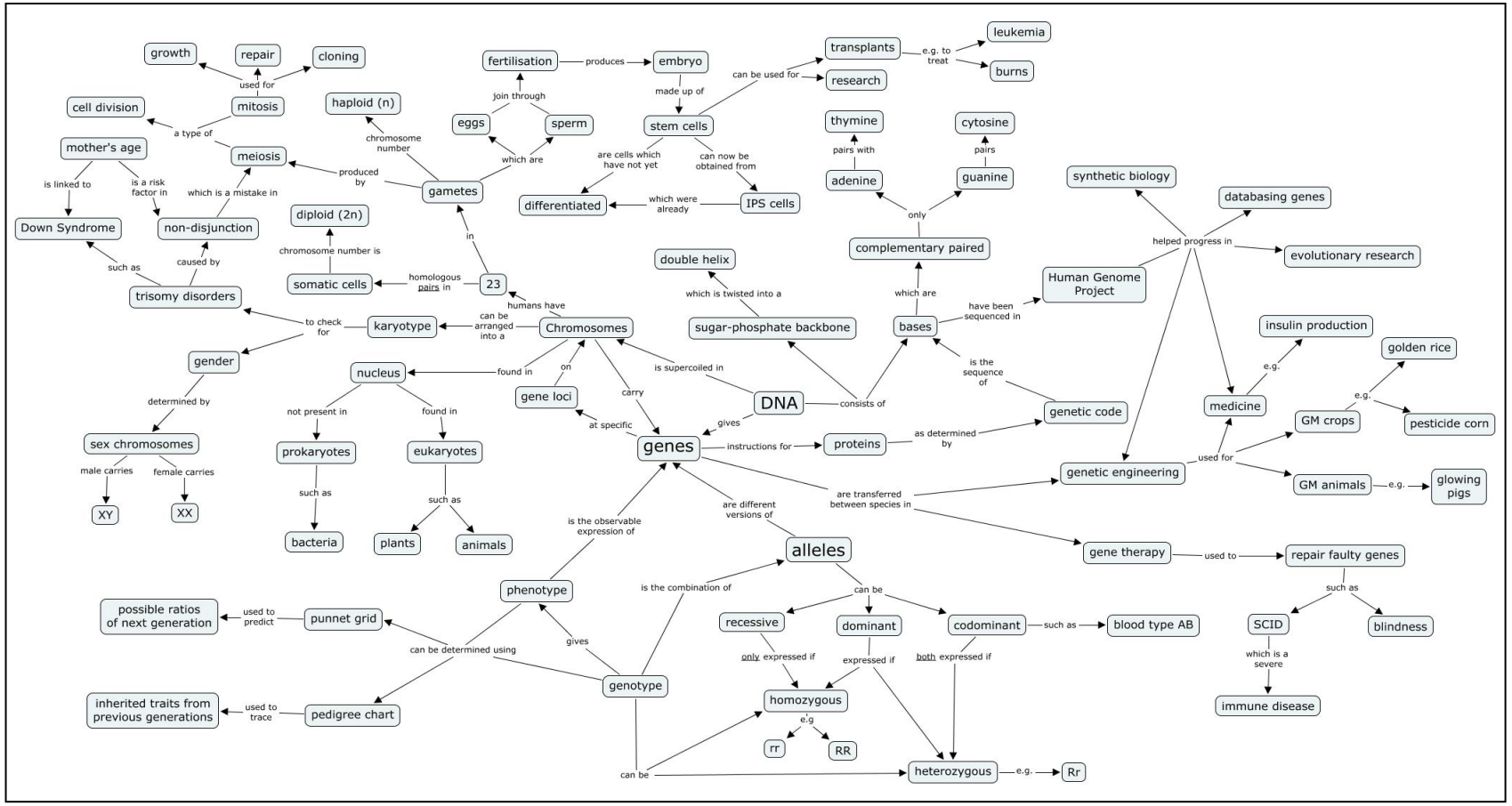
Genetics Laboratory Manual

Fall 2011



Skvirsky RC, Kesseli RV, Kelly MH. Genetics Laboratory Manual. Boston, MA: University of Massachusetts. Fall 2011.
The authors readily acknowledge the many contributions by individuals who have participated in the teaching of these labs over the years. Specific recognition goes (in alphabetical order) to J. Bagarova, J. Chang, M. Gammons and J. Grimsby
http://i-biology.net/about/online-study-tools/

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**Descriptions of labs in italics relate to Lab 3 Trihybrid Cross in Drosophila*

Introduction

1. Grading. The laboratory grade constitutes 25% of the total course grade. It will be based on your laboratory notebook, laboratory reports and worksheets, and the laboratory instructor's evaluation of your performance. The latter includes attendance, participation in discussion, work habits, and evidence that you are seriously attempting to understand the experiments and problems. The instructor will give you more detailed information about what is expected from you.

2. **Cleanliness.** It is important to keep the laboratory clean. You will be maintaining long-term cultures of fruit flies, which are susceptible to mold infections. You will also be handling microorganisms under the usual aseptic conditions demanded of microbiology.

Wash your hands before working with cultures.

Leave cultures open to the room air as briefly as possible.

Leave laboratory tables and benches clear so that they can be wiped down after every session.

3. **Safety.** The instructor will review safety procedures. On the few occasions when toxic chemicals are used, they will be handled in the hood in the prep room next door. Food and beverages may not be consumed in the lab, and should never be placed on the lab benches.

4. **Keeping track.** Label all cultures, plates, and tubes with enough information so that you know what's in them, and can identify them again. Write down this information in your notebook. Keep dated, neat, day-by-day records in a laboratory notebook. All laboratory scientists keep such notebooks. They function something like diaries so that one can go back and find out exactly what was done when. Remember that part of your lab grade will be based on how well you keep your notebook. (See next page for details).

5. **Working arrangements.** Students will work in groups of 3 that will be assigned randomly.

The fruit fly experiment is long-term, and it will be necessary to collect data outside of regular laboratory sessions. (Access to the laboratory in the evening, on weekends, and during holidays will be provided by campus security). It is much more efficient to work in pairs, so that manipulation of cultures and data collection can be divided up. Accuracy is also increased with this arrangement. Nevertheless, each individual must be responsible for the data presented in reports, and for the analysis of data. Each student must write his/her own lab report; lab reports cannot be shared or written together with another student.

6. **Laboratory reports.** Use the standard Biology Department format for reports, described in "How to Write a Lab Report" in this manual. If you have questions, ask the instructor. Reports must be prepared using a computer. You can get instruction and access to the computers in the library, if needed.

How to Keep a Laboratory Notebook

Lab notes in all branches of science are a permanent record of what the scientist actually did and observed, written down as it happened. Your notebook should not be put together later based on what you remember (or think you remember) about the experiment. The notebook should be well organized, with a neat table of contents at the front. PLEASE put your name and telephone number on the cover so that your work doesn't get lost. All work conducted during the lab must be recorded during the lab. You should summarize what you accomplished during the lab at the end of the period.

What to include

Your notebook is the place to write questions and comments when reading the manual before a particular lab. Also use it for any notes you take on your instructor's introduction to the lab. Especially, use it to record what you do while carrying out each experiment or exercise, step by step; your thoughts and calculations along the way; and all of your data, preferably organized in clear, well-organized tables. If you use specific protocols from the manual, you should state that clearly in your lab notebook and in any lab report. But you must record any changes that you make in the procedure and any problems that you encounter. You should also record all observations, unusual circumstances, and any doubts about the validity of your data. Always keep in mind that you will have to go back and use this information for writing up reports. You will find that task much easier if your data entries are neat and well annotated.

Every lab should be in the notebook. Some labs will need only a short entry; the lab on *Drosophila* as an Experimental Organism, in which we practice distinguishing male and female flies, is straightforward. For that lab, take a page to write down what you learned, and any problems you had. Other labs, such as the long *Drosophila* gene mapping experiment, will require a lot of writing and analysis. For each of these labs, you should record in detail all relevant information such as what crosses you set up on what dates; numbers of flies of each sex and phenotype put into each vial; the dates you cleared and counted the flies, etc. Ideally, another experimenter should be able to use your notes to repeat the experiment exactly as you performed it. The questions at the end of specific labs are to help you understand the experiment and should be answered fully in your notebook. Similarly, the predictions you make for *Drosophila* gene mapping lab should be worked out in your notebook.

