmates.

of -CH₃'s required _____

logP _____

5) The shape of a molecule can also have an effect on its hydrophobicity. Create a pair of isomeric molecules. (4 pts)

- Both members of this pair should have the same formula (the same number of carbons, hydrogens, nitrogens, etc.); check this with the formula that jlogp calculates.
- One member should have a 'long and thin' shape; the other should be 'short and fat'; this makes them structural isomers.

Draw your two molecules and label them with their logP values. Discuss the results with your classmates.

Bio 111 Pre-Lab for Lab #08: Name_____

Protein Structure I TA & Sect._____

1) Choose any two amino acids from Figure 5.17 of Campbell, give their names and characterize their side chains by checking the boxes as appropriate.

<u>Amino acid</u>	<u>'phobic/'philic</u>	<u>Bonds it can make*</u>
Name:	<input type="checkbox"/> 'phobic <input type="checkbox"/> 'philic	<input type="checkbox"/> H-bond(s) <input type="checkbox"/> ionic bond(s) <input type="checkbox"/> 'phobic interaction <input type="checkbox"/> van der Waals
Name:	<input type="checkbox"/> 'phobic <input type="checkbox"/> 'philic	<input type="checkbox"/> H-bond(s) <input type="checkbox"/> ionic bond(s) <input type="checkbox"/> 'phobic interaction <input type="checkbox"/> van der Waals

*Assuming that a suitable partner exists.

2) Which interaction(s) are possible between the side chains of the two amino acids you selected?

- ☐ H-bond(s)
- ☐ ionic bond(s)
- ☐ 'phobic interaction
- ☐ van der Waals
- ☐ none

Protein Structure I

Objectives:

In this lab you will work in groups to map out the primary, secondary, and tertiary structure of a small protein, the enzyme lysozyme. This will be an application and a demonstration of the principles discussed in lecture.

Introduction:

This problem deals with the structure of the protein lysozyme. Lysozyme is a single polypeptide chain 129 amino acids long.

Lysozyme is found in many organisms. It is an enzyme that specifically breaks down the cell wall of certain types of bacteria. Lysozyme binds to the cell wall molecule (called the substrate) and breaks one of the covalent bonds that hold this molecule together. Breaking down the cell walls makes the bacteria fragile and they rapidly burst open and die. Lysozyme is thus an example of the non-specific immune system. The lysozyme you will be looking at was obtained from hen's egg whites; it protects the developing chick from bacterial infection.

In order to look at the structure of lysozyme, we will use the molecular viewing program Jmol. Jmol allows the user to rotate a 2-dimensional representation of a 3-dimensional molecule, as well as highlight particular regions and groups.

Procedure:

Part I: Anatomy of Lysozyme

- 1) You will work in groups of three people per computer. You may want to take turns using the computer.
- 2) To find the program, you must first get to the Dock and click on the Safari icon.
- 3) Go to the OLLM for this lab and click on the "Explore Lysozyme" link for this lab.

You should then see this:

This shows the molecule under study. In this window, you can:

- rotate the molecule by holding the mouse button down while dragging the cursor in the window.
- Zoom in or out by holding the shift key while dragging up or down.
- Identify atoms in the molecule by clicking on them. Information on the last atom clicked on can be found in Safari's "Status Bar" located at the bottom of Safari's window. For example: amino acid #77, which is an asparagine (Asn). The "O" in "OD1" means that you

These are the controls for changing the views as required for different problems.

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

This is the color scheme for the atoms in the display.

4) To see the lysozyme molecule and the substrate it is binding to, click the button marked "Show Substrate in purple". You should get a picture like this:

The thin lines represent the **covalent** bonds in the lysozyme molecule. They are colored according to

This is the substrate that the lysozyme is breaking down.

You may want to rotate the image to get a better view.

5) There are 129 amino acids in the lysozyme protein chain; not all of them are interesting from our point of view. Each lab group will be responsible for analyzing one set of 5 consecutive amino acids. Your TA will tell you the 5 amino acids that you are responsible for:

Side-chain Location	Interior (In)					
	Exterior (Out)					
	Substrate-Binding (Sub)					
	Can't Tell (?)					
Side-chain type	Hydrophobic ('phobic)					
	Hydrophilic ('philic)					
2° Structure	α -Helix (helix)					
	Turn (turn)					
	β -Sheet (sheet)					
	Random Coil (random)					
	Amino Acid					

	Number					
--	--------	--	--	--	--	--

Your assignment is to complete this chart for the amino acids assigned to you by your TA.

6) The first task is to determine the location of each amino acid. Is it inside the protein, on the surface, in the substrate binding area, or you can't tell for sure. To do this, you must select each amino acid in turn and look at its position. To select amino acid #45, you pick "show 45 and substrate" from the menu for part (6).

You should get a picture like this in the Main Window:

The small part made of balls is the amino acid you selected.

The large part made of balls is the substrate.

This is the rest of the protein.

Rotate the protein around until you can get a good look at where the amino acid is located. In this case, the amino acid is on the surface of the protein, so you would shade in the box in the "Outside Protein" row above the "Amino Acid" #45. Be sure to look at where the **side chain** is located; you can tell the side chain from the backbone because the backbone parts are connected to the rest of the protein by thick blue lines.

surface of protein						
Unsure						
Inside Protein						
Substrate Binding						
Amino Acid #	43	44	45	46	47	48

If the amino acid was on the inside, you'd shade the "Inside Protein" box.

If the amino acid was in the cleft that the substrate fits into, you'd shade the "Substrate Binding" box.

For some amino acids, it will be difficult to tell for sure where it is, in that case, shade "Unsure".

Do this for each amino acid in that your TA assigned.

7) The next task is to determine whether the side chains of the amino acids in your set are hydrophobic or hydrophilic. Shown below is a list of the amino acid sequence of lysozyme. The amino acids are listed in order from # 1 (the amino terminus) to # 129 (the carboxyl terminus). Look up each amino acid in your set and, using the sheet of amino acid structures on the last page of this handout, determine if each is hydrophobic or hydrophilic. Shade the appropriate box on your sheet.

1	5	10	15	20															
LYS	VAL	PHE	GLY	ARG	CYS	GLU	LEU	ALA	ALA	ALA	MET	LYS	ARG	HIS	GLY	LEU	ASP	ASN	TYR
21	25	30	35	40															
ARG	GLY	TYR	SER	LEU	GLY	ASN	TRP	VAL	CYS	ALA	ALA	LYS	PHE	GLU	SER	ASN	PHE	ASN	THR
41	45	50	55	60															
GLN	ALA	THR	ASN	ARG	ASN	THR	ASP	GLY	SER	THR	ASP	TYR	GLY	ILE	LEU	GLN	ILE	ASN	SER
61	65	70	75	80															
ARG	TRP	TRP	CYS	ASN	ASP	GLY	ARG	THR	PRO	GLY	SER	ARG	ASN	LEU	CYS	ASN	ILE	PRO	CYS
81	85	90	95	100															
SER	ALA	LEU	LEU	SER	SER	ASP	ILE	THR	ALA	SER	VAL	ASN	CYS	ALA	LYS	LYS	ILE	VAL	SER
101	105	110	115	120															
ASP	GLY	ASN	GLY	MET	ASN	ALA	TRP	VAL	ALA	TRP	ARG	ASN	ARG	CYS	LYS	GLY	THR	ASP	VAL
121	125	129																	
GLN	ALA	TRP	ILE	ARG	GLY	CYS	ARG	LEU											

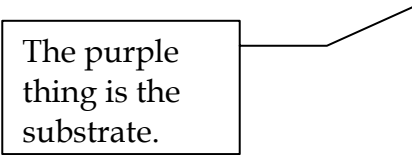
8) The last part of the analysis is to determine the secondary structure of the part of the protein you are looking at. For this, you need to set up the display as follows.

- Make sure that the display is back to normal. If it is in some strange mode, quit the program and re-start it by double-clicking on "cytC.pdb".
- RasMol> select ligand
- select "Spacefill" from the "Display" menu
- RasMol> color purple
- RasMol> select protein
- select "Backbone" from the "Display" menu.
- select "Structure" from the "Colours" menu.

You should get something like this:

The fat line is the backbone of the protein chain. The colors and shapes indicate the secondary structure of the backbone at that point:

<u>Color</u>	<u>Secondary Structure</u>
red	alpha helix (α -helix)
yellow	beta sheet (β -sheet)
pale blue	turn
white	random coil



The purple thing is the substrate.

You can find out which amino acid you are looking at by clicking on the part of the backbone you are interested in. Jmol automatically prints to Safari's "Status Bar" the following info about what you clicked on:

For example, if you clicked on one part of the backbone and got QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

The important information is the "[GLY].126" part. It says that you clicked on amino acid #126 and that amino acid is a glycine.

Using this technique, you can trace the backbone to your set of amino acids and determine their secondary structure. Shade the appropriate boxes.

Once you are all done with your set, go to the computer at the front of the class and enter your data into the "Lysozyme" program that your TA will have set up. Select the appropriate values from the menus for your amino acids.

Once the class has entered all their data, discuss the questions on the next page.

9) Once all groups have finished, you will pool your data. Enter into the “Lysozyme” application as demonstrated by your TA. As a class, answer the following questions:

a) Is there a correlation between whether an amino acid is found on the inside or outside of the protein and its hydrophobicity/hydrophilicity? Does this make sense in terms of what we know about chemistry?

b) Is there any pattern to the secondary structure? (for example alpha helix always follows beta sheet, etc.)

c) The side chains of the amino acids in the inside of the protein are clustered together in the tertiary structure of the protein; are they close together in the primary structure as well?

d) The side chains of the amino acids in the substrate binding pocket are clustered together in the tertiary structure of the protein; are they close together in the primary structure as well?

e) The side chains of amino acids 6 and 124 are very close in the folded protein, but very far apart along the backbone. How is this possible?

f) Amino acid #56 in lysozyme is leucine.

- Mutant lysozymes where position #56 is arginine are non-functional.

Explain this observation in terms of the properties of the amino acids, the location of Leu56 in lysozyme.

g) Amino acid #62 in the lysozyme is tryptophan.

- Mutant lysozymes where position #62 is phenylalanine or tyrosine have virtually the same shape as normal lysozyme. However, these mutant lysozymes are altered in their ability to interact with the substrate.

Explain these observations in terms of the properties of the amino acids, the location of Trp62 in lysozyme.

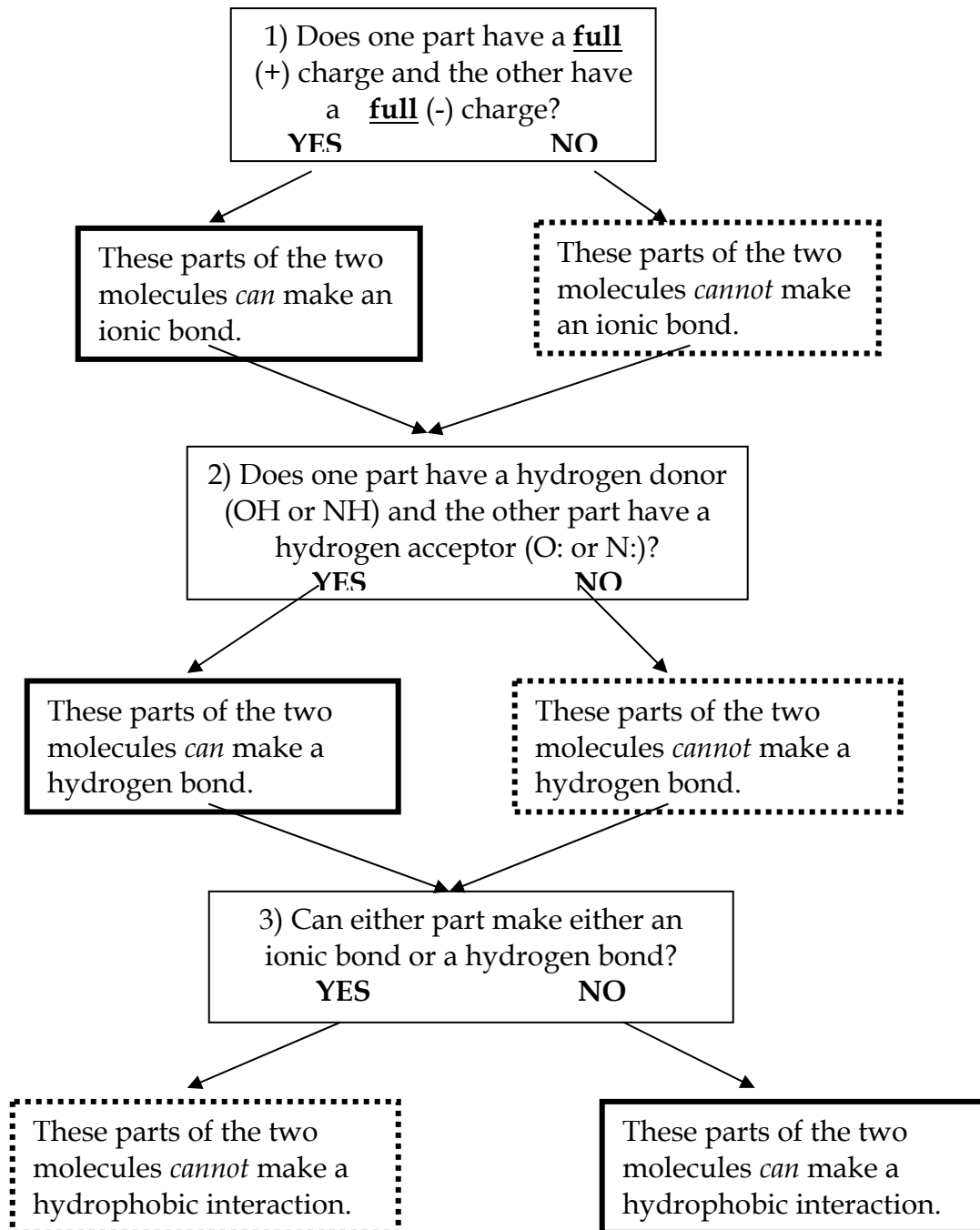
Part II: Side-chain interactions

In this part of the lab, you will examine specific interactions between different side chains of the protein and between side chains of the protein and the substrate.

Part II (a) Interactions between side chains in the protein.

Notes:

- for each of these questions, simply click the button for that part.
- You may find it useful to switch back and forth between the two views:
 - “Ball and stick” - this shows atoms as small balls; the **covalent** bonds connecting them are shown as rods. This is useful for finding out which atoms are covalently bonded and which are not.
 - “Spacefill” - this shows atoms as large spheres at approximately their appropriate sizes. This is useful for figuring out which atoms are near each other.
- The program shows the **covalent** bonds, your job is to infer the presence of various non-covalent interactions using your knowledge of structure and bonding. The chart on the next page outlines this process:



(10) Amino acid #1 is lysine; amino acid #7 is glutamic acid.

a) Given their structures, what type(s) of interactions are *possible* between the side chains of these amino acids?

b) Which of these interactions is the strongest?

c) Highlight these two amino acids in the structure of lysozyme. (Click “select 1 or 7”). Which part(s) of their side chains are closest together (backbone, middle of side chain, tip of side chain, etc.)? (Note that you can click on atoms to find out what side chain they are part of as in part (8) of section I).

d) Draw the structure of the parts of the side chains that are closest together and indicate the bond you named in part (b). Use a “...” to show where the atoms you’ve drawn are covalently bonded to the rest of the molecule.

(11) Use jMol to find other ionic bonds in lysozyme

Researchers often use tools like jmol to find particular types of interactions. If you click the button marked “Show Charged Side Chains”, the side chain atoms of all the (+)-charged (arg, his, and lys) and the (-)-charged (asp and glu) side chains. Using this view, try to find as many possible ionic bonds as you can. You may want to switch back and forth between spacefill and ball and stick views as well.

Hint: it will be easier to look for patterns of color. In general, which atoms have a (+) charge and which have a (-) charge? Look for (+)’s near (-)’s.

You may well ask, “How far apart can they be and still be ionic bonded?” The answer is no farther than one spacefill atom’s diameter (the attraction decreases with the square of the distance).

List possible ionic bonded pairs below:

(12) Amino acid #3 is phenylalanine; amino acid #8 is leucine; amino acid # 55 is isoleucine; amino acid #88 is isoleucine.

a) Given their structures, what type(s) of interactions are *possible* between the side chains of these amino acids?

b) Which of these interactions is the strongest?

c) Highlight these amino acids in the structure of lysozyme. (Click "Show 3, 8, 55, and 88"). Which part(s) of their side chains are closest together (backbone, middle of side chain, tip of side chain, etc.)? (Note that you can click on atoms to find out what side chain they are part of as in part (8) of section I).

d) Are the locations of these side chains relative to one another consistent with the bond you named in part (b)? Why or why not?.

(13) Amino acid #46 is asparagine; amino acid #52 is aspartic acid.

a) Given their structures, what type(s) of interactions are *possible* between the side chains of these amino acids?

b) Which of these interactions is the strongest?

c) Highlight these two amino acids in the structure of lysozyme. (Click "Show 46 and 52"). Which part(s) of their side chains are closest together (backbone, middle of side chain, tip of side chain, etc.)? (Note that you can click on atoms to find out what side chain they are part of as in part (8) of section I)

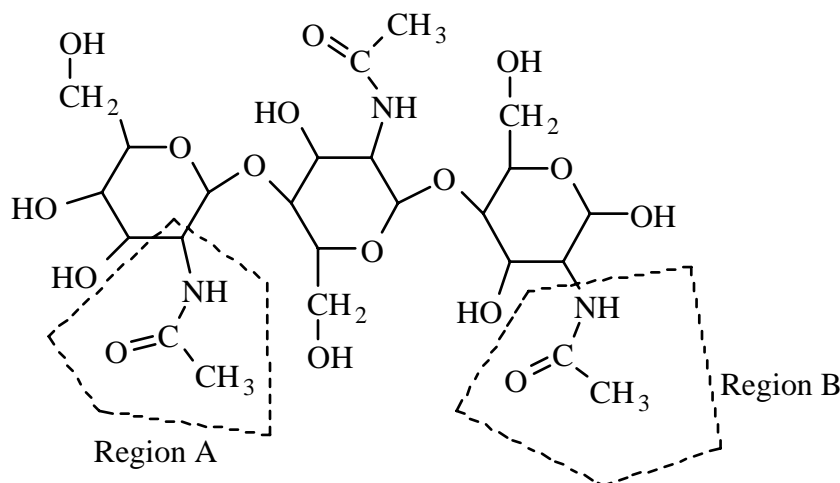
d) Draw the structure of the parts of the side chains that are closest together and indicate the bond you named in part (b). Use a "..." to show where the atoms you've drawn are covalently bonded to the rest of the molecule.

(14) Amino acid #33 is lysine; amino acid #34 is phenylalanine; amino acid #38 is phenylalanine. {this is a challenging one!}

- a) Given their structures, what type(s) of interactions are *possible* between the side chains of these amino acids?
- b) Which of these interactions is the strongest?
- c) Highlight these amino acids in the structure of lysozyme. ("Show 33, 34, and 38"). Which part(s) of their side chains are closest together (backbone, middle of side chain, tip of side chain, etc.)? (Note that you can click on atoms to find out what side chain they are part of as in part (8) of section I)
- d) Sketch (you need not draw every atom, just the general shapes) the structure of the parts of the side chains that are closest together and indicate the type of bond that is most-likely occurring between the side chains of these three amino acids.

Part II (b): Interactions between side chains and the substrate.

The structure of the substrate is shown below, with two important regions circled.



- (15) Amino acid #101 is aspartic acid; it's side chain interacts with region A of the substrate.
- Given their structures, what type(s) of interactions are *possible* between the side chain and this part of the substrate?
 - Which of these interactions is the strongest?
 - Highlight the amino acid and the substrate in the structure of lysozyme. (Click "Show 101 and substrate"). Which part of the side chain is closest to the substrate (backbone, middle of side chain, tip of side chain, etc.)? (Note that you can click on atoms to find out what side chain they are part of as in part (8) of Section I).
 - Draw the structure of the parts of the side chain and the substrate that are closest together and indicate the bond you named in part (b). Use a "..." to show where the atoms you've drawn are covalently bonded to the rest of the molecule.

Lab Report

Your lab report must consist of the following:

- (A) Answers to questions (10) through (15) - including parts (a) through (d) for all questions.
(B) Design a hypothetical binding site.

(B1) Pick a molecule from Purves. It can be any molecule from anywhere in the book, but it must be found in Purves. Hints: don't pick one that is too large or it will be hard to draw. You should also choose a molecule that is capable of making at least one H-bond, ionic bond, and hydrophobic interaction. Give

the name of the molecule and the figure in Purves where you found it.
(B2) Draw the molecule. Circle one hydrophobic part and one hydrophilic part.
Clearly indicate which is hydrophobic and which is hydrophilic.

(B3) Draw the molecule again, this time surrounded by a simple binding site consisting of 3 amino acids of your choosing. Choose your amino acids so that:

- The side chain of one amino acid interacts with your molecule via a hydrogen bond.
- The side chain of another amino acid interacts with your molecule via an ionic bond.
- The side chain of the last amino acid interacts with your molecule via a hydrophobic interaction.

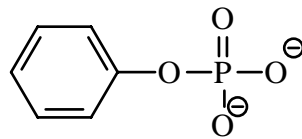
Be sure to clearly indicate the nature of each interaction.

(B4) Choose one of the amino acids from part B3.

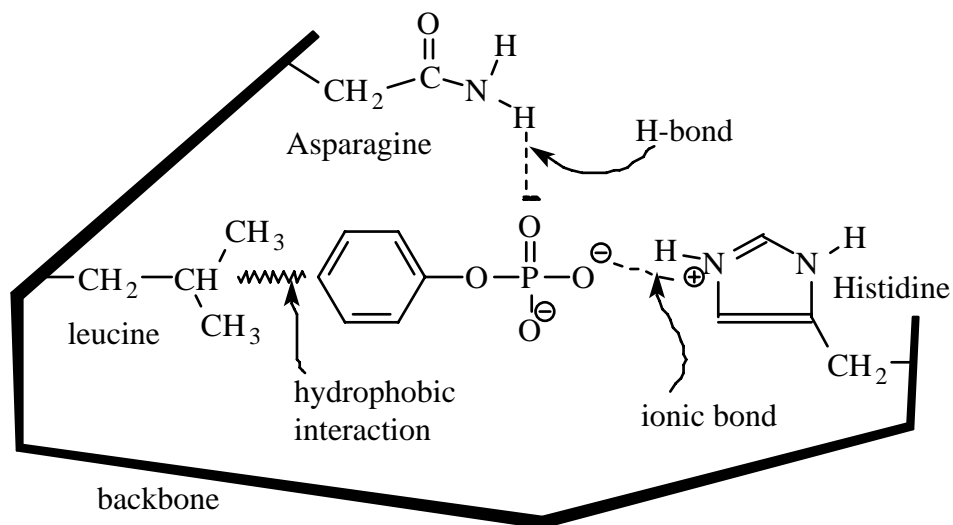
- Describe a mutation that would alter that amino acid. **Note:** mutations only affect one amino acid (there are exceptions that we will learn about later) **and** mutations can only result in the replacement of one amino acid by another (for example, cystine to serine); mutations **never** alter the structure of an amino acid (for example having cystine lose its SH group since the resulting molecule is not one of the 20 amino acids on the chart).
- Describe the effect of that mutation on the binding of your molecule and explain your reasoning.

An example is shown below. Note that the molecule used in this example is not found in Purves.

(B1) Phenyl phosphate.



(B2)

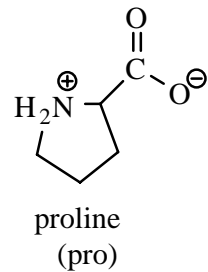
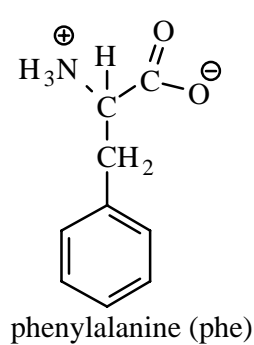
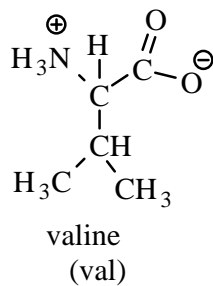
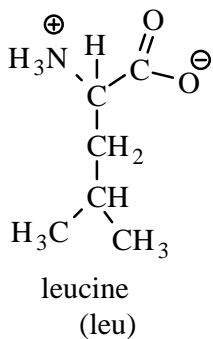
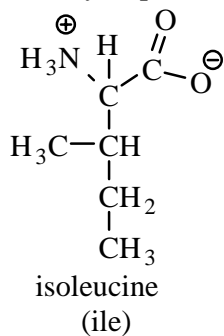


- (B3)
- i) The side chain of the asparagine interacts with the O via a hydrogen bond as shown above.
 - ii) The side chain of the histidine interacts with the O⁽⁻⁾ via an ionic bond as shown above.
 - iii) The side chain of the leucine interacts with the carbon ring via a hydrophobic interaction as shown above.
- (B4) A mutation which changed the histidine to a glutamic acid would reduce the strength of binding of phenyl phosphate to my enzyme. This is because now there would be a (-) charge on the glutamic acid right next to the (-) charge on the phosphate - since like charges repel, the phenyl phosphate would be repelled out of the binding site.

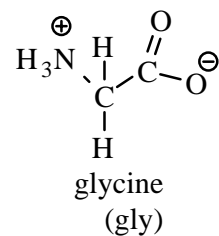
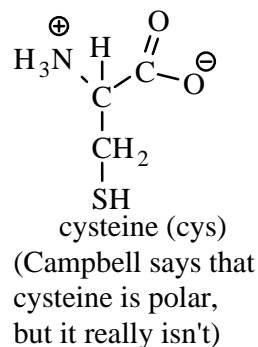
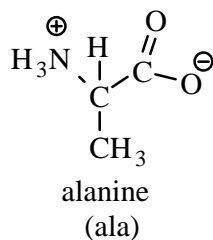
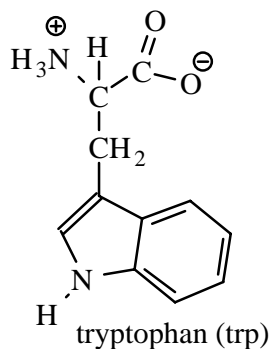
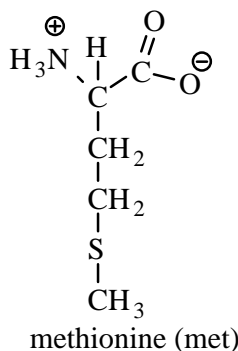
Amino Acids

Non-polar

most hydrophobic

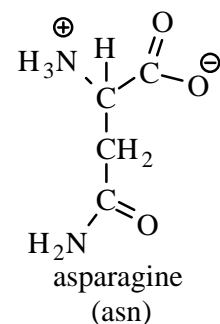
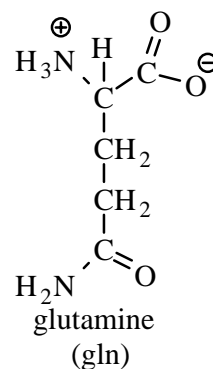
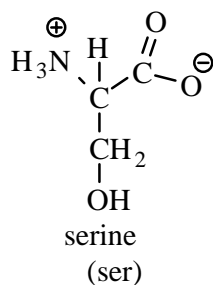
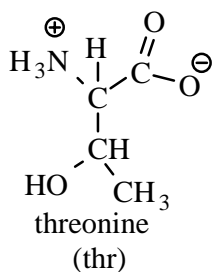
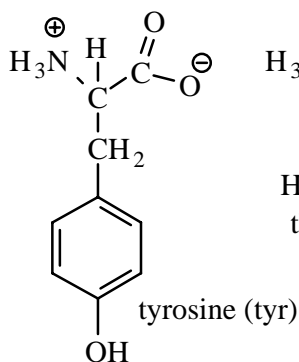


least hydrophobic (but still non-polar)



Polar (but uncharged)

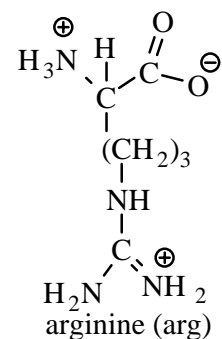
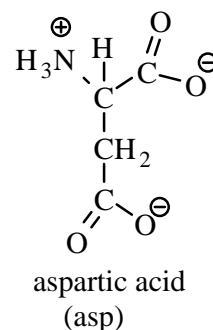
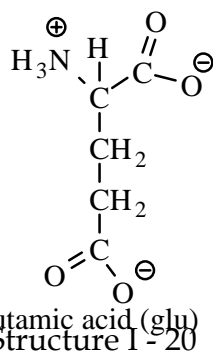
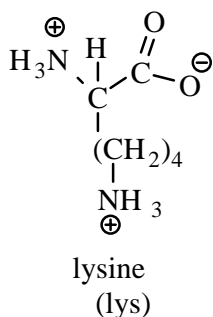
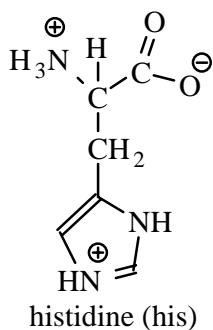
least hydrophilic



most hydrophilic

Charged

least hydrophilic



most hydrophilic

Bio 111 Pre-Lab for Protein Structure II

Name _____
TA & Sect. _____

1) For each of the simplifications listed on page Protein Investigator-4, describe how the simplification differs from real life. That is, if we had said "The protein exists in two dimensions only.", the correct response would be, "Real proteins are 3-dimensional." (2 pts each)

a)

b)

c)

d)

2) Based on the description given in the lab manual, what would you expect the folded shape of the protein N-leu-leu-leu-leu-C to be? Sketch this protein. Note that we will grade this part generously. (2 pts).

Protein Structure II: Protein Investigator

Objectives

- To learn more about the interactions that govern protein structure.
- To test hypotheses regarding protein structure and function.
- To design proteins with specific shapes.

Introduction

In the previous Protein Structure Lab, you explored the enzyme lysozyme. This allowed you to see a protein in its full 3-dimensional form and to make hypotheses about the interactions between various side chains. However, you were not able to test your hypotheses because the program did not allow you to make changes to the protein sequence and observe their effects on the protein's structure and function.

In this lab, you will get an opportunity to interactively explore protein structure and function in a simplified system. 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 reconnected 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 does not model disulfide bonds.
- 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....

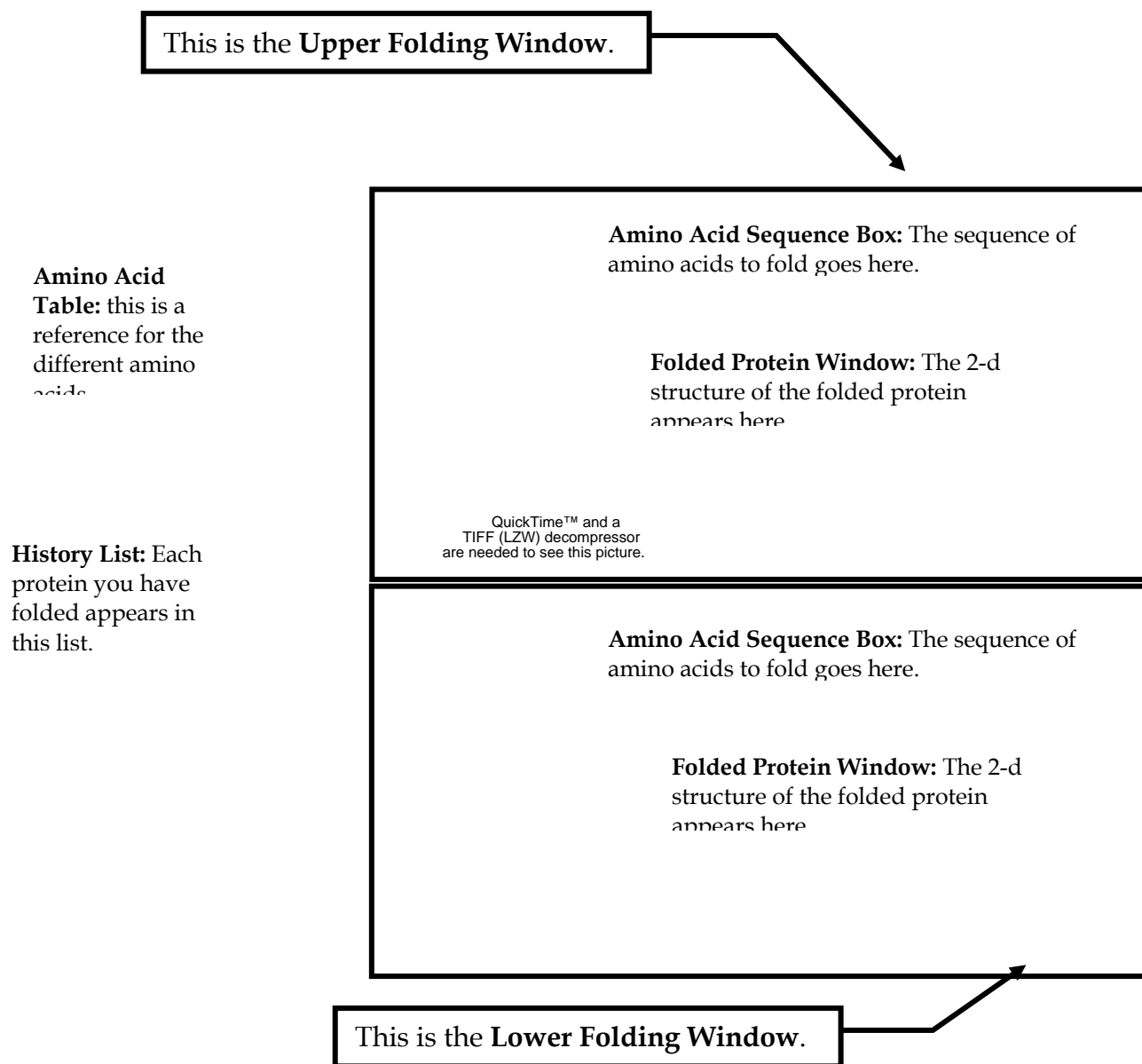
The second reason is educational. As you saw in the lysozyme lab, proteins are complex 3-dimensional molecules. It can be hard to find your way around when inside one. Likewise, it would be very difficult to visually compare two protein molecules to observe the effects of changes to their amino acid 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 in Bio 111 while being simple and fast.

One other note: this is new software. It was written in the Spring of 2005 by a group of Computer Science Graduate Students at UMB and tested in Bio 111 in the Fall of 2005. The first version crashed a lot. The program was extensively revised in the Spring of 2006. You are the first real heavy use testers of the new version. Please be patient and gentle with it and report any bugs and/or suggestions to your TA. You may also want to save your History List from time to time to protect against crashes (see page 6 for how to do this).

Procedure

- 1) Start the Protein Investigator. Click on the ProtInv icon in the Dock. It looks like this:
- 2) ProtInv should start. You should click in the lower right corner and drag the window so that it fills the screen. You will see this:



Using ProtInv

There are some important parts of the program that you should know about:

- **Menus:** there are three menus at the top of the ProtInv window:
 - **File:** this has two items:
 - **Print:** this prints the amino acid sequences and structures of the proteins in the upper and lower Folding Windows.
 - **Quit:** this quits the program.
 - **History List:** this has five items related to the History List:
 - **Save:** this saves the current History List to a .histlist file. You will need to provide a name for this file.
 - **Save As...:** this saves the current History List to a .histlist file you name.
 - **Load:** this clears the History List and replaces it with the contents of a .histlist file you have selected.
 - **Delete Selected:** this deletes the selected item from the History List. This is not un-doable.
 - **Clear:** this deletes all the entries in the History List. This is also not un-doable.
 - **Information:** this has 2 items:
 - **Help:** the help file with instructions on how to use ProtInv.
 - **About:** some information from the authors of ProtInv.
- **Buttons:** there are two buttons in the middle of ProtInv:
 - **> Upper:** clicking this sends the selected entry in the History List into the Upper Folding Window.
 - **> Lower:** clicking this sends the selected entry in the History List into the Lower Folding Window.
- **Combined Color:** this patch shows the color that would result from combining the colors of the two proteins in the Folding Windows. This is useful when attempting the challenge problem on page 15.

Note: the program may tend to crash a lot. When it does, you will lose all the proteins in your **History List**. To protect yourself against this, you should:

- take notes on the proteins you've folded (write down the amino acid sequences and sketch their shapes)
- regularly save your history list to a file on the desktop; here's how:
 1. Choose **Save** from the **History List** menu.
 2. Select the Desktop from the list of files/folders that appears.
 3. Type in a descriptive name for your history list. If you forget to add the .histlist extension, the program will add it for you.
 4. Click "Save". You **History List** will now be saved to this file whenever you select **Save** from the **History List** menu.
- you can re-load a saved **History List** like this:
 1. Choose **Load** from the **History List** menu.
 2. Select the Desktop (or wherever you saved the .histlist file)

3. Select your .histlist file.
4. Click “Open”. The **History List** will now be replaced by the contents of the file you selected.

In most of the next parts, we will ask you to predict what you think will happen before you actually try it. Although you can easily cheat and skip this step, we *strongly* suggest that you take the time to make a prediction. This will give you a better chance to apply and test your knowledge and will prepare you better for the exams on this material.

The One-Letter Amino Acid Code

So far in Bio 111, we have been using the full names of amino acids as well as three-letter abbreviations. There is an even shorter abbreviation for amino acids that uses only one letter for each. Because of its simplicity, you will use this code to *type* protein sequences into ProtInv. This one letter code is used in more advanced biology classes, so it is worth familiarizing yourself with it. Because several amino acids start with the same letter, some of the single letter codes are a little obscure; the mnemonics in the table below will help you learn them. In ProtInv, you *type* the single letter code but the program *shows* you the three-letter code for easier reading. You can use this table or the **Amino Acid Table** in ProtInv for reference.

<u>Amino Acid</u>	<u>3-letter code</u>	<u>1-letter code</u>	<u>Mnemonic</u>
Alanine	Ala	A	<u>A</u> lanine
Arginine	Arg	R	a <u>R</u> ginine
Asparagine	Asn	N	aspara <u>giN</u> e
Aspartic acid	Asp	D	aspar <u>D</u> ic acid
Cystine	Cys	C	<u>C</u> ystine
Glutamine	Gln	Q	<u>Q</u> -tamine
Glutamic Acid	Glu	E	glu-t <u>E</u> -amic acid
Glycine	Gly	G	<u>G</u> lycine
Histidine	His	H	<u>H</u> istidine
Isoleucine	Ile	I	<u>I</u> soleucine
Leucine	Leu	L	<u>L</u> eucine
Lysine	Lys	K	lysin <u>K</u>
Methionine	Met	M	<u>M</u> ethionine
Phenylalanine	Phe	F	<u>F</u> enylalanine
Proline	Pro	P	<u>P</u> roline
Serine	Ser	S	<u>S</u> erine
Threonine	Thr	T	<u>T</u> hreonine
Tryptophan	Trp	W	t <u>W</u> ptophan
Tyrosine	Tyr	Y	t <u>Y</u> rosine
Valine	Val	V	<u>V</u> aline

The Amino Acid Table

In addition to showing the one-letter and three-letter codes for each amino acid, the Amino Acid Table in ProtInv shows more information about each amino acid:

- The circles are colored to indicate the relative hydrophobicities of the side chains. The most hydrophilic are green; the most hydrophobic are orange; yellow amino acids are intermediate.

- The writing on the amino acids is colored to show their charge. Amino acids with positively-charged side chains are shown in blue; negative in red; neutral in black.
- Green amino acids with black writing are hydrophilic and uncharged; they can make hydrogen bonds.

3) Consider the protein N-arg-arg-arg-arg-C.

a) Based on the simplifications described previously, predict how this protein would fold. Sketch your predicted folded protein below.

b) Construct the protein.

- Click in the **Amino Acid Sequence Box** of the **Upper Folding Window** to activate it. You should see a blinking cursor appear.
- Type "RRRR" (without quotes). This is the single-letter code for four arginines.
- You should see "Arg Arg Arg Arg" in the **Amino Acid Sequence Box**. Note that ProtInv shows the three letter code for easy reading. Note also that the border of the **Upper Folding Window** turns pink - this indicates that the protein sequence in the **Sequence Box** has not yet been folded and therefore, the sequences in the **Sequence Box** and the **Folding Window** are no longer the same.
- Click the "**FOLD**" button at the bottom of the **Upper Folding Window**. Wait a few seconds for the computer to do the calculations.
- You will see the folded protein in the **Folded Protein Window**. You will also see a smaller version of the protein appear in a little box in the **History List**. The border of the **Upper Folding Window** will turn gray to indicate that the sequences in the **Sequence Box** and **Folded Protein** are now the same.

c) Does the actual folded shape match your prediction? If not, make sure you understand why it didn't and draw it correctly.

4) Consider the protein N-phe-phe-phe-phe-C.

a) Based on the simplifications described previously, predict how this protein would fold. Sketch your predicted folded protein below.

b) Construct the protein.