There is no need to fit the whole semester's work in the first 20 pages. Give each experiment the space it needs from the start, so you don't have to cram data on 300 flies of 8 phenotypes into half a page, or record one experiment in four different places with other stuff sandwiched in between.

Lab notes should be kept in ink. The notebook must be neat enough for you (and your lab instructor) to read it. You're not graded on handwriting, but please make an effort. The goal is a complete record, including mistakes. (Mistakes should be neatly crossed out, not obliterated, not erased. Sometimes knowing what mistakes you made is as important as reaching a conclusion.) Complete sentences are better than incomprehensible fragments.

Grading

Your notebook counts for 50 points of your lab grade. In terms of your overall course grade, that means it's worth half of an hour exam. Lab notebooks will be collected and graded periodically.

How to Write a Laboratory Report

The ability to write a coherent account of an event is important for people working in any field. A laboratory report in biology should be in part considered an exercise in this art. Great eloquence and elegance of style are not crucial. Aim for clarity and thoroughness as well as good grammar, correct spelling, and proper sentence structure when you write lab reports.

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

Format of a Biology Lab Report:

This format is based on that used in papers published in biological and medical journals.

Introduction: This section should indicate clearly what ideas are being investigated and state specifically the purpose of the experiment or observation. If you do several experiments in one lab, the purpose of each should be given. Do not merely copy what is in the lab manual; present the purpose in your own words. The Introduction need not be lengthy.

Materials and Methods: Ask your lab instructor whether you should present the procedures in detail or can merely state "as in lab manual". If written out, the materials you used and the methods followed should be presented clearly and with enough detail so that another person could perform the experiment based on what you have written. Don't just copy the lab manual methods section: use your own words and perhaps improve on the way the manual explains techniques! This section should not be written as a set of instructions. Rather you should describe what was done. Finally, no results should be included here.

Results: Here you should present your data in tables, graphs, or diagrams that clearly show what happened in the experiment. Descriptive observations may supplement quantitative data. All drawings should show accurately what you saw: they should not be copied from another source. Do not interpret your data in this section. Leave that to the Discussion.

Discussion: This section includes an interpretation of your results and a discussion of what these results mean. If something did not turn out the way you expected, try to explain why. If you think your experiment could have been designed differently for better results, tell how you could improve on it. You can also discuss your results in comparison to others in the lab or in books or published articles.

References: In scientific reports, footnotes are not usual (as they are in history papers, for instance). Instead, we refer to a book or article from which we gained information as follows:

> It is estimated that over 10 million people in this country have some type of genetic disorder (Klug, Cummings, and Spencer, 2006, p. 10).

At the end of your lab report, you would give the full reference to the book or article, and if you used several articles, you would give them in alphabetical order by the first author's last name. Suppose you had used the book cited above by Klug, Cummings, and Spencer. At the end of your lab report you would list the book under the subheading "References."

References:

Klug, W.S., M.R. Cummings, and C.A. Spencer. 2006. Concepts of Genetics. Eighth Ed. Pearson Prentice Hall. Upper Saddle River, N.J.

If you have used an article from a journal, the format would be similar to that above when you refer to it in the text. And in the references at the end you would list it thus:

Rice, G. 2005. "How and why do fireflies light up?" *Scientific American*. Dec; 293 (6):128.

(Note: Journal titles can be abbreviated if certain conventions are followed: *Scientific American* becomes *Sci. Am*.)

Plagiarism: Plagiarism refers to the copying of statements (whether exact or paraphrased words) or the ideas of others without acknowledging where you obtained them. It is a very serious crime in the world of academia. You need to learn how to properly acknowledge your source of information. Often, you will simply acknowledge the source as we did above, showing where we found the details. If you use the exact words of the author you must make that clear by using quotes. For instance,

> Campbell and Reece (2005) state on page 268 that "For those with a family history of Huntington's Disease, the availability of this test poses an agonizing dilemma."

Usually, though, you should not directly quote others. Put the idea into your own words. You show how well you understand a concept when you do that.

Plagiarism may also refer to the copying of papers or lab reports. Lab partners should never copy each other's lab reports. You may do an experiment together, but each partner should be individually responsible for all data. Furthermore, the analysis and presentation of results should be done individually, and lab reports should clearly reflect your own attempt to make sense out of what you did in lab. Of course students may consult with each other when they are trying to analyze data.

To cite the lab manual

To cite the lab manual in the reference section of your lab reports, use the following form including the specific pages you are referencing:

Skvirsky RC, Kesseli RV, Kelly MH. Genetics Laboratory Manual. Boston, MA: University of Massachusetts. Spring 2011. p. xx-xx.

LAB 1: Mitosis and Meiosis: Modeling with Beads

OBJECTIVE: To understand and commit to memory the processes of mitosis and meiosis; to understand and become familiar with the following terms: chromosomes, chromatids, centromeres, haploidy, and N number.

BACKGROUND: In order to understand the genetics of eukaryotic organisms, it is essential to understand (and memorize) the essential features of mitosis and meiosis. Now is the time to fix these fundamental activities of eukaryotic cells in your long-term memory. Before carrying out this lab exercise, you should have read the section on cell division in your genetics textbook. The lab offers 2 more ways to grapple with the patterns of cell division: 1) detailed charts that illustrate the successive stages of mitosis and meiosis, and 2) bead models that allow you to simulate mitosis and meiosis. When you have questions, ask the instructor.

A. Chromosome Structure. The DNA of eukaryotic cells is organized as long, linear molecules (associated with proteins) called chromosomes. The typical chromosome consists of 1 or 2 chromatids. At some point along the length of each chromosome is a specialized region called the centromere. In our chromosome models, plastic beads will represent the gene-bearing arms of the chromatids; the centromeres are represented by magnets.

1) Make a chromatid from the red beads. Make one arm of 12 beads, the other of 5 beads. Insert the ends of each arm into a centromere (Fig. 1). Make another identical chromatid from red beads, and join the two chromatids together at the magnetic centromeres. Now you have a chromosome consisting of 2 sister chromatids.

2) Using yellow beads make another chromosome that is identical to the first except for color. Now you have 2 homologous chromosomes. Such homologs are usually identical in length and position of the centromere, and each carries the same genes in the same order. However, the specific DNA sequences of the corresponding genes may vary. That is, each homolog may carry a different form of a particular gene. These different forms of a gene are called alleles.

3) Make 2 more homologous chromosomes as above. This time make one arm 10 beads long, and the other 3 beads long. Now you have 2 sets of homologous chromosomes. Let us call the long homologs chromosome number 1, and the short homologs, number 2. Chromosome 1 and 2 differ in length and position of the centromere, and also carry different sets of genes (Fig. 2).

4) Take small bits of tape to designate particular genes and alleles on your chromosomes as illustrated in Fig. 2.

B. Ploidy and N number. The nucleus of a germ cell (egg or sperm in animals) contains a fixed number of chromosomes that is typical of the particular species. This is called the haploid number (N).

In *Drosophila*, for instance, N = 4; in humans N = 23. A haploid set of chromosomes contains one set of genes for constructing the organism. In animals, somatic cells (body cells) usually contain 2 sets of chromosomes, and therefore 2 sets of genes. They are diploid (2N). The complete 2N set of human chromosomes is shown in Fig. 3, first as photographed directly from a mitotic cell, and then arranged as paired homologs. The situation is more complex in plants and fungi which have life cycles of alternating N and 2N generations, but the principles are the same.

Cluster the chromosomes you have made on the table in front of you. What is represented is the complement of chromosomes in the somatic cell of an organism for which 2N = 4. It has 2 pairs of homologous chromosomes, designated 1 and 2.

C. Mitosis. The function of mitosis is to replicate exactly the total complement of chromosomes in each cell division, so that all cells of the body will contain the same set of 2N chromosomes.

Refer to the diagram of mitosis in your text, or on the wall chart, and simulate the process of mitosis. Line up the four chromosomes as in metaphase, in any order. Note the orientation of the chromosomes relative to the metaphase plate. Pull the chromatids apart at each centromere, separating them into two daughter cells. (What is this part of mitosis called?)