- Click in the **Amino Acid Sequence Box** of the **Lower Folding Window** to activate it. You should see a blinking cursor appear.
- Type “FFFF” (without quotes); this is the one-letter code for four phenylalanines.
- you should see “Phe Phe Phe Phe” in the **Amino Acid Sequence Box**.
- Click the “**FOLD**” button in the **Lower Folding Window** and wait a few seconds.

c) Does the actual folded shape match your prediction? If not, make sure you understand why it didn't, and draw it correctly.

5) Consider the protein N-phe-arg-arg-arg-arg-phe-C.

a) Based on the simplifications described previously, predict how this protein would fold. Sketch your predicted folded protein below.

b) Construct the protein.

- Click in the **Amino Acid Sequence Box** of the **Lower Folding Window** to activate it. You should see a blinking cursor appear.
- You can build this protein by selecting the entire protein sequence, hitting delete or backspace to clear the text, and then typing "FRRRRF".
- Alternatively, you can edit the existing sequence as described below:
 - Click to put the cursor anywhere in the third "Phe".
 - Hit delete or backspace twice; it should delete two of the Phe's to give you "Phe Phe" with the cursor in the middle. Notice that hitting the delete key deletes an entire amino acid (not just one letter of its name as you might expect).
 - Type "RRRR".
 - The sequence should now read "Phe Arg Arg Arg Arg Phe"
- Click "**FOLD**" and wait a few seconds.

c) Does the actual folded shape match your prediction? If not, make sure you understand why it didn't and draw it correctly.

6) Consider the protein N-ile-phe-met-gln-ser-arg-thr-asp-ala-ala-C. (IFMQSRTDAA)

a) Based on the simplifications described previously, predict how this protein would fold. Roughly sketch your predicted folded protein below.

b) Construct the protein. You should clear one of the **Polypeptide Windows** and start over.

c) Does the actual folded shape match your prediction? If not, make sure you understand why it didn't and draw it correctly.

d) Suppose a mutation in the gene for this protein caused the Arg in the middle (amino acid #6) to be replaced by a Glu. How might this effect the shape of the protein? Sketch your prediction below.

e) Make the mutant protein, either by editing the sequence (you can move the cursor to the right of any amino acid to delete it with the backspace key and then type in the new one) or by clearing and starting over. The mutant protein is: N-ile-phe-met-gln-ser-glu-thr-asp-ala-ala-C (IFMQSETDAA). Click "FOLD". Does the actual folded shape match your prediction? If not, make sure that you understand why it didn't.

You can easily compare two different proteins by clicking on the inactive **Folding Window** then double-clicking on the protein you want to see in the **History List**. It will appear in the **Folding Window** that you just activated.

f) Consider a different mutation, one that causes the same Arg (#6) to be replaced by an Ile. Follow the same procedure as for the mutation in part (e). The mutant protein is: N-ile-phe-met-gln-ser-ile-thr-asp-ala-ala-C (IFMQSITDAA). You may find it useful to have the normal protein in one **Folding Window** and the mutant protein in the other **Folding Window** for comparison purposes (select it in the **History List** and click one of the ">Upper" or ">Lower" buttons as appropriate).

g) Starting from the original protein from step (6a), make a mutation that has **little or no effect** on the shape of the protein. Explain why this mutation has such a small effect.

h) Starting from the original protein from step (6a), make a mutation that has **a large effect** on the shape of the protein. Explain why this mutation has such a large effect.

7) Design and construct a protein of at least 6 amino acids that has approximately the following shape:

Amino acid sequence_____

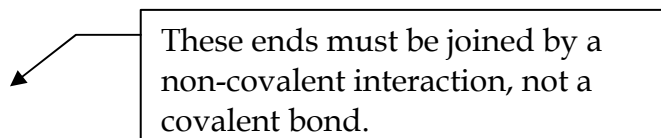
You will need to explain to your TA why your protein has this shape for a check-off.

8) Design and construct a protein of at least 6 amino acids that has approximately the following shape:

Amino acid sequence_____

You will need to explain to your TA why your protein has this shape for a check-off.

9) Design and construct a protein with approximately the following shape:



Amino acid sequence_____

You will need to explain to your TA why your protein has this shape for a check-off.

10) For a real challenge, fold this protein:

N-ser-leu-gln-leu-asn-ile-thr-met-glu-val-asp-phe-trp-N (SLQLNITMEVDFW)

It will have this cool shape:

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

It will be colored orange - you can see this in the little colored square next to the "Color:" label. Try to figure out how the color is determined. You don't have to do this for the lab but if you can, you will really be well-prepared for the exam.

Try these proteins as well to help you figure out how color is formed:

FFFFFFFRRRRRR

KKKKKKYYYYYYY

EEWWWWWWWEEE

Lab Report

There is no lab report for this lab.

Glycolysis & Fermentation

Objectives:

- To familiarize you with the reactions of glycolysis & fermentation.
- To give you a sense of the chemical transformations that enzymes can carry out.
- To explore a biochemical pathway.

Procedure

You will work in groups of 3. You should get a chemical model set for your group. You will switch back and forth between the models and a class discussion.

(I) Going through glycolysis.

You will build a model of glucose and process it through glycolysis to 2 molecules of pyruvate. You will then convert the pyruvate to carbon dioxide and ethanol; the overall process from glucose to ethanol and carbon dioxide is fermentation – specifically, fermentation as carried out by yeasts as they make wine or beer.

Each group will be assigned one of the 6 carbons in glucose to trace through the pathway.

You will be following glucose and its descendants through the reactions of fermentation to ethanol and carbon dioxide. Depending on which carbon atom you are following, your atom will end up in the carbon dioxide or the ethanol. In biochemical terminology, your molecule of glucose will be ‘labeled’ at a particular carbon atom assigned by your TA.

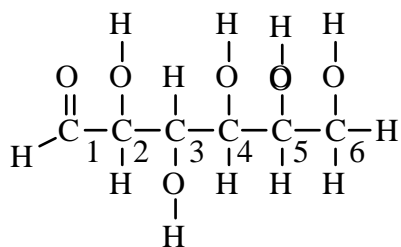
Once your TA has assigned you your carbon atom, you should build glucose using the following code:

- H = little white balls
- O = red or blue
- C = black (except your labeled carbon atom; use gray for this one)

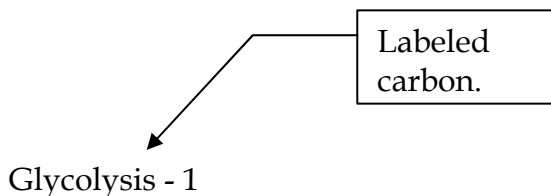
Here is the structure of glucose with the carbon atoms numbered:

(don't worry about keeping bond lengths the same)

Build it, making sure to use the gray atom for your labeled carbon atom.



A picture of glucose labeled at the #3 carbon is shown below in the same orientation as the molecule above:



- Some other details of the simulation:

- NAD⁺ & NADH: NAD⁺ is a complicated molecule so you won't have to build it. You will need to take it into account when doing the reactions, though. You will model the NAD⁺ part with a styrofoam block labeled "NAD". When NAD⁺ reacts with a molecule, an H and the 2 electrons in the bond are transferred to NAD⁺ making NADH - model this by sticking an H and it's bond into the styrofoam. You should see that, by the time you've gotten to ethanol and carbon dioxide, the two NAD⁺'s you started with have been converted to NADH and the restored to NAD⁺. This is shown in the photo below:

- H-atoms: some H⁺ will be produced in some steps and then used in other steps; keep these in a safe place when they are not attached to a molecule.

- ATP, ADP, & P_i : Similarly, ADP is a complex molecule, so we will model it as a styrofoam block labeled "ADP". The phosphate will be modeled as a loose orbital. ATP is modeled by sticking the orbital into the ADP block. This is shown below:

ATP

ADP

P_i

The phosphate can be connected to other molecules by a bond – for example, glucose-6-phosphate is modeled by connecting the orbital to the O on carbon 6 of glucose. This is shown below:

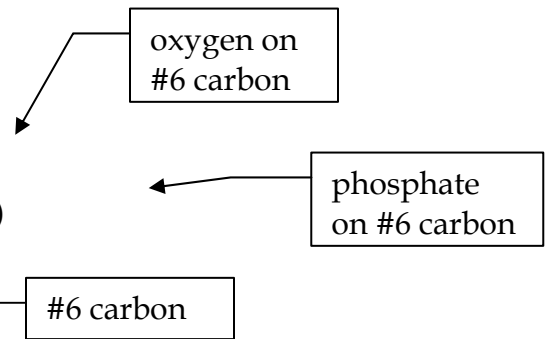
Setup:

– you’ll need:

- your glucose model with the labeled carbon
- 2 ATP’s (2 ADP blocks, each with an orbital stuck on)
- 2 ADP’s (2 ADP blocks)
- 2 P_i’s (unattached orbitals)
- 2 NAD⁺ blocks
- 2 extra oxygen atoms (you’ll use them temporarily – in real life, they would be water molecules exchanged in different reactions)

– Take out the bonds from the kit & close it up. You should not need to take any more atoms out or

put any back in.



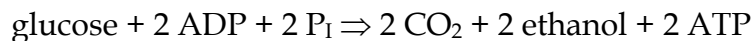
– The next pages show the reactions of glycolysis and fermentation. Starting with glucose, transform your model from one intermediate to the next until you have converted your glucose to 2 molecules of carbon dioxide and 2 molecules of ethanol.

At the end:

– you should have:

- 2 CO₂ & 2 ethanol molecules
- 2 NAD⁺ - they’ve been recycled back to their original state
- 2 free O atoms
- 4 ATP (it cost 2 to start so the net yield is 2 ATP)

– Thus, the overall reaction (not counting things that are the same at the start and end) is:



Questions for discussion (you should keep these in mind while going through the glycolysis reactions):

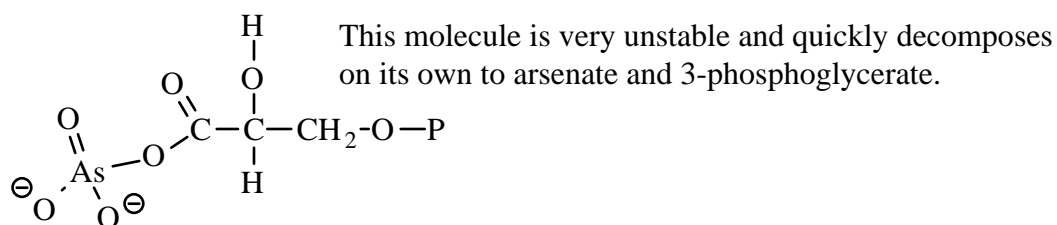
1) Pool the results of your labeling studies. Which carbon atoms of the original glucose molecule end up in CO₂ and which in ethanol (use the numbers on the original glucose molecule)?

2) Describe briefly the chemical transformation at each step of the pathway.

3) Five of the reactions are phosphate transfers – a phosphate is moved from one part of a molecule to another part of the same molecule or to a different molecule. Which steps are these? How are they different?

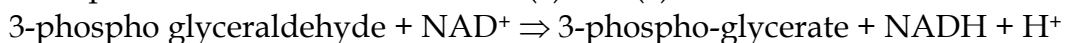
4) Two of the reactions involve exchange of a C=O and a C-OH group. Which are they?

5) Arsenate ion (AsO_4^{3-}) is very similar to phosphate (PO_4^{3-}). If you add arsenate to cells that are carrying out fermentation, arsenate substitutes for phosphate in reaction (6). Thus, in the

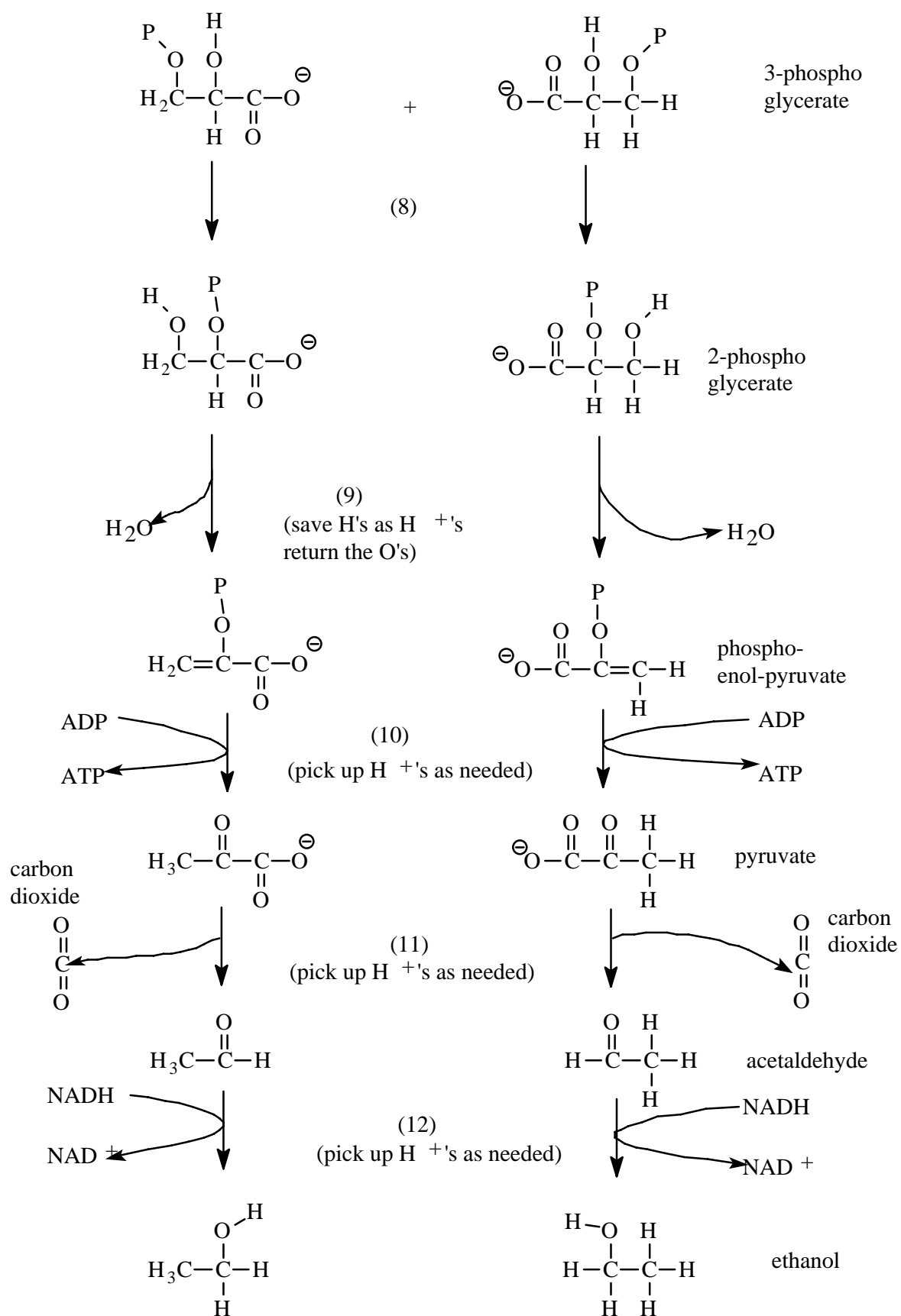


presence of arsenate, the product of reaction (6) is:

Thus, in the presence of arsenate, reactions (6) and (7) become:



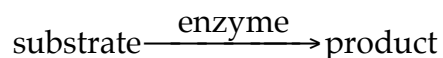
Under these conditions, how many ATP would the cell get per molecule of glucose?



Bio 111 Pre-Lab for Lab #10: Name_____

Catalase TA & Sect. _____

1) Enzymatic reactions involve a substrate which is converted by the enzyme to the product.



a) What are the substrate, enzyme, and product for the reaction you will be studying?

b) For the reaction you have written, is the ΔG greater than, less than, or equal to zero? Explain your reasoning.

c) What are we actually measuring with the disks of filter paper (circle all that apply)?

- | | | |
|-------------------|-------------|----------|
| • accumulation of | • product | • enzyme |
| • consumption of | • substrate | • water |

Enzymes: Catalase

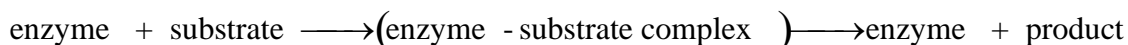
Objectives

- To explore the phenomenon of enzymatic catalysis using the particular enzyme, catalase.
- To see the conservation of enzyme *function* in different organisms
- To see the variation in enzyme *properties* in different organisms

Introduction

Enzymes are biological catalysts that constitute the largest and most highly specialized class of protein molecules. Enzymes act as catalysts, to increase the rates of chemical reactions, but they do not cause a reaction to occur that would not proceed spontaneously without the enzyme. The reactions of metabolism would occur at extremely slow rates at normal body temperature in the absence of enzymes. An appreciation of the catalytic efficiency of enzymes can be gained by realizing that, under optimal conditions, most enzymatic reactions proceed 10^8 to 10^{11} times more rapidly than the corresponding non-enzymatic reactions. Without the enzymes in our digestive tract, for example, it would take us about 50 years to digest a single meal!

Enzymes participate in the reaction which they catalyze, but they emerge unchanged at the end of the reaction, i.e., they are not altered or used up. Thus, a few enzyme molecules can go a long way. This can be represented by the following generalized equation:



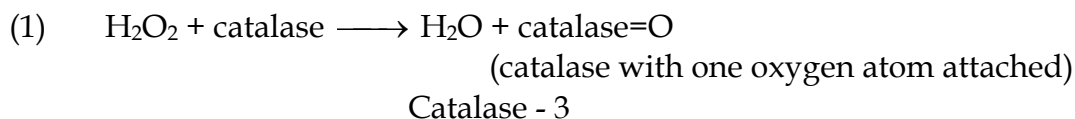
One very important property of enzymes is their specificity. Any one enzyme will only catalyze a single class of chemical reactions. In this lab you will be studying a single reaction carried out by one enzyme. Many thousands of enzymes exist, each catalyzing a different chemical reaction. Many of these enzymes have been isolated, purified and crystallized: the amino acid sequences of many have also been determined.

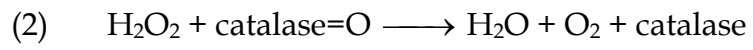
Because enzymes are proteins, they are affected by environmental conditions. Many molecules act as *inhibitors* of enzyme function. These inhibitors bind to the enzyme and render it inactive. Many drugs act by inhibiting important enzymes.

We shall demonstrate the activity of one enzyme in this lab. The enzyme, catalase, catalyzes the conversion of hydrogen peroxide (H_2O_2) to oxygen (O_2) and water (H_2O) via the following reaction (this was described in lecture):



Actually, the reaction proceeds in two steps:





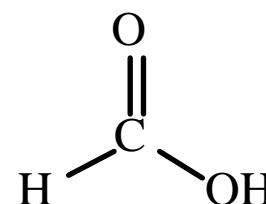
Note that, in each case, the catalase is unchanged in the overall reaction.

Catalase is present in all organisms that live in an oxygen atmosphere. In these organisms, catalase serves to de-toxify the hydrogen peroxide that is produced by the reverse of the overall reaction above; this reverse reaction is stimulated by radiation, etc. Hydrogen peroxide is highly reactive and can damage proteins and DNA (and cause mutations), so it must be rapidly converted to less-toxic O_2 . Certain bacteria that live anaerobically (without oxygen) lack this enzyme; many of these bacteria are killed by exposure to O_2 .

An enzyme that catalyzes the breakdown of H_2O_2 into H_2O and O_2 can be found in virtually all organisms, thus the *function* of catalase is said to be conserved in all these organisms. This conservation is the result of evolution; once an ancestral organism evolved the first catalase enzyme, it was passed to its evolutionary descendants because it provided an advantage - detoxification of hydrogen peroxide. However, since these descendants continue to evolve, the catalase enzyme in different organisms, while still catalyzing the same reaction, will have slightly different amino acid sequences. The more closely-related two organisms are, the more similar their catalase proteins will be (you will see more of this in Bio 112). Although these differences typically do not effect the reaction that catalase catalyzes, they do effect its *properties* - sensitivity to temperature and inhibitors, for example.

In this lab, you will see both the conservation of catalase *function* (catalase from widely different organisms catalyzes the same reaction) and the variation in the *properties* of catalase (catalase from different organisms will be differently sensitive to the inhibitor, formic acid).

Formic acid (FA) is the simplest carboxylic acid. Its structure is: FA is known to bind to several places on the surface of catalase as well as at the active site. Binding of FA to the active site would prevent the usual substrate (hydrogen peroxide) from binding to the active site, making the enzyme inactive. In addition, binding of FA to the surface of catalase could change its shape so that it is no-longer functional.



Although catalase from all organisms is subject to inhibition by FA, because the catalase molecules from different organisms have slightly different amino acid sequences, the catalase from one organism may be more or less sensitive to FA.

In order to assess this differential sensitivity to FA, you will expose your catalase to different levels of FA. We expect that catalase from different sources will be inhibited by different FA levels. The most useful units for measuring the "level of FA" is to measure its concentration in moles per liter (also known as "molarity"; symbol M). Since FA is a relatively strong inhibitor, we will measure its concentration in millimolar (1/1000 M; symbol mM).

How will we study this enzyme? We will measure the rate of the reaction it catalyzes. In this case, we will measure the rate of production of oxygen (O_2). Our measuring technique, or assay, is based on the fact that H_2O_2 is a liquid which dissolves in water, while O_2 is a gas which does not dissolve in water to any great extent. Thus, as the catalase catalyzes the reaction, bubbles of O_2 gas will be produced.

We will measure this production using small disks made of filter paper. You will dip the disks in a solution containing catalase and drop them in a beaker of H_2O_2 and water. (The

hydrogen peroxide is the same as the hydrogen peroxide you can buy at the drugstore: at the drugstore, it is 3% H_2O_2 ; we use 1% in the lab.) The disk, being wet, will sink to the bottom. As the reaction proceeds, bubbles of O_2 will accumulate, trapped in the fibers of the disk. When enough bubbles have accumulated, the disk will float to the surface. Since the amount of O_2 required to float a disk is roughly the same for all disks, the faster the reaction, the less time it will take for the disk to float. This is shown in the pictures that follow:

First, you drop the disk that has been soaked in catalase into the hydrogen peroxide solution.

Start Your Stopwatch.

The reaction starts and the disk then sinks to the bottom. Soon, you may see a small column of oxygen bubbles rising from the disk.

Bubbles of O₂ accumulate in the disk and, eventually, it begins to float. Sometimes, it floats part-way up, sheds some bubbles that were stuck on, and sinks back down. This is called “burping”, it is not the end of the reaction.

If “burping” occurs, you should wait until the disk goes to the surface and stays there before stopping your stopwatch.

Eventually, the disk floats to the surface and stays there.

Stop Your
Stopwatch &
Record the time.

Today, you will extract catalase from a sample you have brought in and measure its activity in the presence of different levels of the inhibitor formic acid.

Procedure

(I) Quick test for catalase activity

Before going to all the trouble to extract catalase from your sample, you should be sure that catalase is actually present. Cut off a chunk of your sample, about the size of a pencil eraser. Drop this in a beaker of 0mM FA {COLORLESS} hydrogen peroxide solution. Look for a small stream of tiny bubbles rising from your chunk. If you see bubbles, they are O₂, and you have catalase in your sample; go on with the lab. If you don't, there is no catalase in your sample and you should get a piece of another lab group's sample that shows catalase activity.

(II) Prepare your extract

In biochemical terms, an extract is the solution that results from grinding something up. This extract contains enzymes, etc. Typically, you prepare an extract using a particular solution which is designed to stabilize the enzyme; these solutions are called extraction buffers.

1) Start with a chunk of your sample roughly 3/4 to 1 inch on a side. Cut your sample in to small pieces (smaller than a pencil eraser) and put in to the mortar and pestle. See below:

2) Pour on enough extraction buffer to almost cover your sample. The less buffer you use, the more enzyme per unit volume, the faster your reactions will go. The buffer contains a mixture of sodium phthalates at pH 4.0 to stabilize the enzyme and a small amount of phenylthiourea to inhibit competing enzymes which might be present in your extract.

3) Holding the mortar and pestle as above, grind your sample into as fine a mush as you can. Note: don't use the pestle in a circular motion as though you were stirring soup; use a crushing motion against the curved part of the mortar near the bottom as though you were mashing potatoes. Do it gently so you don't splash.

4) Remove the pestle and pour the liquid part of your extract into a test tube. Don't worry about getting it all, you won't need much more than 1/2 test tube full. Try to avoid getting any remaining chunks of your sample in the test tube.

5) Let the smaller particles in your extract settle out for 5 minutes or so. Then, very carefully, suck off some of the clearer extract at the top of the test tube to another test tube - this is the "clarified extract". Use a plastic pipette with attached squeeze-bulb. The trick to using these carefully is to squeeze out the air in the bulb **before** putting the tip in the liquid. You might want to practice this. If you stir up your extract too much, just wait another 5 minutes for it to settle down and try again.

6) Take the tube of clarified extract flick it gently to mix well, and pour it into the corner of a plastic petri dish. Try to keep it from spreading as much as you can. Now you are ready to measure the activity of your clarified extract.

(III) Measure the activity of catalase from your sample in the absence of FA (0mM)

- 1) Prepare a 30ml beaker with roughly 20ml 'hydrogen peroxide' (actually, it is pH 4 buffered 1% H_2O_2).
- 2) Pick up a fresh disk of filter paper with tweezers. Dip it in the puddle of clarified extract for about 5 seconds.
- 3) Touch it to a clean paper towel to blot the excess extract off.
- 4) Drop into the beaker with hydrogen peroxide in it. START YOUR STOPWATCH.
- 5) When the disk touches the surface, STOP YOUR STOPWATCH.
- 6) Record the time it took.
 - If this time is less than 5 seconds, your extract is too concentrated. Add a roughly equal volume of extraction buffer to your puddle and mix well by sucking up and down in a pipette. Try assaying again to be sure that your time is more than 5 seconds.
 - If this time is more than 1 - 2 minutes, your extract is too dilute. Re-make your extract with more sample and less extraction buffer. Re-measure the time to be sure it is less than a minute or two.

Do a total of 5 measurements using a new disk each time and record the times. You should discard the hydrogen peroxide after each set of 5 and get fresh solution.

(IV) Measure the sensitivity of your enzyme to FA

There will be 5 different hydrogen peroxide solutions with different concentrations of FA:

0 mM {COLORLESS}
5 mM {RED}
10 mM {ORANGE}
20 mM {GREEN}
40 mM {BLUE}
100 mM {VIOLET}

Using a fresh disk for each measurement and a freshly-washed beaker of hydrogen peroxide for each concentration of FA, measure the time to floating for your enzyme 5 times at each concentration of FA.

If nothing seems to be happening after waiting 10 times longer than the time it takes at 0 mM, you can stop counting and record "> xxx seconds". For example, if it took 25 seconds to float at 0 mM, you can stop waiting if it hasn't floated after 250 seconds (4 minutes 10 seconds) and put "> 250" in your data table.

(V) Calculations

The goal of these calculations is to produce a graph of reaction rate as a function of FA concentration.

1) Organize your data. Here is a typical data table; the numbers in the boxes will be the number of seconds it took the disk to float.

measurement #	TIME TO FLOAT (seconds)					
	0 mM	5 mM	10 mM	20 mM	40 mM	100 mM
1						
2						
3						
4						
5						

2) Convert the time data to rate data. Since rate is inversely proportional to time (faster rate means shorter time), calculate rate = . This gives a second data table like the one you made on day 2.

3) Average your data for each concentration of FA.

4) Graph your data. Graph all your data points, including the average. Note that the concentrations are not equally-spaced. Draw a line through the average points.

5) Clean up. You can discard the test tubes and plastic pipettes. Wash out the mortar and pestle carefully with soap, rinse well, and dry carefully.

Lab Report:

- Must be typed; handwritten reports will not be accepted. Graphs can be hand-drawn, but they must be legible. We will be very strict about the format of your graphs; check with your TA for details.
- See syllabus for due date. This is a firm deadline.
- Although you will perform these experiments as a group, each member of the group must turn in an individual lab report.

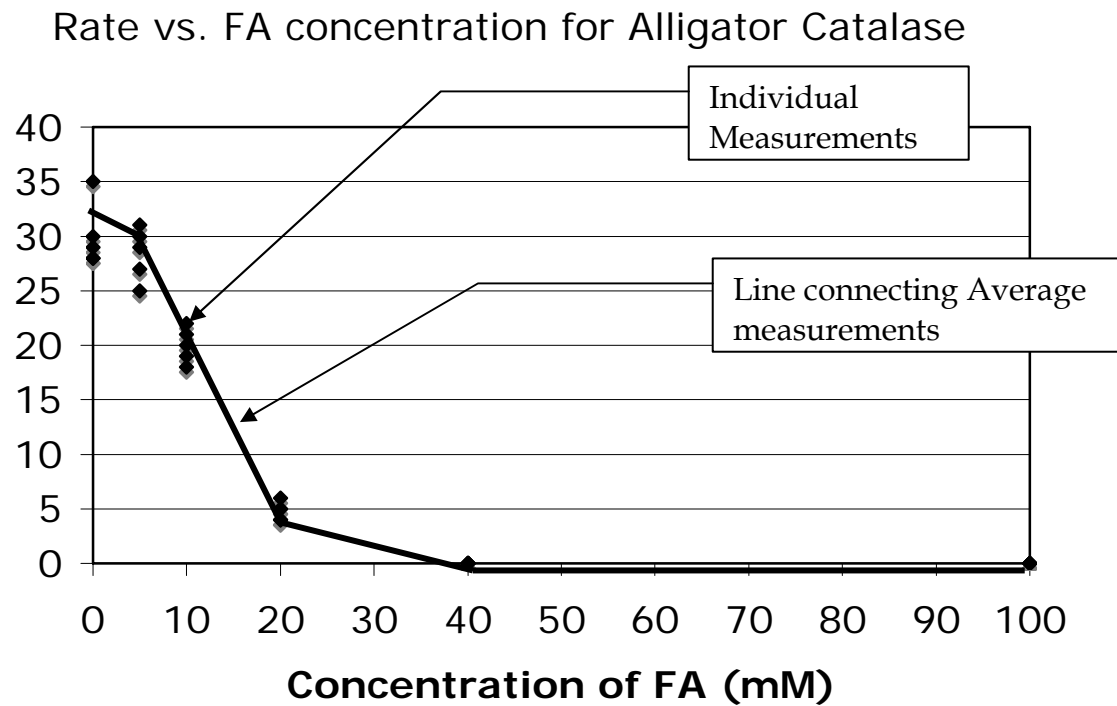
Your lab report must consist of the following :

1) Data table:

- “time to float” at 0, 5, 10, 20, 40, and 100 mM FA for catalase from your sample; this should include all your measurements (5 measurements at each concentration of FA)
- rate at 0, 5, 10, 20, 40, and 100 mM FA for catalase from your sample; this should include all your measurements (5 measurements at each concentration of FA)
- average rate at 0, 5, 10, 20, 40, and 100 mM FA for catalase from your sample; this should include the averages only (1 number at each concentration of FA)

- 2) A graph of rate vs. concentration of FA for your catalase (0, 5, 10, 20, 40, and 100 mM FA)
- this graph must include:
 - 6 points at each concentration of FA = 5 different measurements & the average
 - a line drawn through the average points only
 - the axes must be clearly labeled and the graph must have a descriptive title.

Here is a completely hypothetical example:



3) A **brief** description in words of what the graph shows. For the example above, "Catalase from alligator is partially inhibited as 10 and 20mM FA and completely inhibited above 40mM."

LEGO Molecular Biology Lab

Purpose:

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

- rules for DNA & RNA structure
- base-pairing
- DNA replication
- transcription including promoters & terminators
- translation including start & stop codons

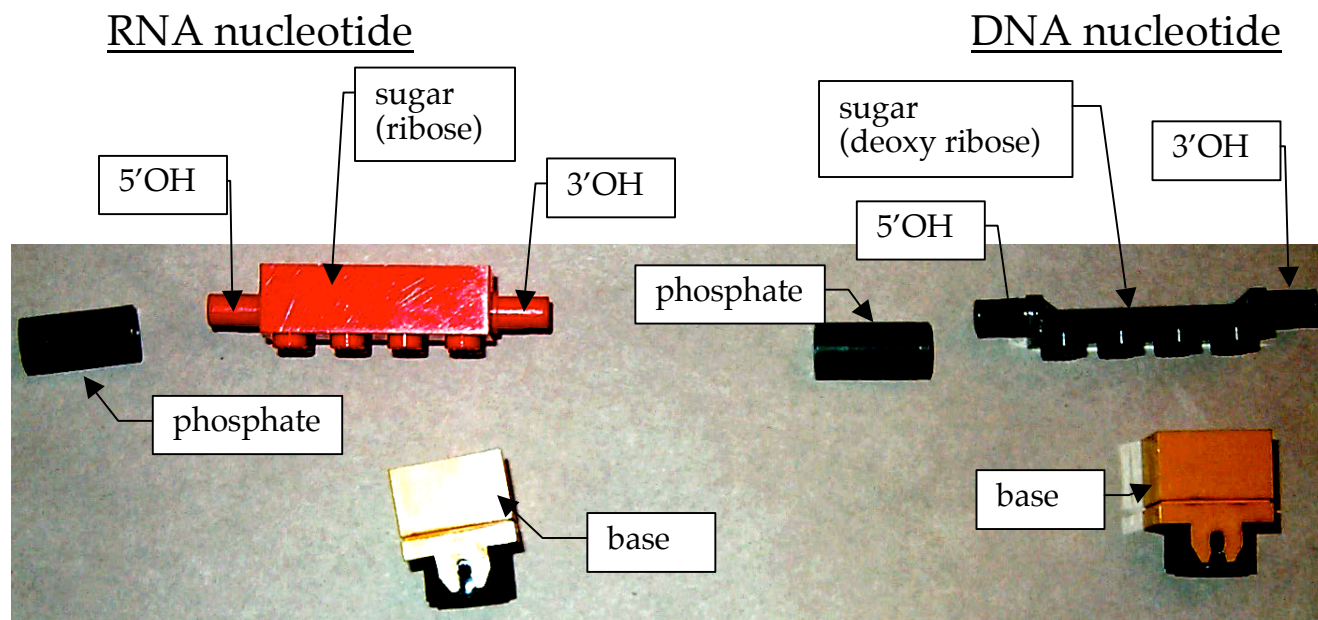
Introduction:

Later on in lab, you will be working through molecular biology problems on paper. In order to thoroughly understand these more complex problems we will work through some simpler problems using LEGO DNA models this week.

Part I: LEGO DNA

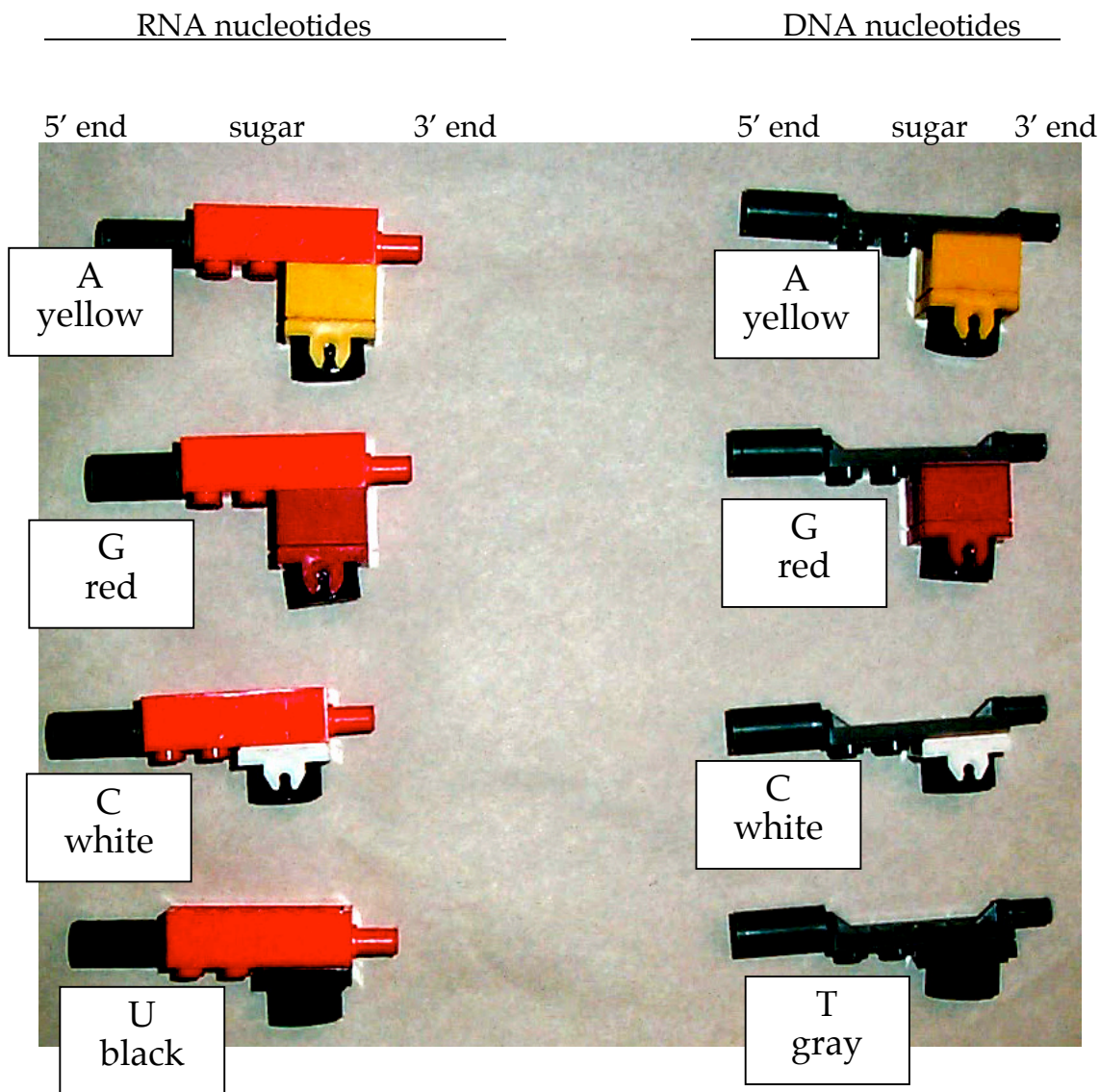
The LEGO models DNA and RNA as follows:

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

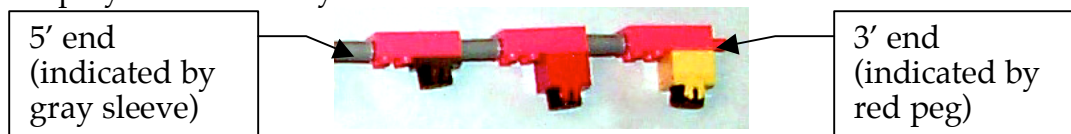


Important note: Unlike the LEGO Mitosis lab, we will not spell out all the details of what you have to do in each step of this lab. We do this on purpose - we want you to figure out some of the details with the help of your classmates and your TA. As you figure these out, the details will become clearer.

When nucleotides are assembled, they look like this:



- The correct bases pair via hydrogen bonds simulated by the black magnets on each base.
- The backbone is connected by covalent bonds simulated by the plug on the 3' end and the socket on the phosphate on the 5' end.
- Since each nucleotide has a 5' and a 3' end, if they are connected 5' to 3' in a long polymer, this polymer will always have a 5' and a 3' end. This is shown below:



The sequence of this small RNA molecule would be: 5'-UGA-3'

Procedure:

1) Check your kit. You should have:

- 12 DNA A's (yellow base)
- 12 DNA G's (red base)
- 12 DNA C's (white base)
- 12 DNA T's (black base)
- 6 RNA A's (yellow base)
- 6 RNA G's (red base)
- 6 RNA C's (white base)
- 6 RNA U's (gray base)

They should be set up as in the pictures above. That is, the phosphate (gray sleeve) should be on the 5' end - the end farthest from the base.

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

5' -ACGGTACGCTAT-3'

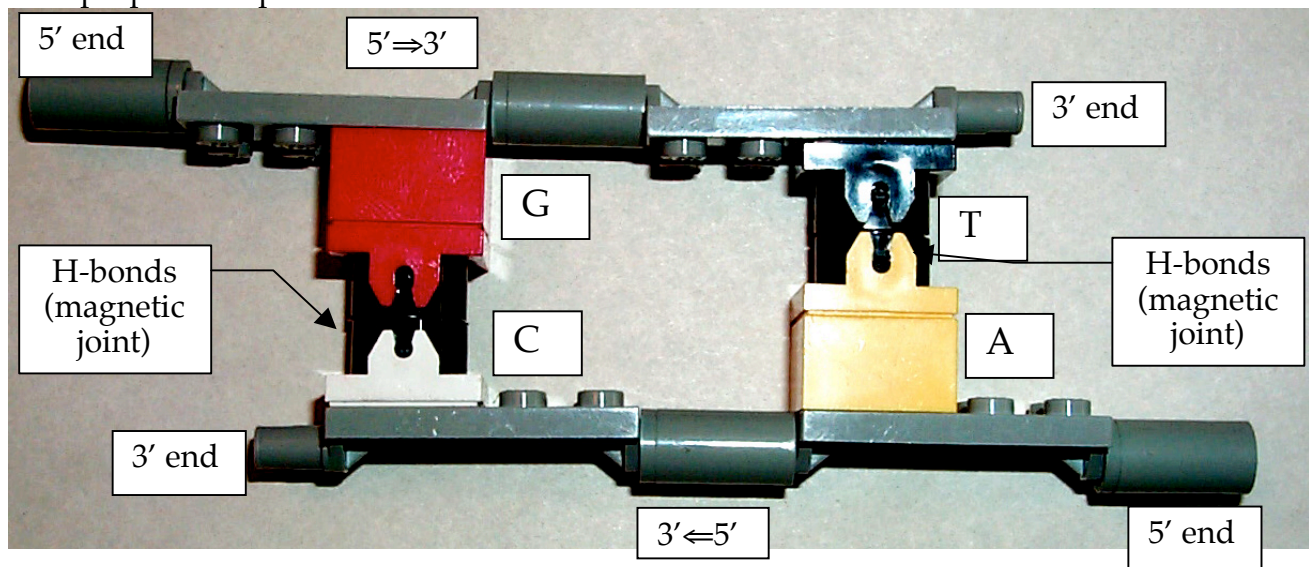
Notice that all the sugars run in the same direction.