If you want, you can tie strings to the centromeres, as in Figs. 4 and 5, to represent the fibers of the mitotic spindle that provides the motive force for the separation of chromatids. Look closely at your daughter cells. (What phase did they just go through?) If each one contains a complete set of 4 chromosomes, then your mitosis went correctly. (*What is the consequence of a missing or extra chromosome? How does this arise?)* If a chromosome has been lost or added to a cell, then something went wrong, and the cell will probably die or malfunction.

Note that just before mitosis, each chromosome consists of 2 identical (sister) chromatids joined at the centromere. Just after mitosis, each chromosome consists of 1 chromatid. Before the next round of division, each chromatid will be copied again, in the process of DNA replication, during the S phase of the cell cycle (see text). If you wish, you can simulate DNA synthesis and go through another round of cell division, ending up with 4 identical sets of chromosomes in 4 cells.

D. Meiosis. Meiosis occurs only in the formation of germ cells. It consists of 2 sequential cell divisions. Meiosis results in 4 genetically unique cells in which the chromosome number is reduced to N. Egg and sperm cells must be haploid (N) because they unite in fertilization to produce a new diploid (2N) organism.

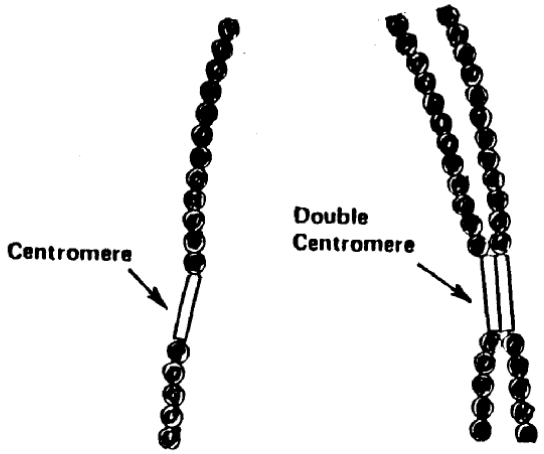
Start again with a complete set of 4 chromosomes, each with 2 sister chromatids. In the first meiotic division, homologous chromosomes pair, crossing-over occurs, and then they separate. Line up the homologs in pairs (Fig. 5). At this stage chromatids belonging to each homolog can exchange pieces, in a process called crossing-over. Do such a recombination of genetic material for each pair of chromosomes, as illustrated in Fig. 5. Now pull the homologous chromosomes apart into daughter cells. At the end of the first meiotic division (meiosis I), the chromosome number has been reduced from 2N to N. Each chromosome still consists of 2 chromatids, but in segments where crossing-over has occurred, the chromatids may be genetically different.

The second meiotic division (meiotic division II) resembles mitotic division. Line the chromosomes up in each of the cells generated by division I. Now pull them apart at the centromere. You should now have 4 cells. Each cell should contain one copy of chromosomes 1 and 2. If any cell lacks one of the chromosomes, something has gone wrong. It will lack a set of essential genes and will probably die.

You should see that because of crossing-over, each of the 4 cells resulting from meiosis is genetically unique. As a result of fertilization (the union of an egg and a sperm cell), the new diploid organism receives one set of chromosomes from its mother and one from its father. Let us say that in the organism you have been simulating, the red chromosomes were maternal, the yellow chromosomes paternal. Note that crossing-over mixes up (recombines) the paternal and maternal genes. Thus the essential function of meiosis, crossing-over and fertilization, and the whole complicated business of sexual reproduction, is thought to be genetic recombination. The alleles within a population are reshuffled at each generation. Considering only the alleles A, a, B, and b, see if you can figure out how many genetically different germ cells can result from the meiosis you just simulated.

Clean up:

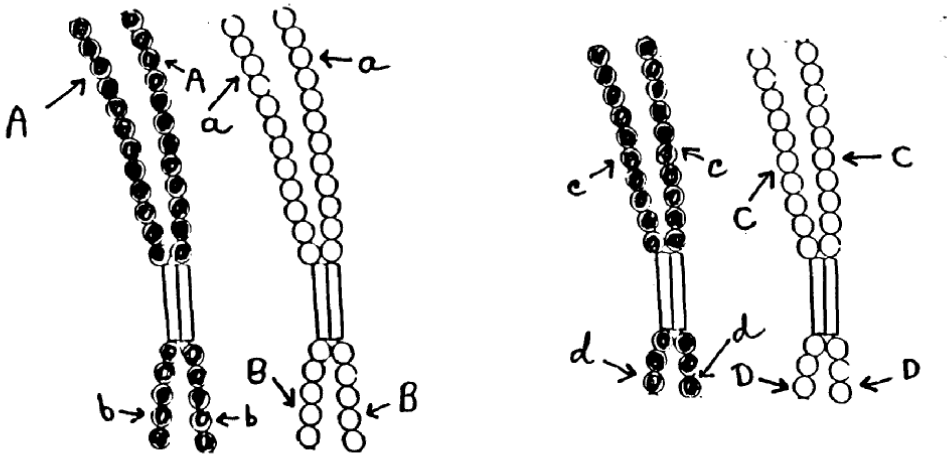
Please remove any tape from the beads and place them back on the tray in the configuration that they were in when you arrived.



LABORATORY 1

Mitosis and Meiosis

Fig. 1. Model of chromosome with one (left) and 2 (right) chromatids.

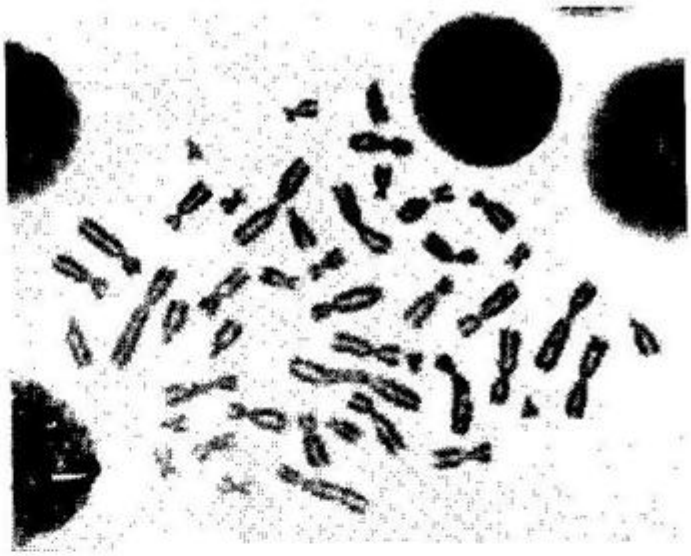


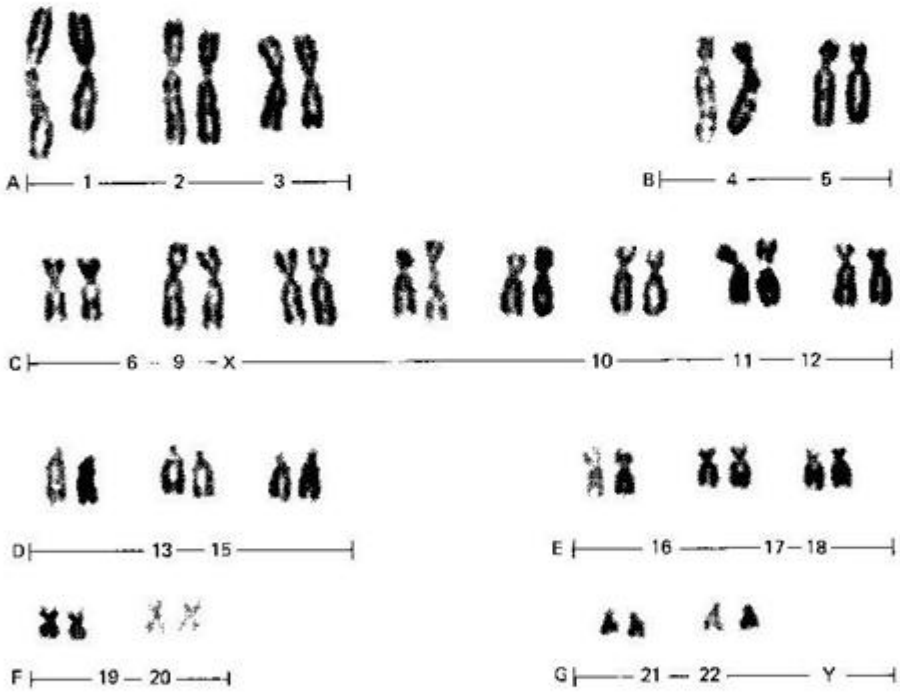
chromosome no. 1 chromosome no. 2

Fig. 2. Homologous chromosomes.