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

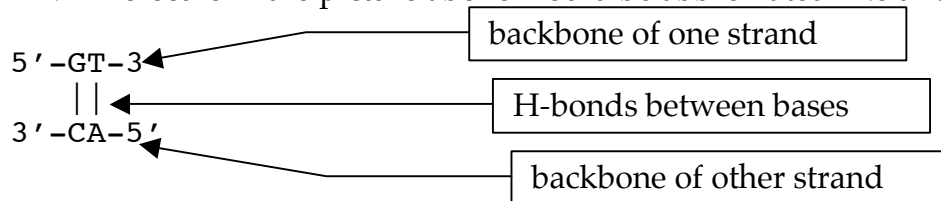
Note:

- the strands must be anti-parallel (run 5' \Rightarrow 3' in opposite directions)
- large bases (A and G - purines) pair with small bases (C and T - pyrimidines); NEVER pair a large with a large or a small with a small (the magnets might let you do this, but it is biologically impossible).
- 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:

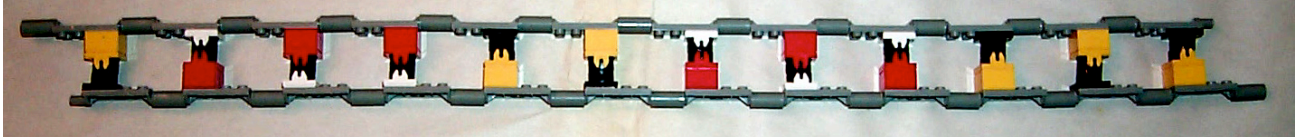


The sequence of the DNA molecule in the picture above would be abbreviated like this:



LEGO DNA - 3

Your molecule should look something like this:



Try twisting the molecule to make a double helix.

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

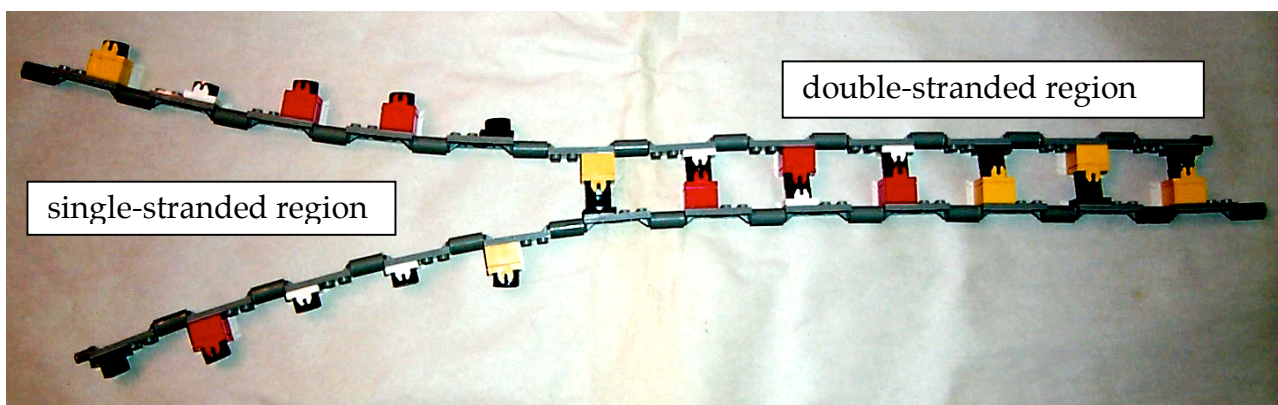
5'-_____ -3'

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

DNA Replication

You will now simulate the replication of this DNA molecule.

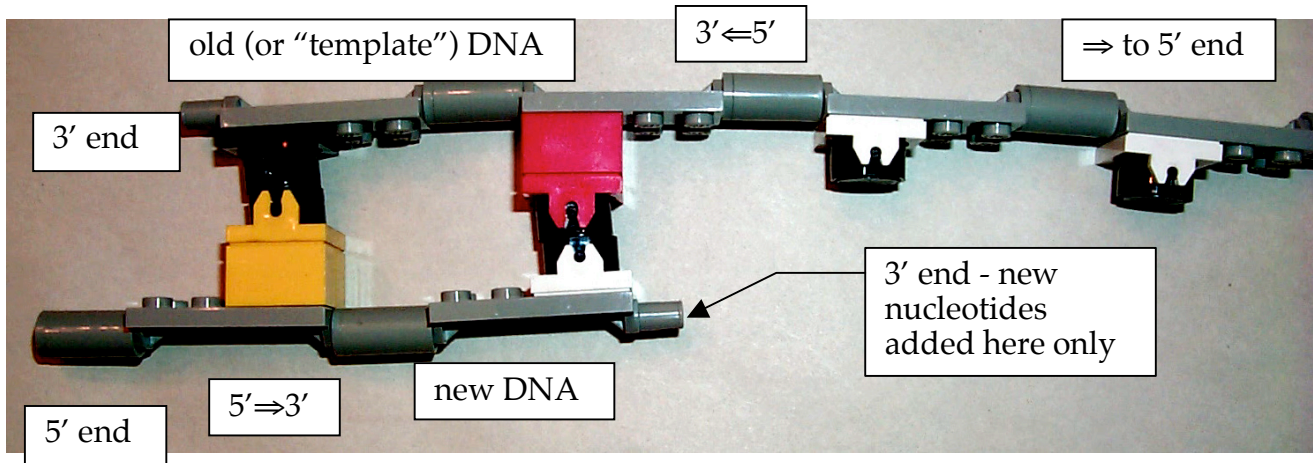
4) Prepare the left-hand end of the molecule for replication. Un-zip (break the hydrogen bonds - simulated by separating the magnets) the 5 base-pairs at the left end of your DNA molecule to make a region of single-stranded DNA. You will have to turn the bases to face out from the center or they will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



5) Start replicating DNA on one of the single-stranded regions of your DNA molecule.

Remember to follow the rules:

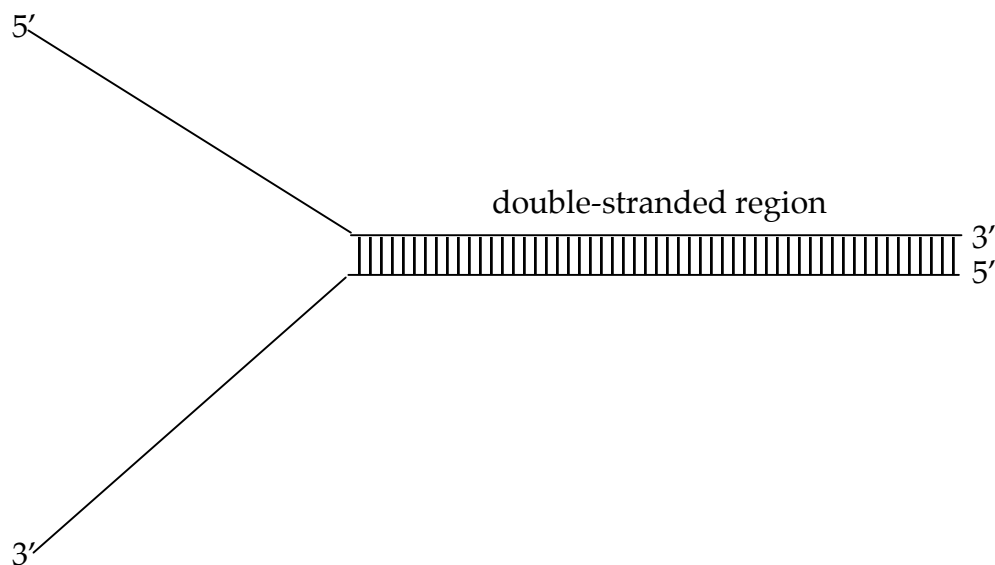
- the strands must be anti-parallel (run $5' \Rightarrow 3'$ in opposite directions)
- A pairs with T (yellow with black) the magnets won't let you pair it any other way
- G pairs with C (red with white) the magnets won't let you pair it any other way
- DNA polymerase can only add nucleotides to a $3'$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



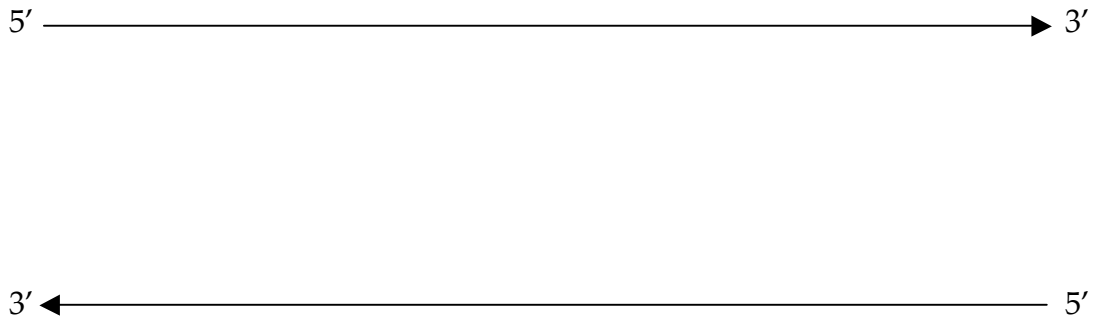
6) Continue replicating this strand until you have to stop - either because you've reached the end of the template strand or you've run into the double-stranded region.

7) Replicate the other strand in the single-stranded region. Keep in mind the rules from step (5). You will notice an important difference between the two strands.

8) 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. ($5' \Rightarrow 3'$)



9) Unzip the remaining base-pairs in the double-stranded region and finish replicating the DNA strands. On the drawing below, draw the new DNA strands; the solid lines represent the template DNA. Use a wavy line for the DNA you made in step (8) and a dotted line for the DNA you made in step (9). Be sure to indicate the 5' and 3' ends as appropriate.



List the differences between the replication on the two strands:

Leading strand:

Lagging strand:

* You will note that there are gaps in the backbone of the new DNA on the lagging strand. These are also present in the new DNA on the lagging strand in real cells. An enzyme called DNA ligase seals these breaks.

10) 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:



A Small Gene

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

11) 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'

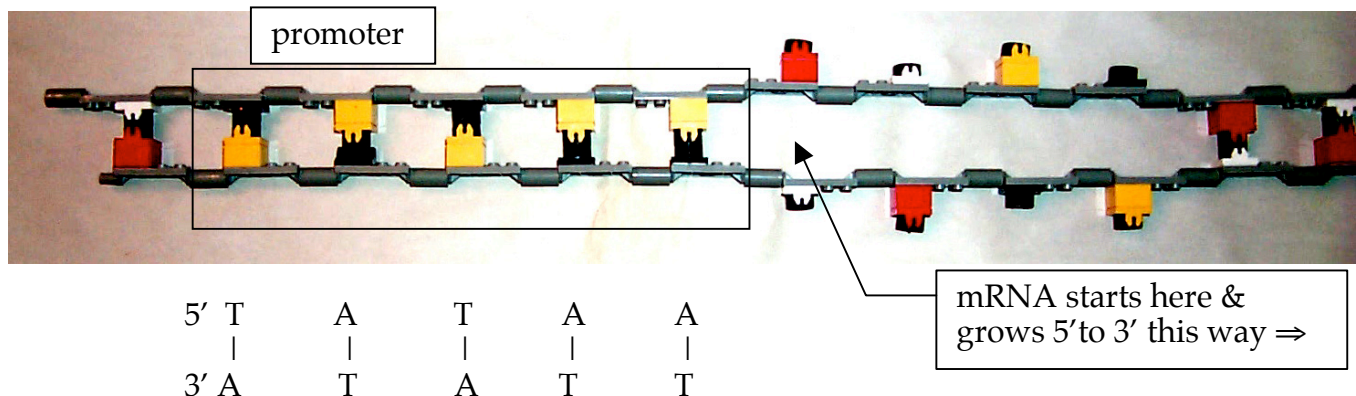
12) Build the corresponding other strand of DNA. If you have got the sequence exactly right, you will use up all of your DNA nucleotides.

Transcription

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

<u>DNA bases</u>	<u>LEGO Colors</u>
5' -TATAAx.....-3'	(B = black (T), Y = yellow (A)) 5' -BYBYYx.....-3'
3' -ATATTy.....-5'	3' -YBYBBY.....-5'

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



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

<u>DNA bases</u>	<u>LEGO Colors</u>
(C) 5' -xGGGT.....-3'	(B = black (T), Y = yellow (A), R = red (G), W = white 5' -xRRRB....-3'
3' -yCCCA.....-5'	3' -yWWY....-5'

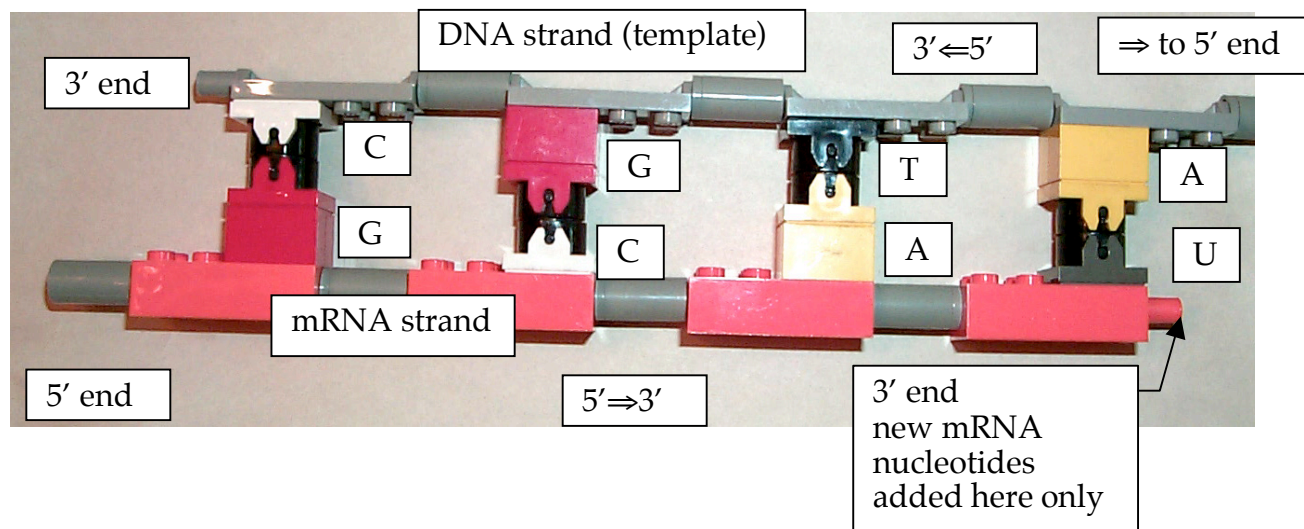
The 3' end of the mRNA ends with base pair x-y.

13) 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.

14) Make the mRNA using the following rules:

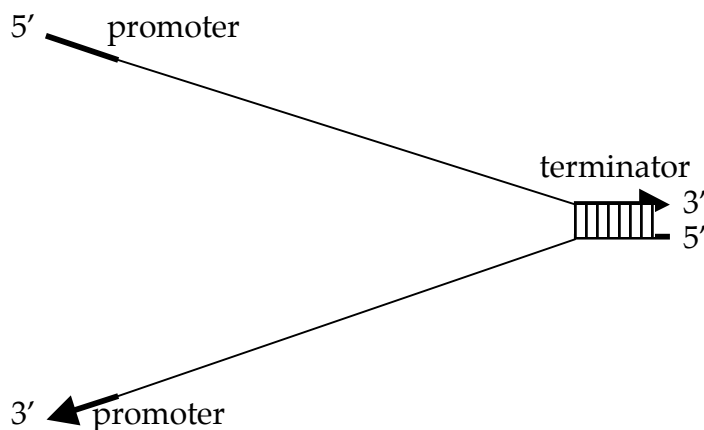
- the strands must be anti-parallel (run $5' \Rightarrow 3'$ in opposite directions)
- A pairs with U (yellow with gray) the magnets won't let you pair it any other way
- G pairs with C (red with white) the magnets won't let you pair it any other way
- RNA polymerase can only add nucleotides to a $3'$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

Correct RNA-DNA base-pairs are shown below:



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

The solid lines in the diagram below represent the DNA strands. Draw in the mRNA that you just made. Be sure to indicate the 5' and 3' ends.



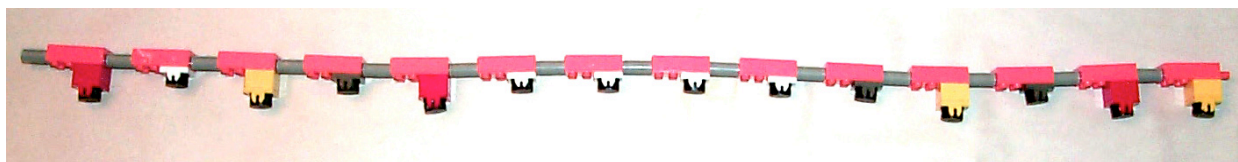
Why is it not possible to make any other mRNA? Which rule(s) would this other mRNA break?

What is the sequence of this mRNA?

5'-_____ -3'

Translation

15) Your mRNA should look something like this:



You will now act like a ribosome and read this mRNA 5' to 3' to produce a protein.

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 at the end of this section of the lab manual.

Acting as a ribosome, translate this mRNA.

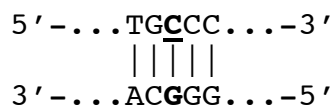
What is the resulting protein sequence?

N-_____ -C

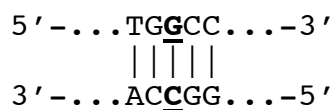
A mutant gene

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

16) 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:



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



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

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

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

Part II: Problems on paper

You will now apply what you have just learned to problems lie those you might see on an exam. You should work through problem 4.1.2 (a) through (e) from Chapter 3 of *A Problems Approach to Introductory Biology*.

Lab Report: "Designer Genes"

This lab report is due at the start of next week's lab. Labs will not be accepted late. This lab report must be typed; handwritten labs will not be accepted.

If you use a font like Courier that spaces all letters equally, it will be much easier to keep your DNA strands lined up.

This lab must be your own work.

Your lab report will be worth 10 points and must consist of answers to the following questions:

Make up a protein that is 5 amino acids long. You will then design a gene from the hypothetical organism used in this lab to produce that protein. You should use promoters, terminators, start codons, and stop codons as necessary. This gene should be fully functional in this organism; that is, if you built the gene as you did in step (11) and followed steps (12) through (15), it would produce the protein desired.

- 1) Give the sequence of your protein. Be sure to indicate the N and C termini.
- 2) Give the sequence of the mRNA that would encode your protein. Note that more than one answer is possible here; give only one. Remember that there are often parts of the mRNA that are not translated. Be sure to indicate the 5' and 3' ends.
- 3) Give the structure of the double-stranded DNA molecule that would produce this mRNA. Use the format:

```
5' -GT-3'  
  ||  
3' -CA-5'
```

Be sure to indicate the 5' and 3' ends of both strands. Be sure to include in the DNA the sequences required for the DNA to be transcribed.

Gene Explorer Lab

Part I: Warm-up on paper

Work through problem 4.1.2 g, h, and i in Chapter 3 of *A Problems Approach to Introductory Biology* to go over different types of mutations.

Part II: Gene Explorer Lab

Work through problem C2 in Chapter 3 of *A Problems Approach to Introductory Biology*.

Lab Report

- Must be typed; handwritten reports will not be accepted.
- Due next week at the start of the lab session you are currently in. This is a firm deadline.
- Although you have worked as a group, each member of the group must turn in an individual lab report.

Your lab report must include:

An entirely new gene that you have invented. Using the new Gene Explorer, this gene must:

- produce a protein of at least 5 amino acids (including the N-terminal met)
- contain at least one intron

Your report can consist entirely of a printout from the Gene Explorer showing your gene (with your name on it, of course...).