FIGURE 3A:

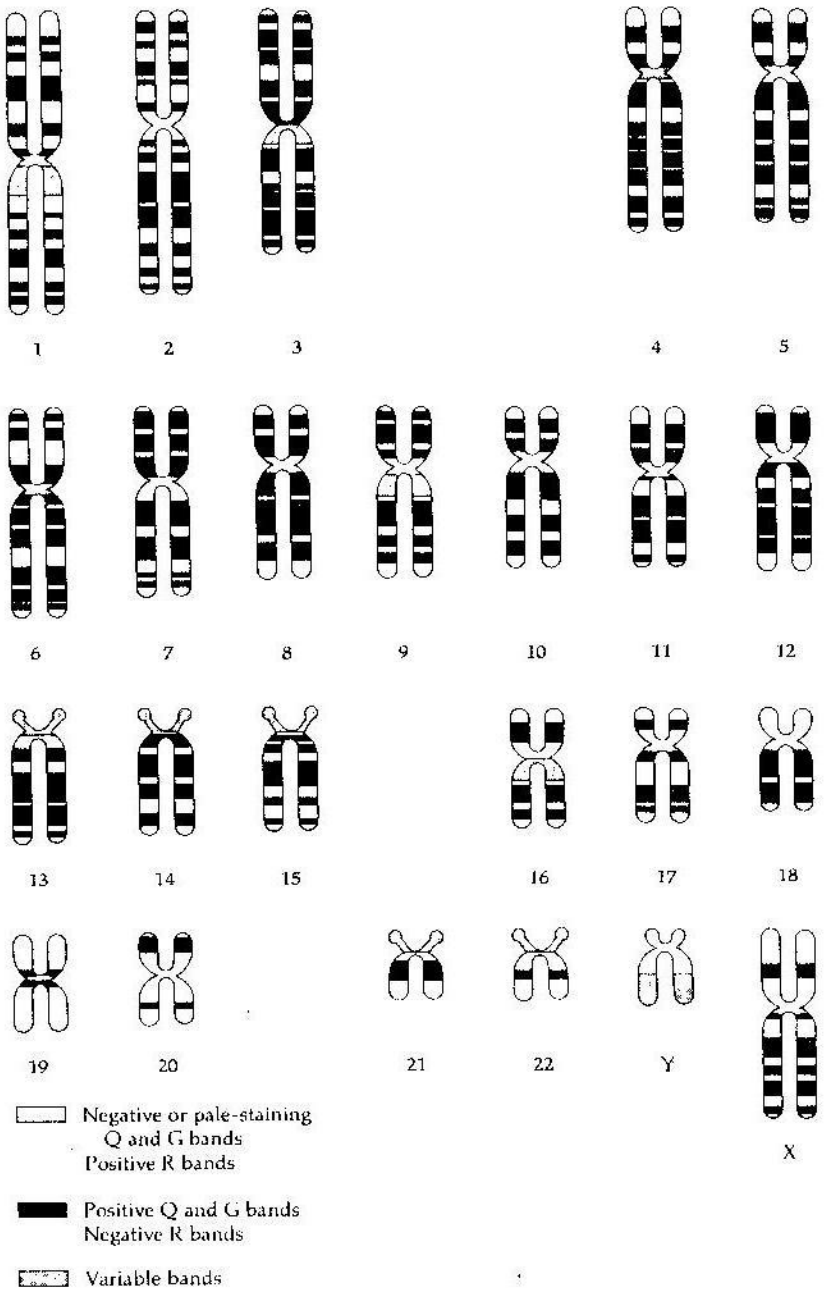
The chromosomes of a normal human female. In this case the chromosomes have been stained with an ordinary dye that simply darkens the chromosome. (Courtesy of R. E. Magenis, University of Oregon Health Sciences Center.)





The chromosomes of Figure 1-5 arranged by pairs according to size and shape in a standard karyotype. (Courtesy of R. E. Magenis, University of Oregon Health Sciences Center.)

FIGURE 3B:



Banding pattern of human chromosomes obtained as a composite of various special staining procedures. G bands are Giemsa bands (see Figure 2.6); R bands are reverse Giemsa bands (R bands are heavy where G bands are light and light where G bands are heavy); and Q bands are quinacrine fluorescent bands (see Figure 2.5).

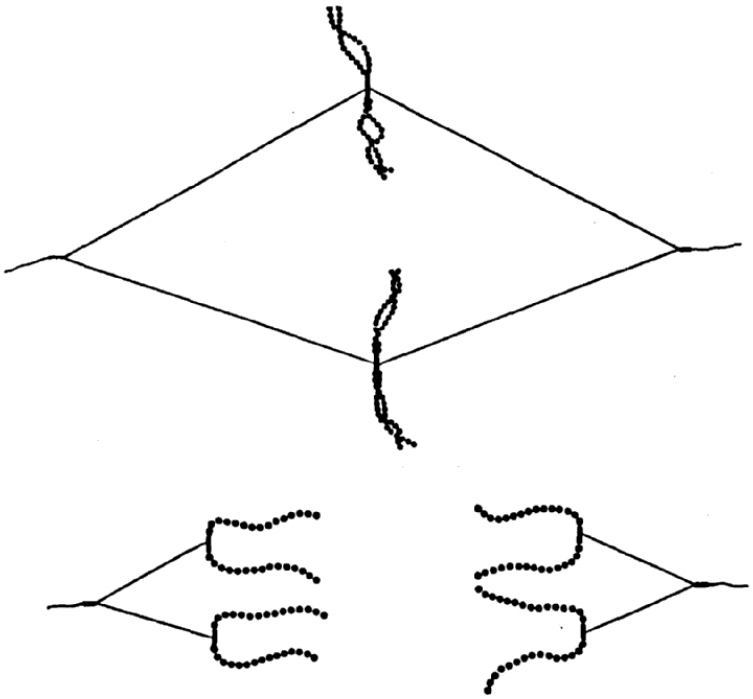


Fig. 4. A way to simulate the mitotic apparatus with strings. Mitotic metaphase above; anaphase below.

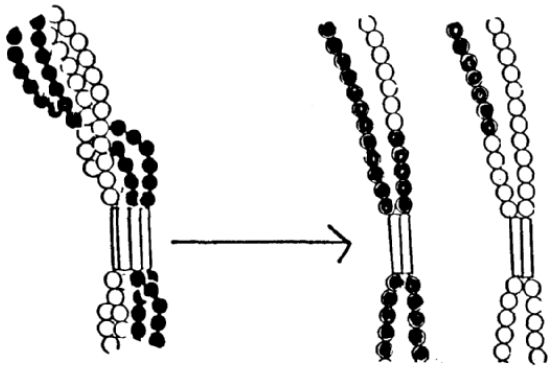


Fig. 5. To the left, Homologues of chromosome no. 1 (see Fig. 2) paired in meiotic prophase I. C indicates a chiasma (point of crossing over). To the right, the homologues as they pull apart after metaphase, showing the result of recombination.

Worksheet

To test your understanding of mitosis and meiosis, fill in the tables below. If you make a mistake, or find some aspect confusing, discuss the issue with other students or with the instructor.

The somatic cells of the mouse contain 40 chromosomes. With regard to the mouse, fill in the following blanks and tables with the appropriate numbers.

1) Diploid chromosome number: _____ Haploid chromosome number:_____

2) Write in the number of chromatids per chromosome at the end of each stage of cell division:

Mitosis

	prophase	metaphase	anaphase	telophase
chromatids per chromosome				

Meiosis I

	prophase I	metaphase I	anaphase I	telophase I
chromatids per chromosome				

Meiosis II

	prophase II	metaphase II	anaphase II	telophase II
chromatids per chromosome				

3) Write in the total number of chromosomes and chromatids per cell at the end of each stage of the cell cycle or cell division:

	G1	end of S	G2
chromosomes			
chromatids			

Mitosis

	prophase	metaphase	anaphase	telophase
chromosomes				
chromatids				

Meiosis I

	prophase I	metaphase I	anaphase I	telophase I
chromosomes				
chromatids				

Meiosis II

	prophase II	metaphase II	anaphase II	telophase II
chromosomes				
chromatids				

Lab 2: Dihybrid Crosses with Corn (*Zea mays)* and *Brassica* ("Wisconsin Fast Plants")

OBJECTIVE: To predict the outcome of several dihybrid crosses in *Zea mays* and *Brassica* using Mendel's Laws and to test the predictions against actual crosses using the chi square method.

BACKGROUND: Maize or corn (*Zea mays*), like *Drosophila*, has been a very important organism for the study of genetics. Species that make good genetic model systems often have several features in common. Different lines should be easy to cross (mate) and large progenies should be produced. The species should also have traits that can be scored easily and quickly. Maize has these features. For traits, many can be viewed early in the kernels or in the seedlings. We will discuss several of these traits in both kernels and seedlings and then you will examine three crosses segregating for a few of these traits. Research on Maize has not only aided our understanding of fundamental genetic processes, but has also contributed to the improvement of corn as an agricultural product.

Kernel Traits. Many corn mutants that have been isolated affect the kernels. A mature kernel has three major parts: the pericarp, endosperm and embryo (Figure 1). The pericarp, the transparent outer layer, is derived from the maternal ovary wall and is therefore genetically identical to the maternal plant. The endosperm and embryo develop after fertilization and therefore represent the next generation. The enzymatically active outer cell layer of the endosperm is the aleurone. This specialized layer of cells is capable of becoming pigmented while the inner endosperm is generally only white or yellow. Genetic experiments involving kernel mutants are convenient because it is easy to view many characteristics of the progeny without growing the plant. The endosperm, aleurone and embryo, formed from fertilized cells, express both maternal and paternal genes.

Several morphological markers along with descriptions and locations are listed below.

Locus	Location[a]	Name, Phenotype
a1	3L-149.0 (111)	anthocyanin-less: colorless aleurone, green or brown plant, brown pericarp with P1-RR
a2	5S-35 (15)	anthocyaninless: like *a1* , but red pericarp with P1-RR
a3	3L-132	anthocyanin: recessive intensifier of expression of R1 and B1 in plant tissues
$C1^{i}$	9S-26	Aleurone Color Inhibitor
R1	10L-61 (57)	colored: red or purple color in aleurone and/or anthers, leaf tip, brace roots, etc.
sh1	9S-29 (29)	shrunken: inflated endosperm collapses on drying, forming smoothly indented kernels
sh2	3L-149.2 (111.2)	shrunken: inflated, transparent, sweet kernels collapse on drying, becoming angular and brittle
su1	4S-66 (71)	sugary: endosperm wrinkled and translucent when dry; sweet at milk stage; starch debranching enzyme
su2	6L-58 (57)	sugary: endosperm glassy, translucent,
y1	6L-17 (17)	yellow endosperm: carotenoid pigments in endosperm; some alleles affect chlorophyll in seedlings
y3	2S-near *al1*	white endosperm: like *y1*

y8	7S-18 (18)	white endosperm: pale yellow endosperm
y9	10S-24	white endosperm: pale yellow endosperm, slightly viviparous; green to pale green

a Location refers to chromosome number (1-10), arm (Long or Short) and the current recombination distance from one end (older recombination distance listed in your map, Figure 2).

Points to note are: 1) Several loci located in different parts of the genome affect the same character. Can you give some simple explanations for this occurrence? 2) Some phenotypes which may appear similar (e.g. *sh* and *su*) are caused by different types of changes. Can you tell the difference between these genes? 3) The phenotypes that we are observing affect different tissues (e.g. endosperm vs. aleurone). How might changes in the aleurone affect your ability to view changes in the endosperm? You will need to think about this question in order to evaluate Cross # 2.

Seedling Traits. Several mutants are also known to affect seedling characteristics. Similar to embryo and endosperm traits, mutations expressed in seedlings can be viewed early in development and are thus easy to score quickly and to assess heritable patterns. Traits expressed in adults such as flowering and fruit development require more space for growing larger plants and more time before scoring. You may notice that Mendel had far greater sample sizes (> 7000) for the seed traits that are expressed in early development.

Locus	**Location**	**Mutant Phenotype**	**Wildtype Phenotype**
d3	9S-62	dwarf	normal height
d5	2S-34	dwarf	normal height
D8	1L-128	Dwarf (dominant)	normal height
gl1	7L-36	glossy leaves sprayed water forms droplets	normal leaves sprayed water does not form droplets
gl2	2S-30	glossy leaves	normal leaves
gl3	4L-118	glossy leaves	normal leaves
lg1	2S-11	liguleless (the ligule is a short sheath projecting from the leaf where it separates from the stem)	ligule is present
lg2	3L-83	liguleless	ligule is present
Lg3	3S-46	Liguleless (dominant)	ligule is present
lw1	1L-128	white seedling	green seedling
lw2	5L-50	white seedling	green seedling
oy	10S-12	yellowish - green seedling	green seedling
w2	10L-73	white seedling	green seedling
w3	2L-111	white seedling	green seedling
wt	2S-60	white tip of leaf	no white tip - green seedling

Wisconsin Fast Plants Seedling Traits

Locus	Mutant Phenotype	Wildtype Phenotype
ros	dwarf	normal height
anl	non-purple stem	purple stem

PROCEDURE

A. You will be given either cobs to score kernel traits, germinated kernels to score seedling traits, or Wisconsin Fast Plants (WFP) seedlings to score seedling traits. Predict the phenotypic ratios in the following dihybrid crosses, based on Mendel's Laws and the location information provided. You should predict the expected ratios of each locus separately (*e.g.* red and colorless for the R1/r1 locus in the first cross) and then the two loci together (e.g. the red and sugary phenotypes for the R1/r1 and the Su_1/su_1 loci together). Also predict phenotypes and the genotypes of the inbred parents for each F1 of your crosses (The inbred parents of this generation are often called P1 and P2).

Cross 1: For the WFP seedling traits, the following cross was made.

1) F_1 was intercrossed to produce F_2

Ros/ros Anl/anl X Ros/ros Anl/anl

Cross 2: For the corn kernel traits, the following cross was made.

1) F_1 was intercrossed to produce F_2

$Y_1y_1\ R_1r_1$ X $Y_1y_1\ R_1r_1$

Cross 3: For the corn seedling traits, the following crosses were made.

1) F_1 was testcrossed to homozygous recessive

$Gl_2\ gl_2$ *Wt wt* X $gl_2\ gl_2$ *wt wt*

B. Test your predictions by scoring the cobs or seedlings distributed in the lab. Try to disturb the seedlings as little as possible as you count them because other labs will use the same seedlings. Return the boxes of seedlings to the appropriate area.

C. Use the chi square test to evaluate your data. What is your null hypothesis in each case? If there is linkage involved, calculate the recombination distance between loci.

Chi Square Analysis

The chi square test [x^2, pronounced "kye (rhymes with sky) square"] is a statistical test commonly used in genetics. It determines the "goodness of fit" of a data set which means it compares the *observed* frequencies of an experiment to the *expected* frequencies of an experiment. For example, the ratio of expected phenotypic frequencies of a monohybrid cross is 3:1; three out of four offspring will show the dominant phenotype and one out of four offspring will show the recessive phenotype. If we have an experiment of 1000 offspring and find 782 dominant phenotypes and 218 recessive phenotypes, can we statistically state that those offspring numbers are close enough to the expected numbers of 750 and 250 that they fit the 3:1 ratio? Are the differences in numbers due to chance? Or is another factor likely affecting the observed numbers? Chi square analysis will tell us.

The formula used in chi square analysis is:

$$X^2 = \sum \frac{(o - e)^2}{e}$$

In this equation, *o* is the observed value, *e* is the expected value and ∑ (sigma) is the "sum of". Therefore, the chi square value will be the sum of the observed values minus the expected values squared, divided by the expected value for each category. In our example we have 2 categories, dominant phenotype and recessive phenotype. A chart is a good way to organize the calculations.