Tips

- You can use Gene Explorer as a web page or as an application.
- Use the “Enter New DNA Sequence” button and delete the starting sequence from the entry blank.
- Type in a promoter, a little DNA and a terminator and be sure your RNA is made.
- Click on your gene and add start codon, coding region, and stop codon; be sure your protein is made. Type slowly so the program can keep up.
- Similarly, add an intron in the coding region and be sure your gene works.
- If you are using a PC, you should “Print in B&W”, it will look better than in color.

The Genetic Code in mRNA

	U	C	A	G	
U	UUU phe UUC phe UUA leu UUG leu	UCU ser UCC ser UCA ser UCG ser	UAU tyr UAC tyr UAA STOP UAG STOP	UGU cys UGC cys UGA STOP UGG trp	U C A G
C	CUU leu CUC leu CUA leu CUG leu	CCU pro CCC pro CCA pro CCG pro	CAU his CAC his CAA gln CAG gln	CGU arg CGC arg CGA arg CGG arg	U C A G
A	AUU ile AUC ile AUA ile AUG met	ACU thr ACC thr ACA thr ACG thr	AAU asn AAC asn AAA lys AAG lys	AGU ser AGC ser AGA arg AGG arg	U C A G
G	GUU val GUC val GUA val GUG val	GCU ala GCC ala GCA ala GCG ala	GAU asp GAC asp GAA glu GAG glu	GGU gly GGC gly GGA gly GGG gly	U C A G

Bio 111 Exam #1

Part I; Version A

10/13/00

Your Name: _____ TA's Name: _____

Write your initials on every page in the space provided.

This exam has 5 pages including this coversheet.

Check that you have pages 1-5.

This exam has three questions.

Read all questions before starting to write.

Make your answers as clear and precise as possible.

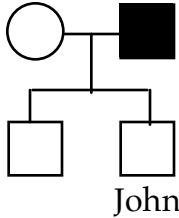
Answer all questions in the space provided.

Question	Value	Score
1	12	_____
2	20	_____
3	18	_____
TOTAL:	50	_____

Question 1: Chromosomes, Mitosis, Meiosis, & Fertilization (12 points)

Cystic Fibrosis is an autosomal recessive human genetic disease. Therefore,

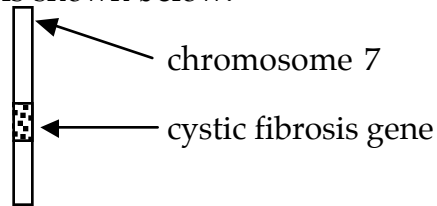
Allele	contribution to phenotype
A	normal; dominant
a	cystic fibrosis; recessive



Key:		
	Affected	unaffected
male		
female		

a) Using the allele symbols defined above, what is John's genotype? _____ (4 pts)

b) The gene for cystic fibrosis is found on chromosome number 7. A sketch of one copy of chromosome 7 is shown below:

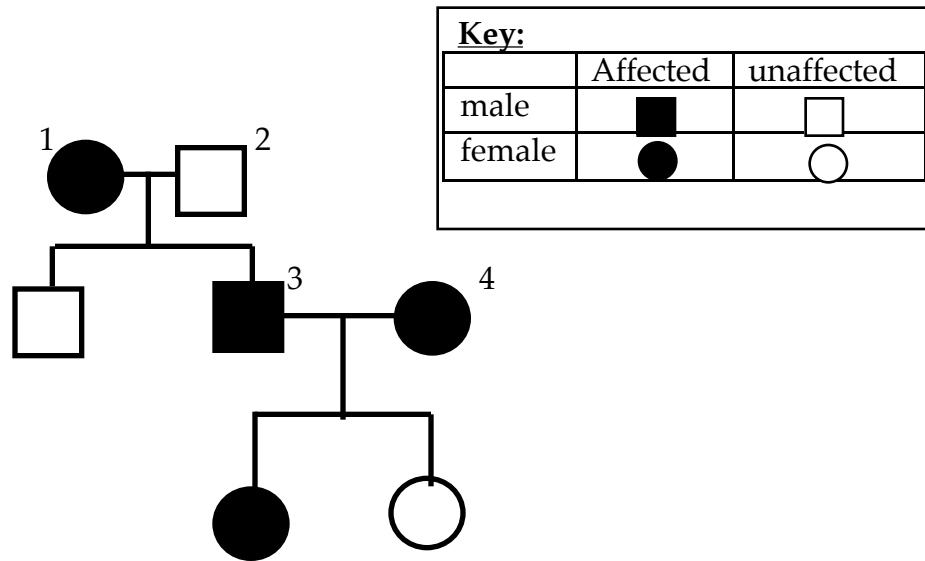


i) Using a diagram similar to the one above, draw the copy(ies) of chromosome 7 that would be found in the sperm produced by John's father that resulted in John. Be sure to indicate any allele(s) that are present. (4 pts)

ii) Using a diagram similar to the one above, draw the copy(ies) of chromosome 7 that would be found in the egg produced by John's mother that resulted in John. Be sure to indicate any allele(s) that are present. (4 pts)

Question 2: Pedigrees I (20 points)

Consider the following pedigree for a rare human genetic disease.



a) What is the most likely mode of inheritance of this disease? Circle one: (5 pts)

- ☐ autosomal recessive
 ☐ autosomal dominant
 ☐ sex-linked recessive

b) Based on your model of part (a), define appropriate allele symbols using the table below: (2 pts)

<u>allele</u>	<u>contribution to phenotype</u>
---------------	----------------------------------

c) Using your symbols from part (b), give the genotypes of the following individuals: (8 pts)

#1 _____
 #2 _____
 #3 _____
 #4 _____

d) If 3 and 4 have another daughter, what is the chance that this daughter will be affected? Justify your answer. (5 pts)

Question 3: Blood-type (18 points)

You are working in the maternity ward of a major Boston hospital. There are 3 babies in your care:

<u>Baby</u>	<u>Blood-type</u>
Cathy	A
Steve	B
Rodger	O

One of the doctors has lost all the information matching the babies with their parents. Your job is to match the babies with their parents. So far, you only have blood-type data on two couples:

- Couple #1: **Tom** (type AB) and **Ann** (type A)
- Couple #2: **Peter** (type B) and **Sally** (type B)

The other couple has not yet given you blood samples. With the data you have, you try to match babies and parents.

a) Given the information above, can you rule out any of the couples as the parents of any of the babies? Explain your reasoning. (6 pts)

Question 3, continued:

b) You learn that Ann's parents have blood-types AB and AB. Based on the information you have so far can you rule out any of the couples as the parents of any of the babies? Explain your reasoning. (6 pts)

c) You learn that Peter's parents have blood-types B and O and that Sally's parents have blood-types AB and AB. Based on the information you have so far can you rule out any of the couples as the parents of any of the babies? Explain your reasoning. (6 pts)

Initials_____

Bio 111 Exam #1

Part II; Version A

10/16/00

Your Name: _____ TA's Name: _____

Write your initials on every page in the space provided.

This exam has 6 pages including this coversheet.

Check that you have pages 1-6.

This exam has two questions.

Read all questions before starting to write.

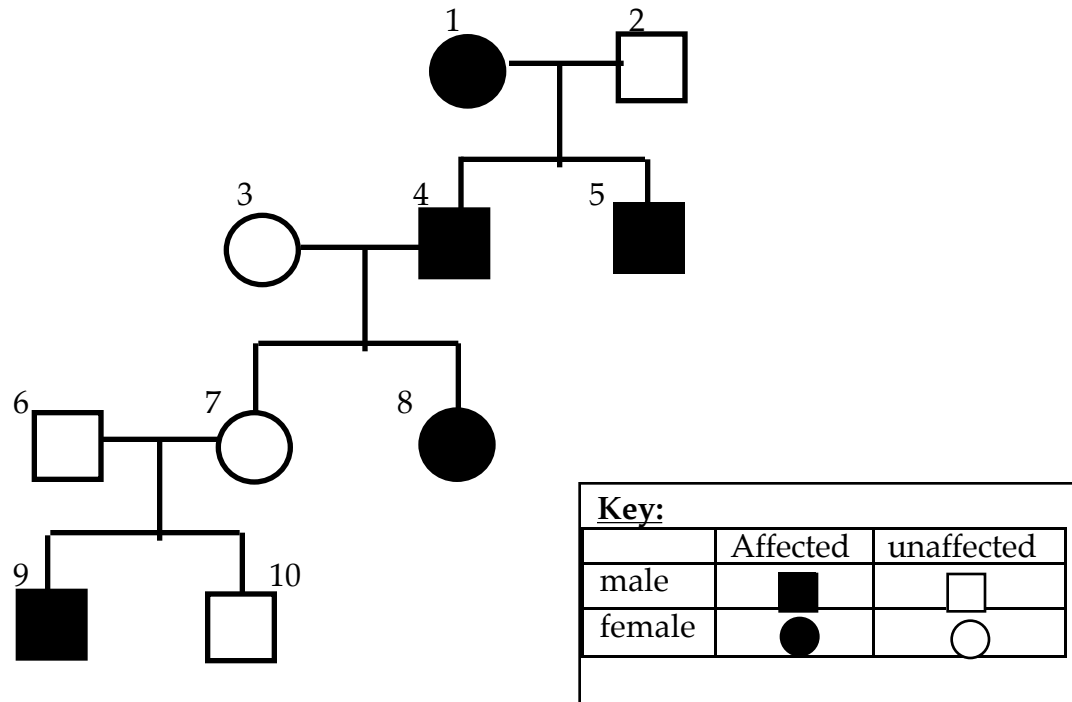
Make your answers as clear and precise as possible.

Answer all questions in the space provided.

Question	Value	Score
4	25	_____
5	25	_____
TOTAL:	50	_____

Question 4: Pedigrees II (25 points)

You are studying a rare human genetic disease. You collect the following pedigree:



Determine the **most likely** mode of inheritance of this disease and answer the questions that follow.

- a) Is the **most likely** mode of inheritance of this disease **autosomal recessive**? yes no
- if **yes**, define appropriate symbols and give the genotypes of the individuals listed:
 allele contribution to phenotype (7 pts)

#1 _____ #3 _____ #5 _____ #7 _____ #9 _____

- if **no**, explain your reasoning. Be as specific as you can and identify individuals by number. If there is more than one reason, give only one.

Question 4, continued:

- b) Is the **most likely** mode of inheritance of this disease **autosomal dominant**? yes no
- if **yes**, define appropriate symbols and give the genotypes of the individuals listed:
 allele contribution to phenotype (7 pts)

#1_____ #3_____ #5_____ #7_____ #9_____

- if **no**, explain your reasoning. Be as specific as you can and identify individuals by number. If there is more than one reason, give only one.

- c) Is the **most likely** mode of inheritance of this disease **sex-linked recessive**? yes no
- if **yes**, define appropriate symbols and give the genotypes of the individuals listed:
 allele contribution to phenotype (7 pts)

#1_____ #3_____ #5_____ #7_____ #9_____

- if **no**, explain your reasoning. Be as specific as you can and identify individuals by number. If there is more than one reason, give only one.

Question 4, continued:

d) Based on the most likely mode of inheritance that you chose, if 6 and 7 have another son, what is the chance that he will be affected? Justify your answer. (4 pts)

Question 5: Alien Plants (25 points)

You are studying a strange (and hypothetical) population of alien plants; these plants are diploid. You find plants in two colors: purple and blue. You do some crosses to find out how color is inherited:

<u>Cross 1</u>			<u>Cross 2</u>		
P:	purple X	blue	P:	purple X	blue
		↓			↓
F₁:	247 purple		F₁:	460 purple	
	262 blue				

Based on this information only, give a genetic model of color inheritance in these plants:

- a) Define appropriate allele symbols and give their contribution to phenotype: (6 pts)
- | | |
|---------------|----------------------------------|
| <u>allele</u> | <u>contribution to phenotype</u> |
|---------------|----------------------------------|

Question 5, continued:

You find some red plants and do some more crosses:

Cross 3
purple X purple
↓
3:1 purple: blue

Cross 4
purple X purple
↓
3:1 purple:red

Cross 5
blue X red
↓
all blue

Based on all the information given in this question, give a genetic model of color inheritance in these plants:

b) Define appropriate allele symbols and give their contribution to phenotype: (9 pts)

allele contribution to phenotype

c) Using the symbols you defined in part (b), give the genotypes of: (2 pts each)
If more than one genotype is possible, give all possibilities.

- Cross 1 purple parent _____
- Cross 2 purple parent _____
- Cross 3 purple parent _____
- Cross 4 purple parent _____
- Cross 5 blue parent _____

Initials_____

Bio 111 Exam #2

Version A

11/13/00

Your Name:_____

TA's Name:_____

Lab Section_____

Write your initials on every page in the space provided.
This exam has 9 pages including this coversheet.
Check that you have pages 1-9.

This exam has five questions.
You may want to read all questions before starting to write.

Make your answers as clear and precise as possible.
Answer all questions in the space provided.

Question	Value	Score
1	27	_____
2	18	_____
3	25	_____
4	15	_____
5	15	_____

TOTAL: 100 _____

Initials _____

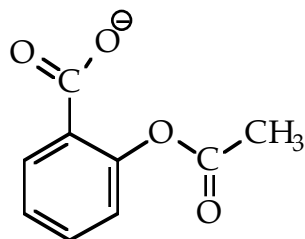
Question 1: Chemistry (27 points)

Note that a summary of chemistry can be found on page 8 of this exam.

Consider the following three molecules:

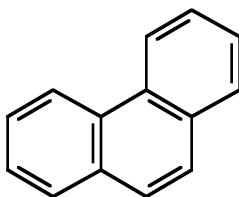
Molecule #1

aspirin



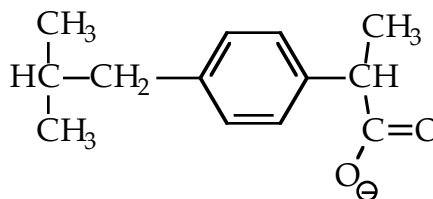
Molecule #2

phenanthrene



Molecule #3

advil



a) Rank the three molecules in order from the most hydrophobic to the most hydrophilic. Note that they are not amino acids. Write your answers in the blanks below (9 pts.)

Most
Hydrophobic

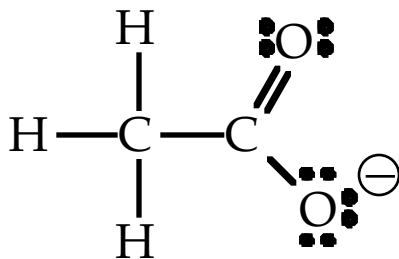
Most
Hydrophilic

b) Draw a molecule in the space below which is similar to the molecules above and is more hydrophilic than the most hydrophilic molecule you indicated above. Make sure that your molecule obeys the bonding rules described in lecture and includes all hydrogen atoms. Note that there are many possible correct answers here. (8 pts)

c) Explain why your molecule from part (b) is more hydrophilic than the most hydrophilic molecule shown in part (a). (4 pts)

Question 1, continued:

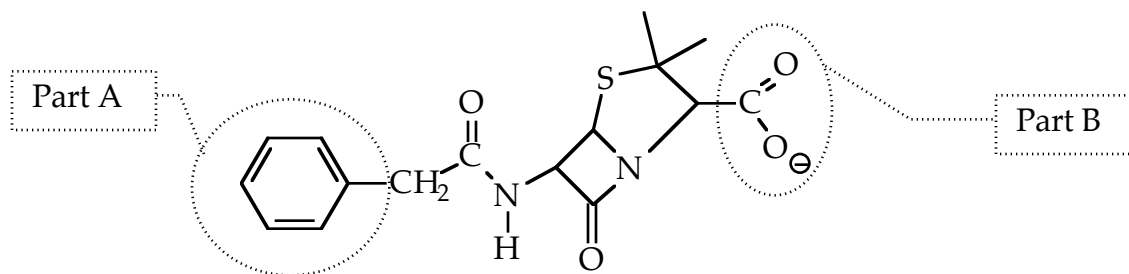
d) Shown below is an acetate ion. Draw a single molecule of water forming a single hydrogen bond to an appropriate part of the molecule. Indicate the hydrogen bond with a **dashed line**. (6 pts)



Question 2: Protein Structure (18 points)

Note that structures of amino acids can be found on page 9 of this exam.

The structure of the antibiotic Penicillin G is shown below with 2 parts circled:



You are designing a protein to bind to Penicillin G.

a) **Consider Part A.**

- i) What is the strongest interaction that the side chain of an amino acid could have with this part of the molecule? (circle one) (3 pts)

ionic bond

hydrogen bond

hydrophobic interaction

- ii) Name an amino acid whose side chain could interact with Part A via the type of bond you circled in part (i). (There may be more than one right answer here; choose only one) (6 pts)

b) **Consider Part B.**

- i) What is the strongest interaction that the side chain of an amino acid could have with this part of the molecule? (circle one) (3 pts)

ionic bond

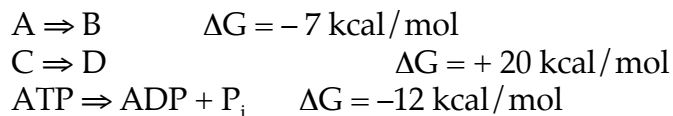
hydrogen bond

hydrophobic interaction

- ii) Name an amino acid whose side chain could interact with Part A via the type of bond you circled in part (i). (There may be more than one right answer here; choose only one) (6 pts)

Question 3: Thermodynamics (25 points)

Given the following information (A, B, C, D, ATP, ADP, and P_i are molecules):



For each of the following questions, circle true or false as appropriate.

- if you circle true, no explanation is required
- if you circle false, explain why the statement is false

a) The reaction $A \Rightarrow B$ is spontaneous. (6 pts) true false
 Explanation if false:

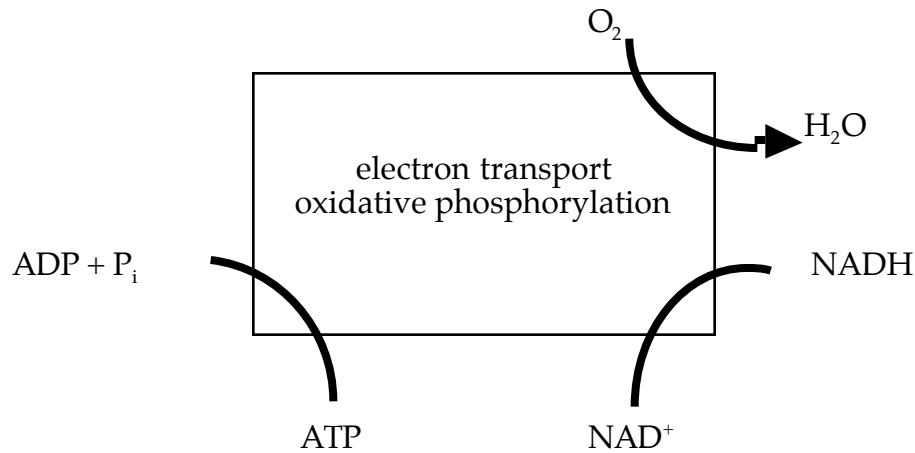
b) The reaction $D \Rightarrow C$ is spontaneous. (6 pts) true false
 Explanation if false:

c) The reaction $A \Rightarrow B$ will proceed at a slower rate than the reaction $\text{ATP} \Rightarrow \text{ADP} + P_i$. (6 pts)
 Explanation if false: true false

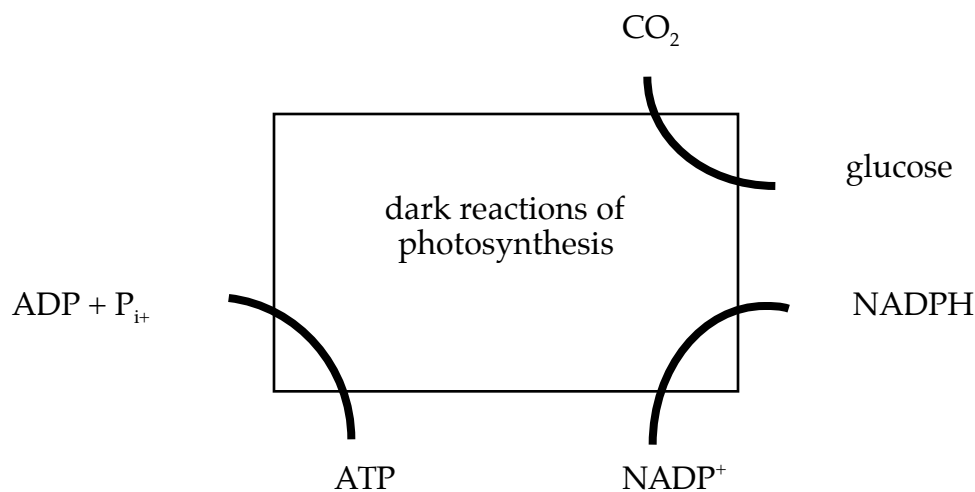
d) The reaction $C + \text{ATP} \Rightarrow D + \text{ADP} + P_i$ is spontaneous. (7 pts) true false
 Explanation if false:

Question 4: Glycolysis & Respiration (15 points)

a) The diagram below shows the major reactants and products involved in electron transport and oxidative phosphorylation. Each of the thick lines should be an arrow indicating the overall flow of the reaction. Complete the arrows by adding arrowheads as appropriate. The arrowhead for the overall reaction $O_2 \Rightarrow H_2O$ is shown as an example. (3 pts per arrow; 2 arrows).



b) The diagram below shows the major reactants and products involved in the dark reactions of photosynthesis. Each of the thick lines should be an arrow indicating the overall flow of the reaction. Complete the arrows by adding arrowheads as appropriate. (3 pts per arrow; 3 arrows).