Monohybrid Cross					
Expected Ratio	Observed (*o*)	Expected (*e*)	Deviation $(o - e)$	Deviation^2 $(o - e)^2$	Deviation^2/ Expected $(o - e)^2/e$
3/4	782	¾ (1000) = 750	782 – 750 = 32	$32^2 = 1024$	1024 / 750 = 1.37
1/4	218	¼ (1000) = 250	218 – 250 = -32	$-32^2 = 1024$	1024 / 250 = 4.10
	Total = 1000				$x^2 = 1.37 + 4.10 = 5.47$

When we begin a chi square analysis we state a *null hypothesis.* The null hypothesis asserts that the data set we are testing has no significant difference from the expected data. If the values of observed and expected data don't match exactly, the difference can be accepted as chance variation. To determine if we can reject the null hypothesis or fail to reject it, we need to find the probability value (*p*) associated with the chi square value. This is done using a chi square probability table.

Another factor needed to find the probability value is the *degrees of freedom (df)*. The degrees of freedom is equal to the number of categories we are using minus one (*n*-1). In this instance, we are using two possible categories (dominant phenotype and recessive phenotype) so our *df* = 2 - 1 = 1. Find the chi square value of 5.47 in the row for 1 *df*, or find the two columns that the value lies between. Determine the probability by reading the value at the head of the column.

Chi Square Probability Table

df	0.995	0.99	0.975	0.95	0.90	0.10	0.05	0.025	0.01	0.005
								***p*-values**		
1	---	---	0.001	0.004	0.016	2.706	3.841	5.024	6.635	7.879
2	0.010	0.020	0.051	0.103	0.211	4.605	5.991	7.378	9.210	10.597
3	0.072	0.115	0.216	0.352	0.584	6.251	7.815	9.348	11.345	12.838
4	0.207	0.297	0.484	0.711	1.064	7.779	9.488	11.143	13.277	14.860
5	0.412	0.554	0.831	1.145	1.610	9.236	11.070	12.833	15.086	16.750
6	0.676	0.872	1.237	1.635	2.204	10.645	12.592	14.449	16.812	18.548
7	0.989	1.239	1.690	2.167	2.833	12.017	14.067	16.013	18.475	20.278
8	1.344	1.646	2.180	2.733	3.490	13.362	15.507	17.535	20.090	21.955
9	1.735	2.088	2.700	3.325	4.168	14.684	16.919	19.023	21.666	23.589
10	2.156	2.558	3.247	3.940	4.865	15.987	18.307	20.483	23.209	25.188

If the *p*-value (probability value) is 0.05 or less, we reject the null hypothesis. Our x^2 value lies between a *p*-value of 0.025 and 0.01 so it is less than 0.05. This means that there is less than a 5% chance that the difference in values is due to chance variation; we *cannot* say that 782 dominant phenotypes and 218 recessive phenotypes fit the 3:1 ratio. Something else is likely affecting those values.

The following chi square analysis of a dihybrid cross produces a *p*-value between 0.9 and 0.1 which is greater than 0.05 and therefore we fail to reject the null hypothesis. We *can* statistically state that there is no difference between the observed values and the expected values and that the values stated below fit the expected dihybrid ratio of 9:3:3:1. Any value differences can be attributed to chance.

Dihybrid Cross					
Expected Ratio	*o*	*e*	$o - e$	$(o - e)^2$	$(o - e)^2 / e$
9/16	552	567	-15	225	0.40
3/16	176	189	-13	169	0.89
3/16	210	189	21	441	2.33
1/16	70	63	7	49	0.78
	Total = 1008				$x^2 = 4.40$ $df = 3$

For a more detailed explanation of chi square analysis, see your text.