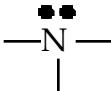
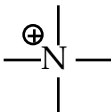
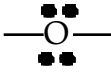
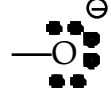
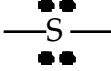


Question 5: Cell Biology (15 points)

Complete the following table by circling “has it” or “doesn’t have it” as appropriate. For example, if an animal cell has DNA anywhere in it, you should circle “has it” in the top box in the “Animal cell” column. (1 pt each)

	<u>Animal Cell</u>	<u>Plant Cell</u>	<u>Bacterial Cell</u>
DNA	<ul style="list-style-type: none"> • has it • doesn’t have it 	<ul style="list-style-type: none"> • has it • doesn’t have it 	<ul style="list-style-type: none"> • has it • doesn’t have it
Protein	<ul style="list-style-type: none"> • has it • doesn’t have it 	<ul style="list-style-type: none"> • has it • doesn’t have it 	<ul style="list-style-type: none"> • has it • doesn’t have it
Lipid	<ul style="list-style-type: none"> • has it • doesn’t have it 	<ul style="list-style-type: none"> • has it • doesn’t have it 	<ul style="list-style-type: none"> • has it • doesn’t have it
Mitochondria	<ul style="list-style-type: none"> • has it • doesn’t have it 	<ul style="list-style-type: none"> • has it • doesn’t have it 	<ul style="list-style-type: none"> • has it • doesn’t have it
Chloroplast	<ul style="list-style-type: none"> • has it • doesn’t have it 	<ul style="list-style-type: none"> • has it • doesn’t have it 	<ul style="list-style-type: none"> • has it • doesn’t have it

Summary Chart:

If you see.. Part of molecule	You should think.... Properties				
	Bond is polar or non-polar	Contributes to making a region hydrophobic or hydrophilic	Can make ionic bonds ^a	Can make hydrogen bonds [‡]	Can make a hydrophobic interaction [*]
C–C	non-polar	'phobic	no	no	yes
C–H	non-polar	'phobic	no	no	yes
C–N	polar	'philic	^a	‡	no
C–O	polar	'philic	^a	‡	no
C–S	non-polar	'phobic	no	no	yes
O–H	polar	'philic	^a	yes	no
N–H	polar	'philic	^a	yes	no
P–O	polar	'philic	^a	‡	no
S–H	non-polar	'phobic	no	no	yes
S–O	polar	'philic	^a	‡	no
	§	'philic	no	yes	no
	§	'philic	yes	no	no
	§	'philic	no	yes	no
	§	'philic	yes	yes	no
	§	'phobic	no	no	yes

Notes:

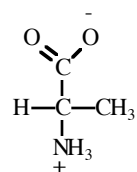
^{*} Assuming a suitable partner is nearby.

^a If the O or N is charged, "yes"; if not "no".

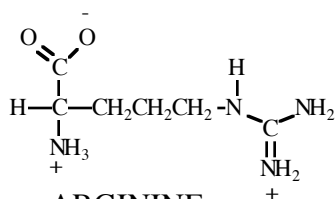
‡ Yes, if the N or O has a lone pair available.

§ Since this is an atom, not a bond, it is neither polar nor non-polar.

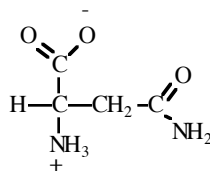
STRUCTURES OF AMINO ACIDS



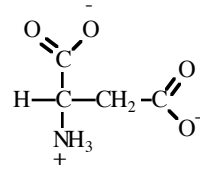
ALANINE
(ala)



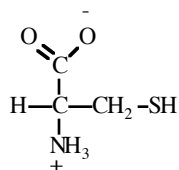
ARGININE
(arg)



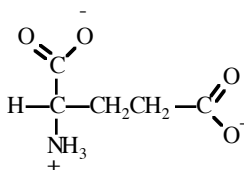
ASPARAGINE
(asN)



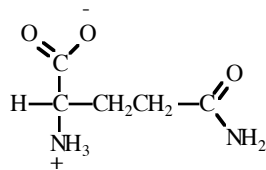
ASPARTIC ACID
(asp)



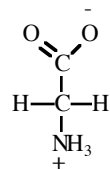
CYSTEINE
(cys)



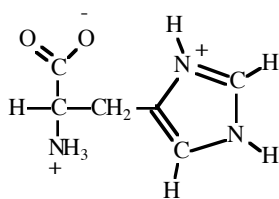
GLUTAMIC ACID
(glu)



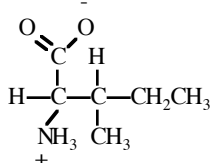
GLUTAMINE
(glN)



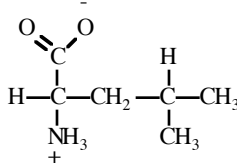
GLYCINE
(gly)



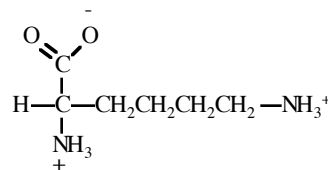
HISTIDINE
(his)



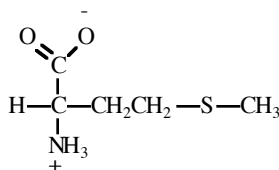
ISOLEUCINE
(ile)



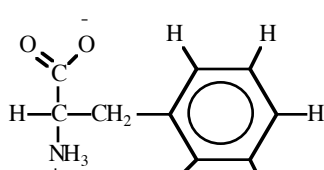
LEUCINE
(leu)



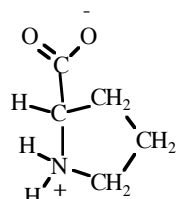
LYSINE
(lys)



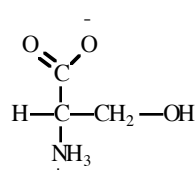
METHIONINE
(met)



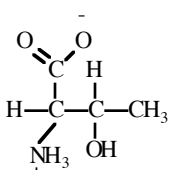
PHENYLALANINE
(phe)



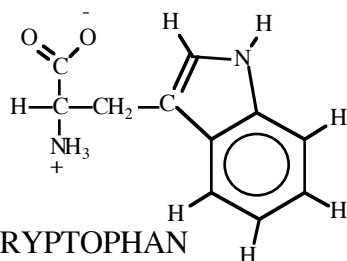
PROLINE
(pro)



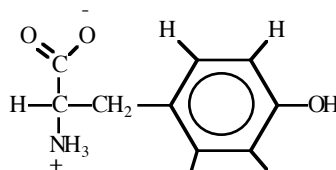
SERINE
(ser)



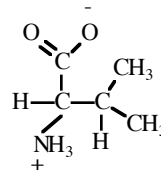
THREONINE
(thr)



TRYPTOPHAN
(trp)



TYROSINE
(tyr)



VALINE
(val)

Initials _____

Bio 111 Exam #3

Version A

12/4/00

Your Name: _____ TA's Name: _____

Write your initials on every page in the space provided.

This exam has 7 pages including this coversheet.

Check that you have pages 1-7.

This exam has four questions.

You may want to read all questions before starting to write.

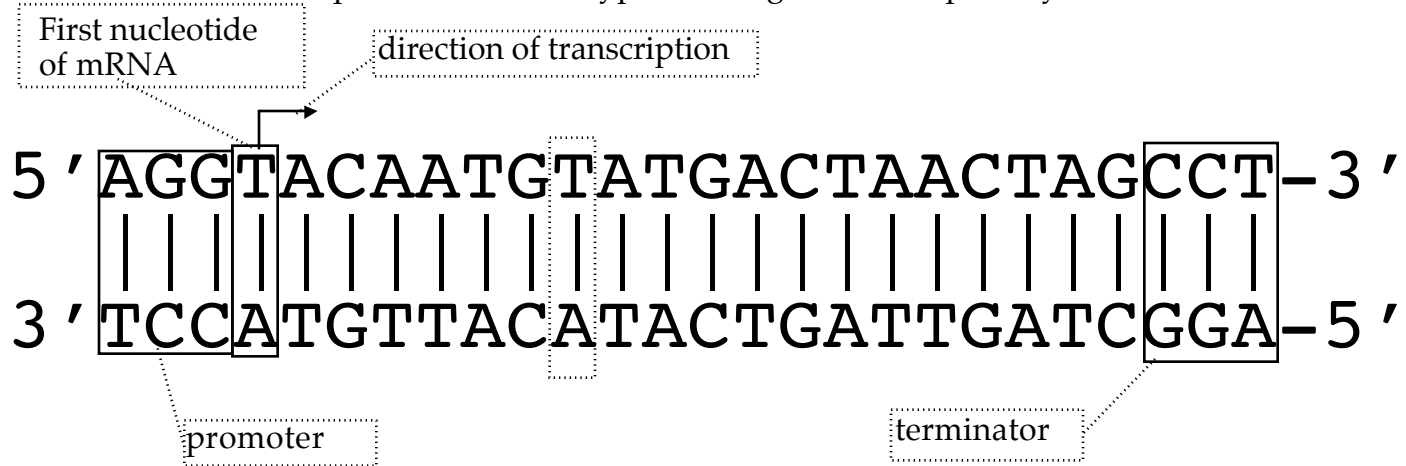
Make your answers as clear and precise as possible.

Answer all questions in the space provided.

Question	Value	Score
1	29	_____
2	28	_____
3	12	_____
4	31	_____
TOTAL:	100	_____

Question 1: Transcription & Translation (29 points)

Shown below is the sequence of a small hypothetical gene from a prokaryote:



a) Give the first 5 nucleotides of the mRNA produced by this gene. Be sure to indicate the 5' and 3' ends. (5 pts)

b) What is the amino acid sequence of the protein produced by this gene? Be sure to indicate the amino and carboxyl ends. A table of the genetic code can be found on page 7 of this exam. (10 pts)

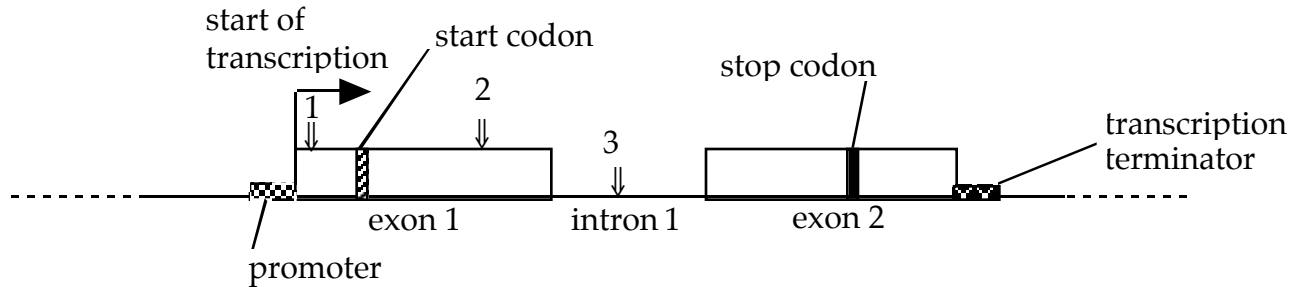
c) What would be the amino acid sequence of the protein produced by this gene if the ^T/_A base pair in the dashed box were deleted? Be sure to indicate the amino and carboxyl ends. (10 pts)

d) What type of mutation is described in part (c)? Circle all that apply. (4 pts)

missense nonsense frameshift promoter no effect on protein sequence

Question 2: Gene Structure (28 points)

Shown below is a diagram of a typical eukaryotic gene which encodes a protein:



i) Could the insertion of three base-pairs at location 2 prevent the formation of normal protein? (circle yes or no) Why or why not? Yes No (7 pts)

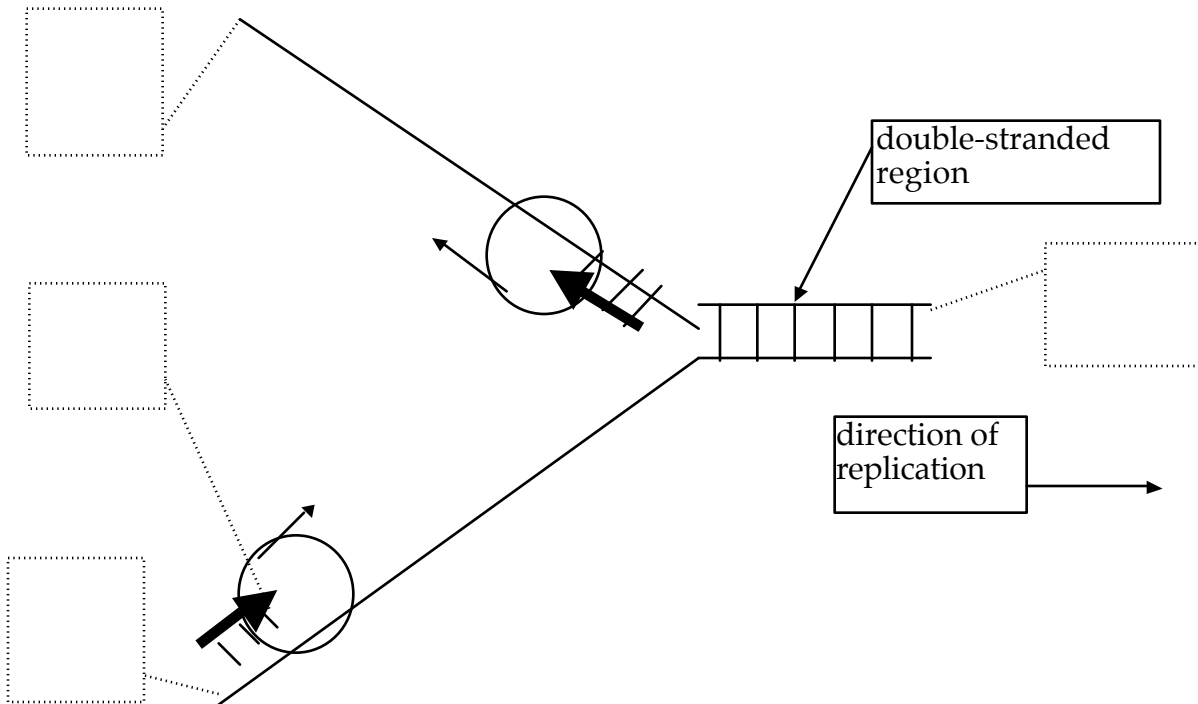
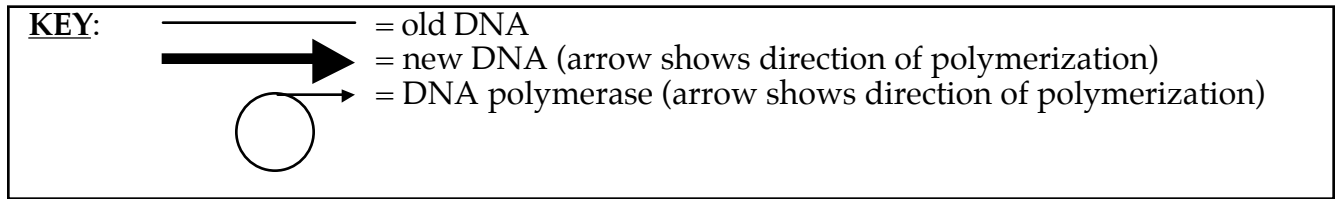
ii) Could the insertion of three base-pairs at location 3 prevent the formation of normal protein? (circle yes or no) Why or why not? Yes No (7 pts)

iii) Using an arrow, indicate a place on the gene above where the insertion of three base-pairs **would be likely to prevent** the formation of normal protein. Explain how your mutation would have this effect. (7 pts)

iv) Surprisingly, the insertion of three base-pairs at location 1 causes a completely abnormal protein to be produced. Provide a plausible explanation for this observation. (7 pts)

Question 3: DNA Structure & Replication (12 points)

a) The diagram below shows a DNA replication fork.



In each of the dashed boxes above, write 5' or 3' as appropriate. (2 pts each)

b) In the figure above, which DNA strand is the leading strand? top bottom
(circle one) (2 pts)

c) In the figure above, which DNA strand is the lagging strand? top bottom
(circle one) (2 pts)

Question 4: HIV/AIDS & Central Dogma (31 points)

a) Listed below are some of the steps in the HIV life cycle. Fill in the boxes with the appropriate missing steps. (3 pts each) **Note** that the numbering here may not be the same as in lecture.

- 1) HIV binds to outside of cell.
- 2) HIV fuses with the cell membrane and is taken into the cell, releasing it's RNA into the cytoplasm.

3)

- 4) Reverse transcriptase makes a second strand of DNA based on the DNA it just made.
- 5) The double-stranded DNA moves into the nucleus of the host cell.
- 6) The DNA integrates into the DNA of the host cell.
- 7) The viral DNA remains latent for some time.
- 8) The viral DNA is transcribed.

9)

- 10) Virus particles are assembled.
- 11) Virus particles leave the cell.

b) You are a doctor treating an AIDS patient. You can treat your patient with either of two drugs:

- Drug X, which binds to ribosomes and prevents them from working.
- Drug Y, which inhibits Reverse Transcriptase and only Reverse Transcriptase.

i) Complete the following table by circling 'yes' or 'no' as appropriate (2 pts each):

	Will this drug prevent the patient's cells from functioning properly?	Will this drug prevent HIV from replicating?
Drug X	<ul style="list-style-type: none"> • yes • no 	<ul style="list-style-type: none"> • yes • no
Drug Y	<ul style="list-style-type: none"> • yes • no 	<ul style="list-style-type: none"> • yes • no

Question 4b, continued:

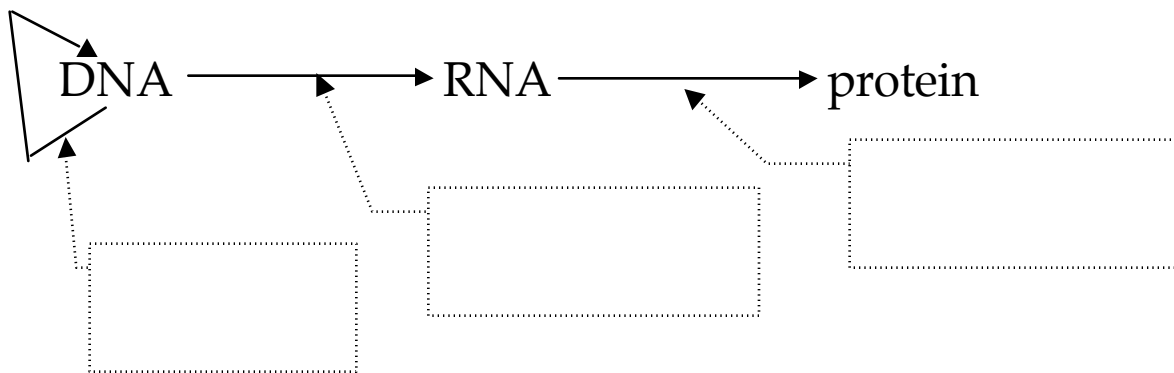
- ii) Based on your responses to part (i), which is the better drug for treating the patient?
Circle one and explain your reasoning. (5 pts)

Drug X

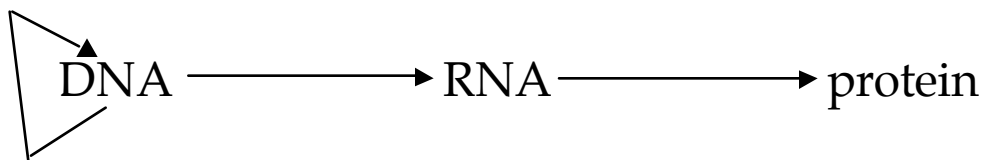
Drug Y

Explanation:

- c) Shown below is a diagram of the “Central Dogma”. The arrows indicate the flow of information.