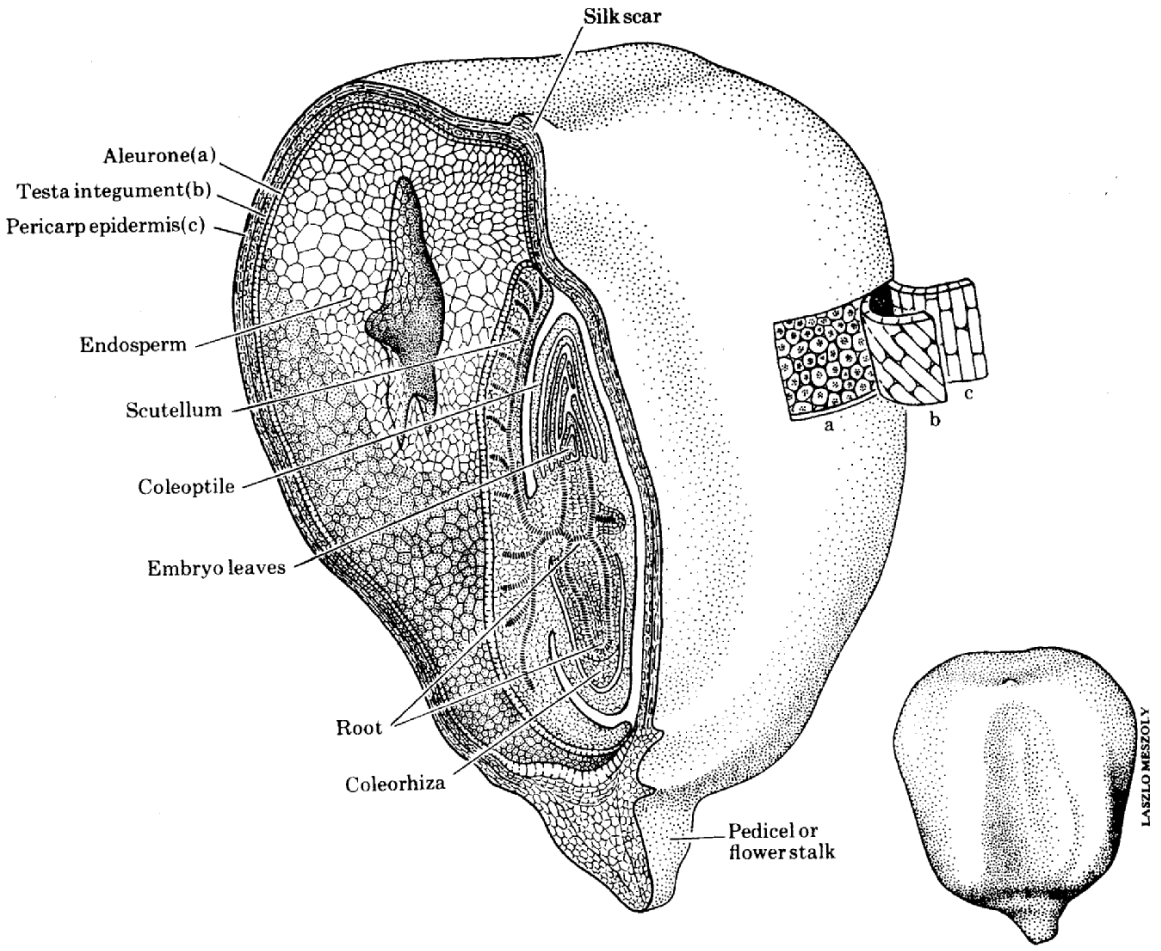
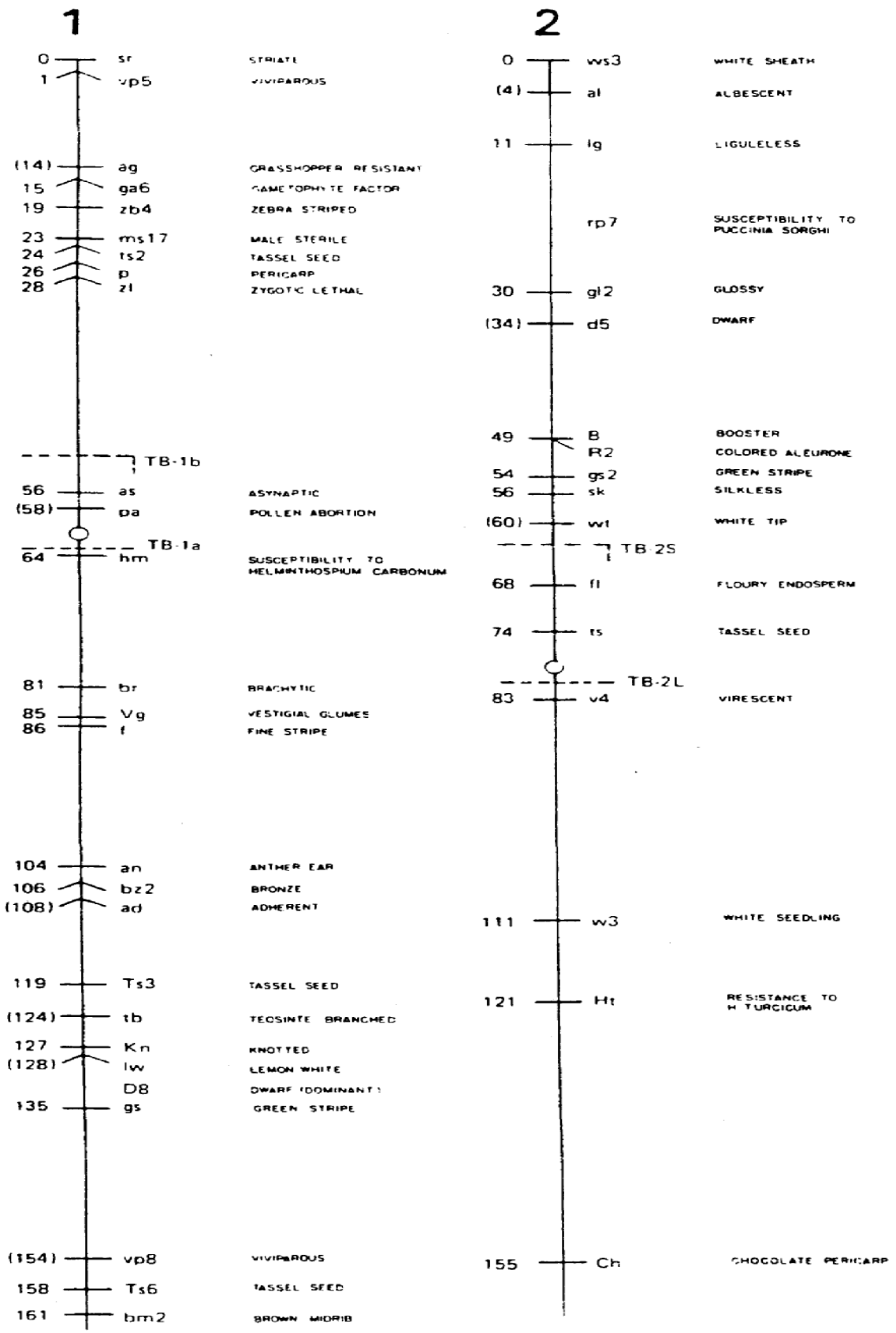
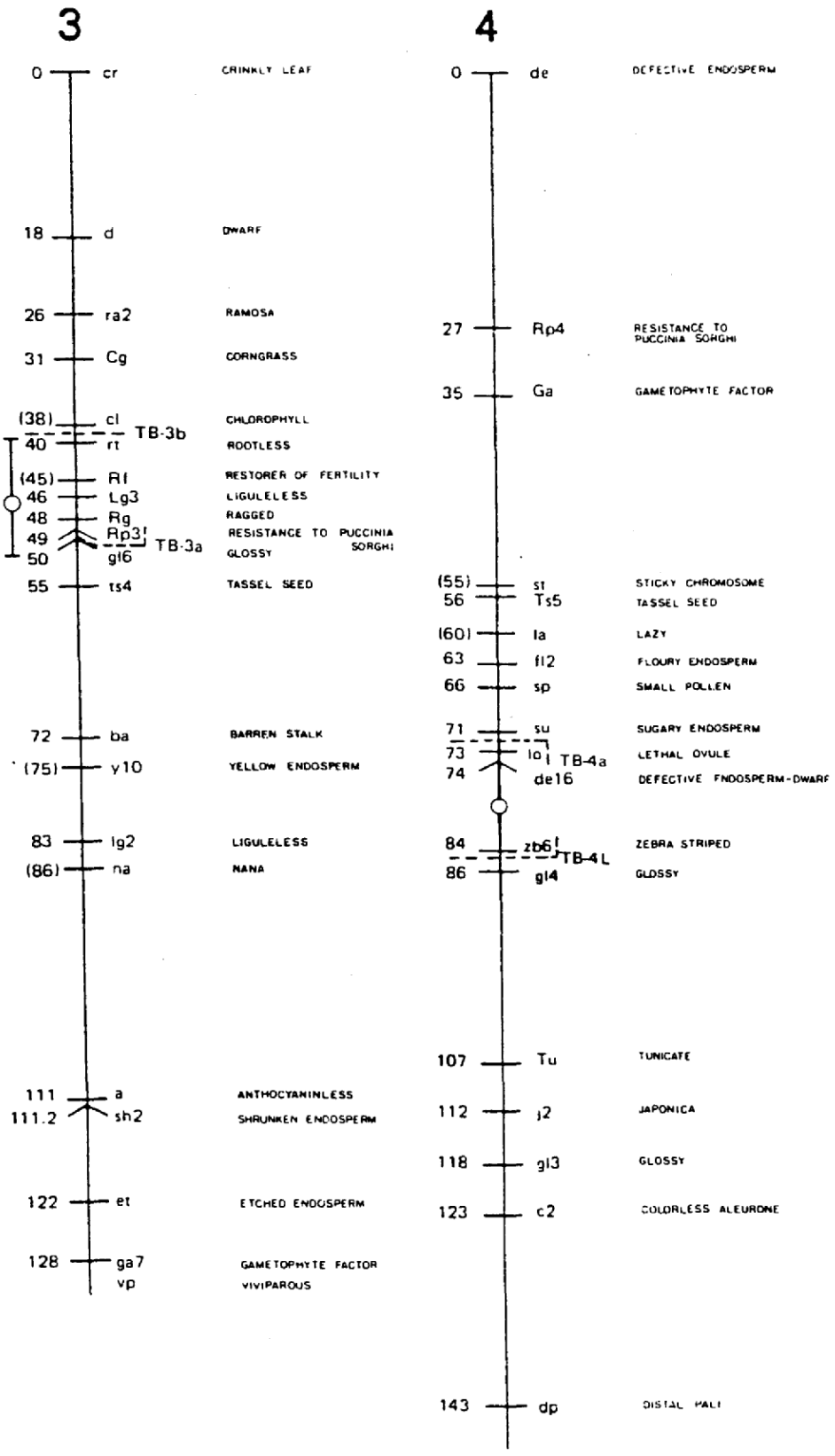
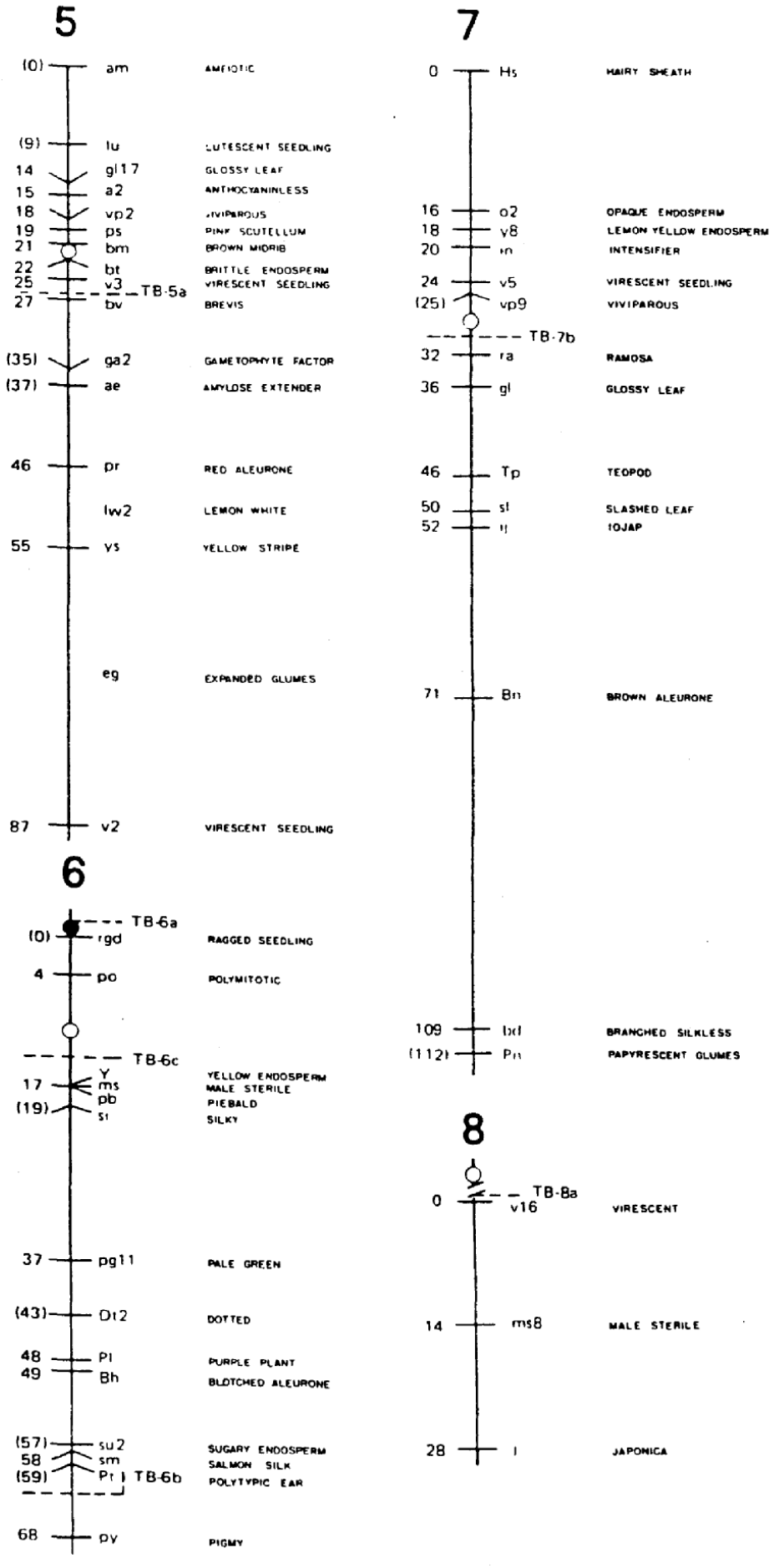