- i) Write the name of each process in the dashed boxes above as appropriate. (3 pts each)
- ii) On the diagram below, draw an arrow that corresponds to the flow of information catalyzed by Reverse Transcriptase. (3 pts)



The Genetic Code

	U	C	A	G	
U	UUU phe UUC phe UUA leu UUG leu	UCU ser UCC ser UCA ser UCG ser	UAU tyr UAC tyr UAA STOP UAG STOP	UGU cys UGC cys UGA STOP UGG trp	U C A G
C	CUU leu CUC leu CUA leu CUG leu	CCU pro CCC pro CCA pro CCG pro	CAU his CAC his CAA gln CAG gln	CGU arg CGC arg CGA arg CGG arg	U C A G
A	AUU ile AUC ile AUA ile AUG met	ACU thr ACC thr ACA thr ACG thr	AAU asn AAC asn AAA lys AAG lys	AGU ser AGC ser AGA arg AGG arg	U C A G
G	GUU val GUC val GUA val GUG val	GCU ala GCC ala GCA ala GCG ala	GAU asp GAC asp GAA glu GAG glu	GGU gly GGC gly GGA gly GGG gly	U C A G

Initials_____

Initials _____

Bio 111 Final Exam

Version A

12/20/00

Write your initials on every page in the space provided.

This exam has 14 pages including this coversheet.

Check that you have pages 1-14.

This exam has four questions.

You may want to read all the questions before starting to write.

Make your answers as clear and precise as possible.

Answer all questions in the space provided.

Name _____

TA _____

Question	Value	Score
1	30	_____

2	28	_____
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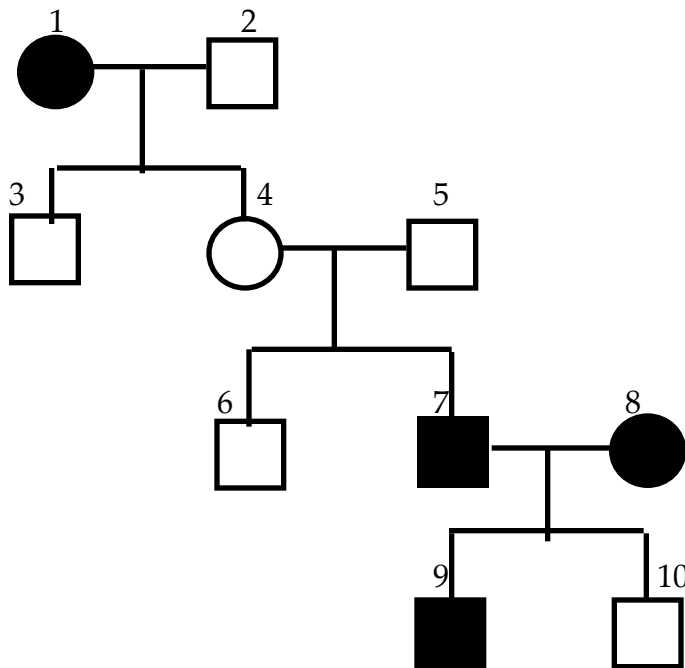
3	18	_____
---	----	-------

4	24	_____
---	----	-------

Total:		_____
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Question 1: Genetics (30 points)

a) You are studying a human disease to see if it is inherited or not. You construct the following pedigree and find that it is not consistent with any of the usual modes of inheritance.



Key:		
	Affected	unaffected
male	■	□
female	●	○

i) Which part of the pedigree is inconsistent with autosomal dominant inheritance? Redraw that portion in the space below and explain why it is **inconsistent** with autosomal dominant inheritance. If more than one part of the pedigree is appropriate you need only draw one part. You may explain in words or using genotypes as you prefer. (5 pts)

Question 1, continued:

ii) Which part of the pedigree is inconsistent with autosomal recessive inheritance? Redraw that portion in the space below and explain why it is **inconsistent** with autosomal recessive inheritance. If more than one part of the pedigree is appropriate you need only draw one part. You may explain in words or using genotypes as you prefer. (5 pts)

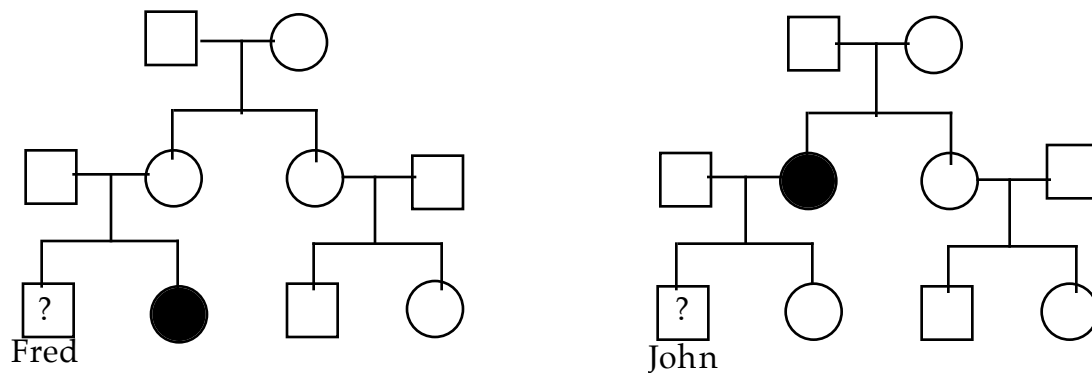
iii) Which part of the pedigree is inconsistent with sex-linked recessive inheritance? Redraw that portion in the space below and explain why it is **inconsistent** with sex-linked recessive inheritance. If more than one part of the pedigree is appropriate you need only draw one part. You may explain in words or using genotypes as you prefer. (5 pts)

iv) If you changed **one** of the unaffected individuals to affected, the pedigree would be consistent with autosomal recessive inheritance. Which individual would you change? If more than one is possible, give only one. (5 pts)

Individual to change from unaffected to affected _____

Question 1, continued:

b) Shown below are two pedigrees for a **rare autosomal recessive** genetic disease. Fewer than 1 in 1000 people are carriers for this disease.



Fred and John are as-yet unborn children of parents who are concerned that they may be affected with the genetic disease.

Based on the above information, which individual, Fred or John, has a greater risk of being affected by the disease. Circle the appropriate name below and explain your reasoning. (5 pts)

Person with higher risk:

Fred

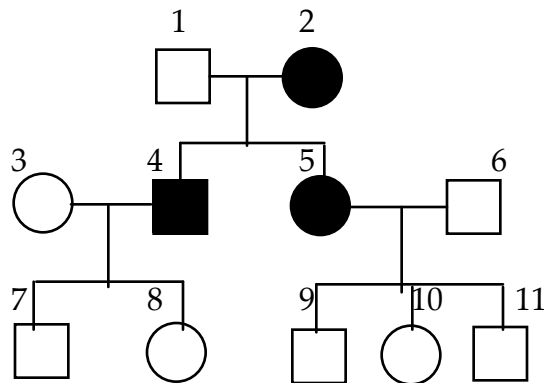
John





Explanation:

Question 1, continued,

c) Mitochondria are essential parts of all human cells. Because mitochondria have their own genome, mutations in the mitochondrial genome are inherited in a different manner from mutations carried on the chromosomes in the nucleus. Mitochondrial mutations are inherited via what is called 'maternal inheritance'. The following facts apply to this situation:

- All the mitochondria in a particular cell are genetically identical. (In real life, it is sometimes more complex than this, but not in this question.)
- All the mitochondria in a particular person are identical.
- All mitochondria are haploid - that is, they carry ONE copy of each of their genes.
- When egg and sperm fuse at fertilization, the mitochondria in the sperm are lost. Thus, a child inherits ALL of his or her mitochondria from his or her MOTHER (hence, maternal inheritance).



Key:		
	Affected	unaffected
male		
female		

Shade in the symbols for individuals 7 through 11 in the pedigree above as appropriate for a mitochondrial maternally inherited disorder as described above (5 pts).

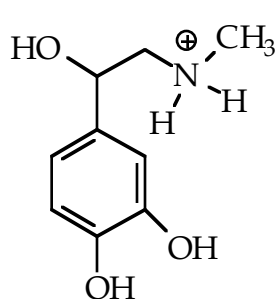
Question 2: Chemistry & Biochemistry (28 points)

a) In the space below, draw a molecule that has the formula C_3H_5NS (that is, it is made of 3 carbons, 5 hydrogens, one nitrogen, and one sulfur. Be sure that:

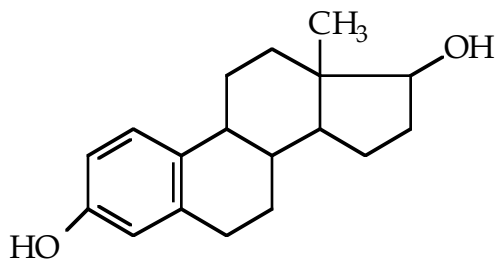
- your structure obeys the bonding rules
- you show all the atoms
- you show any lone pairs
- you show any charges

Your structure need not be that of an actual or chemically-possible molecule. (10 pts)

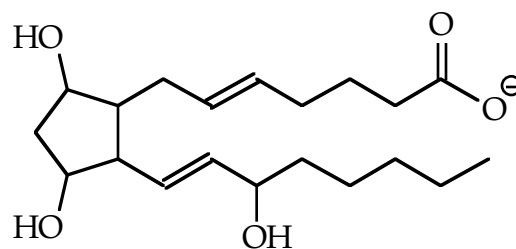
b) Consider the following human hormones:



(1) adrenalin



(2) estradiol
("estrogen")



(3) Prostaglandin

Rank the three molecules in order from the most hydrophobic to the most hydrophilic.

Note that they are not amino acids. Write your answers in the blanks below (6 pts.)

**Most
Hydrophobic**

**Most
Hydrophilic**

Question 2, continued

c) For each of the following pairs of amino acids, select the **strongest** interaction that is possible between their **side chains**. (3 pts each)

i) Aspartic acid and asparagine.

ionic bond

hydrogen bond

hydrophobic
interaction

ii) Histidine and aspartic acid.

ionic bond

hydrogen bond

hydrophobic
interaction

iii) Phenylalanine and alanine. ionic bond

ionic bond

hydrogen bond

hydrophobic
interaction

iv) Serine and tyrosine.

ionic bond

hydrogen bond

hydrophobic
interaction

Question 3: Genetics, Biochemistry, & Molecular Biology (18 points)

Hypertyrosinemia is an autosomal recessive genetic disease characterized by unusually high levels of the amino acid tyrosine (tyr) in the blood. Patients with this disease are unable to break down any excess tyrosine they get from protein in their diet. Untreated hypertyrosinemia patients usually die of kidney and liver failure. Treatment involves severely restricting the dietary intake of tyrosine.

The gene involved in this disease encodes the enzyme FAH, which is required to break down tyrosine. Hypertyrosinemia patients have no active FAH enzyme, leading to a buildup of tyrosine in the blood.

Thus:

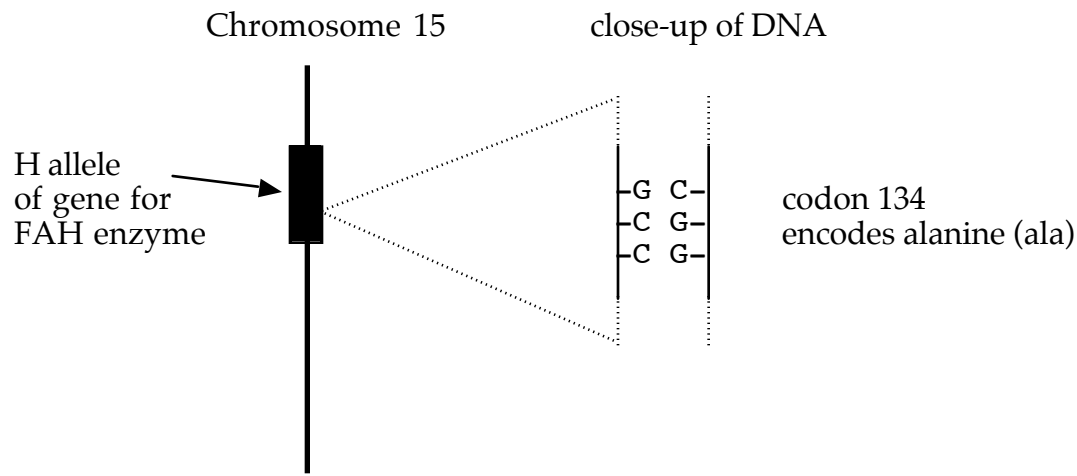
	<u>allele contribution to phenotype</u>	<u>FAH enzyme encoded</u>
H	normal tyrosine breakdown (dominant)	functional
h	hypertyrosinemia (recessive)	non-functional

a) Provide a plausible explanation for why Hh individuals are normal (do not show signs of hypertyrosinemia) in terms of the FAH enzyme produced by these individuals. (5 pts)

b) The normal enzyme encoded by the H allele has an alanine (ala) at position 134 in the protein chain. The non-functional enzyme encoded by the h allele has an aspartic acid (asp) at position 134 in the protein. Based on your knowledge of protein structure, provide a plausible explanation for how this amino acid change could result in a non-functional protein. (5 pts)

Question 3, continued,

c) The gene for FAH is found on human chromosome 15. In the H allele, codon 134 in the DNA is GCC; in the h allele it is GAC. This is diagrammed below:



Using drawing(s) like the one above, draw the copie(s) of chromosome 15 present in a cell from an individual with genotype Hh as they would appear in interphase of mitosis before the chromosomes have duplicated. Be sure to indicate the chromosome(s), gene(s) for the FAH enzyme, the allele(s) and the DNA sequence of codon 134 as shown above. (8 pts)

Question 4: Cancer (24 points)

a) For each of the cells described below, predict whether or not the cell would grow in the presence or absence of growth factor. Circle the appropriate answers and explain your reasoning. A normal cell is shown as an example. (6 pts each)

	<u>Cell</u>	Will cell grow <u>without</u> growth factor?	Will cell grow <u>with</u> growth factor?
	Normal Cell	Yes <input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Yes No
(i)	A cell where one copy of the gene for ras produces a ras protein that is always <u>inactive</u> . And the other copy produces a ras protein that is always <u>active</u> . <u>Explanation:</u>	Yes No	Yes No
(ii)	A cell where both copies of the p53 gene produce a p53 protein which is <u>normal</u> . <u>Explanation:</u>	Yes No	Yes No


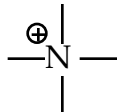
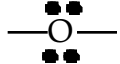
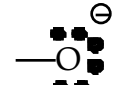
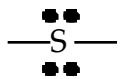
Question 4, continued:

b) Certain individuals inherit a decreased ability to repair damaged DNA. These individuals have a greatly increased cancer risk. Provide a plausible explanation for how the decreased ability to repair DNA can result in an increased cancer risk.

(6 pts)

c) The drug cytarabine is used to treat certain cancers (leukemias). It prevents the enzyme DNA polymerase from carrying out its normal function. Explain how this would prevent the growth of a tumor. (6 pts)

Summary Chart:

If you see.. Part of molecule	You should think....				
	Properties				
	Bond is polar or non-polar	Contributes to making a region hydrophobic or hydrophilic	Can make ionic bonds ^a	Can make hydrogen bonds [‡]	Can make a hydrophobic interaction*
C–C	non-polar	'phobic	no	no	yes
C–H	non-polar	'phobic	no	no	yes
C–N	polar	'philic	^a	‡	no
C–O	polar	'philic	^a	‡	no
C–S	non-polar	'phobic	no	no	yes
O–H	polar	'philic	^a	yes	no
N–H	polar	'philic	^a	yes	no
P–O	polar	'philic	^a	‡	no
S–H	non-polar	'phobic	no	no	yes
S–O	polar	'philic	^a	‡	no
	§	'philic	no	yes	no
	§	'philic	yes	no	no
	§	'philic	no	yes	no
	§	'philic	yes	yes	no
	§	'phobic	no	no	yes

Notes:

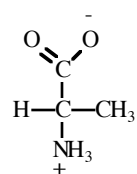
* Assuming a suitable partner is nearby.

^a If the O or N is charged, "yes"; if not "no".

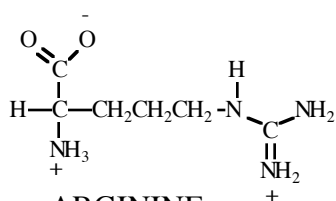
‡ Yes, if the N or O has a lone pair available.

§ Since this is an atom, not a bond, it is neither polar nor non-polar.

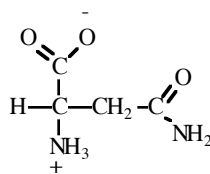
STRUCTURES OF AMINO ACIDS



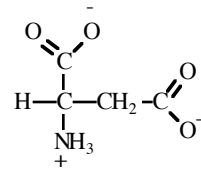
ALANINE
(ala)



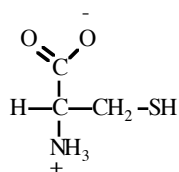
ARGININE
(arg)



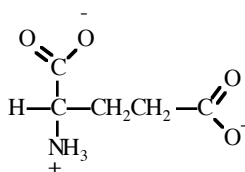
ASPARAGINE
(asN)



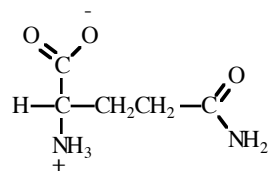
ASPARTIC ACID
(asp)



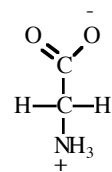
CYSTEINE
(cys)



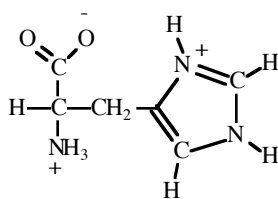
GLUTAMIC ACID
(glu)



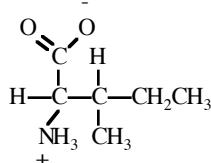
GLUTAMINE
(glN)



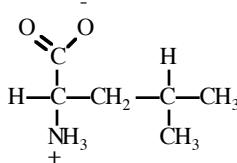
GLYCINE
(gly)



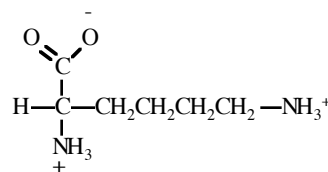
HISTIDINE
(his)



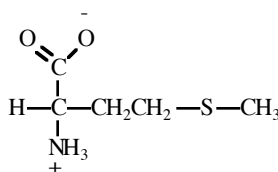
ISOLEUCINE
(ile)



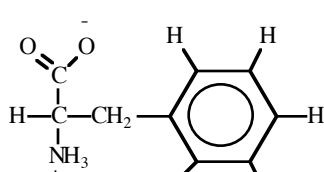
LEUCINE
(leu)



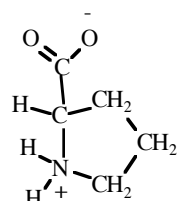
LYSINE
(lys)



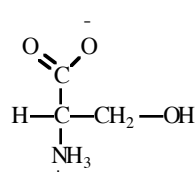
METHIONINE
(met)



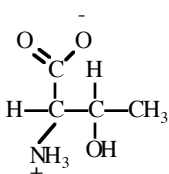
PHENYLALANINE
(phe)



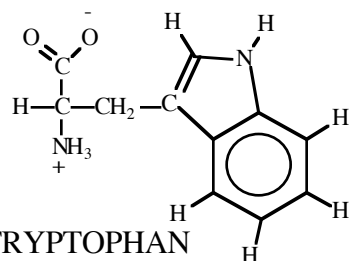
PROLINE
(pro)



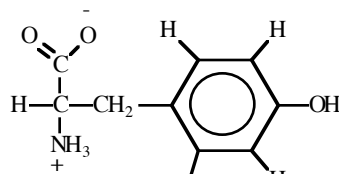
SERINE
(ser)



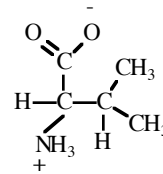
THREONINE
(thr)



TRYPTOPHAN
(trp)



TYROSINE
(tyr)



VALINE
(val)

The Genetic Code:

	U	C	A	G	
U	UUU phe UUC phe UUA leu UUG leu	UCU ser UCC ser UCA ser UCG ser	UAU tyr UAC tyr UAA STOP UAG STOP	UGU cys UGC cys UGA STOP UGG trp	U C A G
C	CUU leu CUC leu CUA leu CUG leu	CCU pro CCC pro CCA pro CCG pro	CAU his CAC his CAA gln CAG gln	CGU arg CGC arg CGA arg CGG arg	U C A G
A	AUU ile AUC ile AUA ile AUG met	ACU thr ACC thr ACA thr ACG thr	AAU asn AAC asn AAA lys AAG lys	AGU ser AGC ser AGA arg AGG arg	U C A G
G	GUU val GUC val GUA val GUG val	GCU ala GCC ala GCA ala GCG ala	GAU asp GAC asp GAA glu GAG glu	GGU gly GGC gly GGA gly GGG gly	U C A G