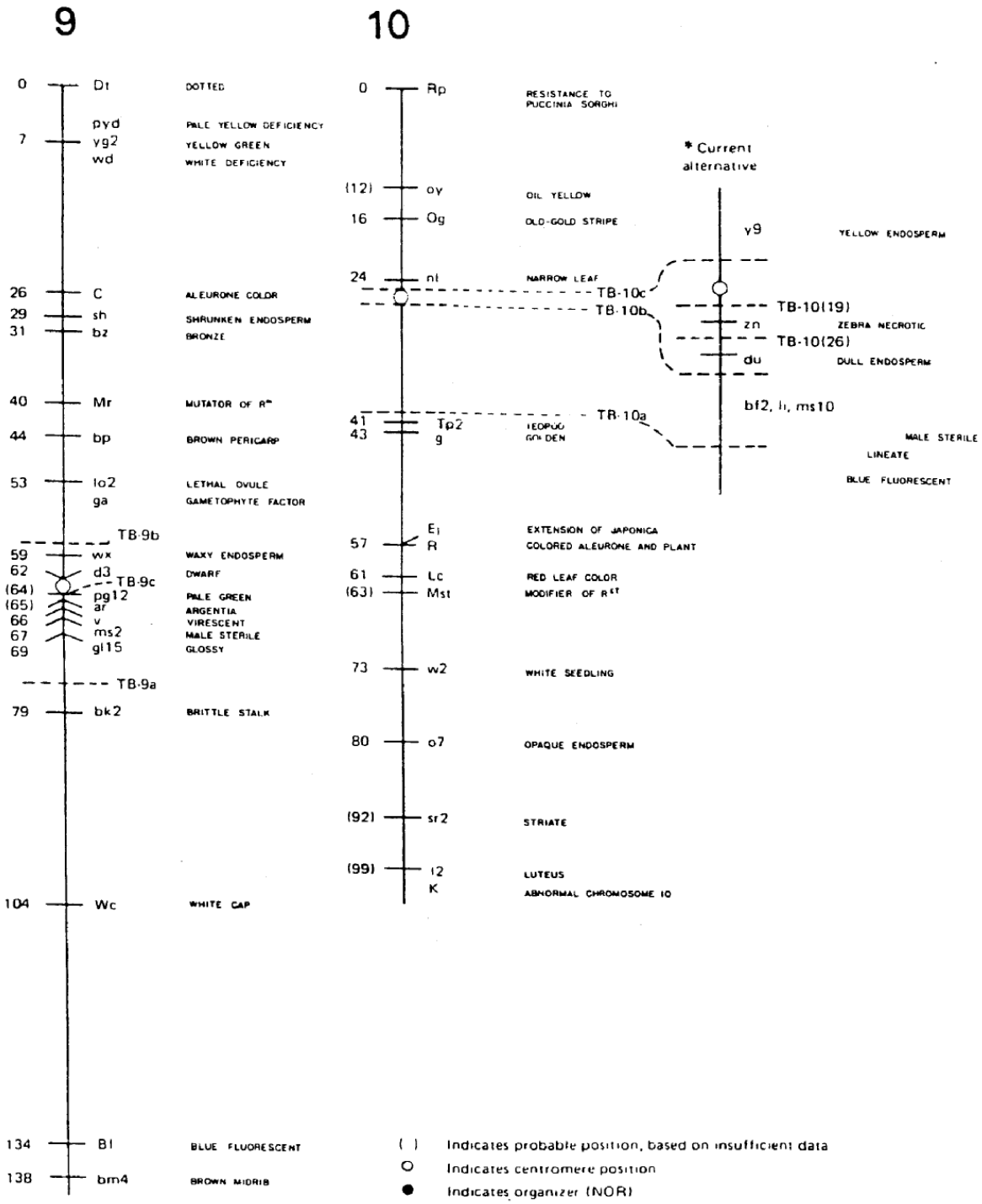
FIGURE 1. **A corn seed is illustrated in the diagram above. The seed is composed of protective tissues (pericarp epidermis and testa integument, which form the seed coat), enzyme-rich digestive tissues (aleurone and scutellum), a carbohydrate-rich storage tissue (endosperm) and a young plant (embryo) rich in oils and protein. The first leaf (coleoptile) and first root (coleorhiza) bring the leaves up to the surface of the soil and push the root down into the soil.**

LINKAGE MAP OF MAIZE




3
0 cr CRINKLY LEAF
18 d DWARF
26 ra2 RAMOSA
31 Cg CORNGRASS
(38) cl CHLOROPHYLL
TB-3b
40 rt ROOTLESS
(45) Rf RESTORER OF FERTILITY
46 Lg3 LIGULELESS
48 Rg RAGGED
49 Rp3 RESISTANCE TO PUCCINIA SORGHI
TB-3a
50 gl6 GLOSSY
55 ts4 TASSEL SEED
72 ba BARREN STALK
(75) y10 YELLOW ENDOSPERM
83 lg2 LIGULELESS
(86) na NANA
111 a ANTHOCYANINLESS
111.2 sh2 SHRUNKEN ENDOSPERM
122 et ETCHED ENDOSPERM
128 ga7 GAMETOPHYTE FACTOR
vp VIVIPAROUS
4
0 de DEFECTIVE ENDOSPERM
27 Rp4 RESISTANCE TO PUCCINIA SORGHI
35 Ga GAMETOPHYTE FACTOR
(55) st STICKY CHROMOSOME
56 Ts5 TASSEL SEED
(60) la LAZY
63 fl2 FLOURY ENDOSPERM
66 sp SMALL POLLEN
71 su SUGARY ENDOSPERM
73 lo LETHAL OVULE
TB-4a
74 de16 DEFECTIVE ENDOSPERM-DWARF
84 zb6 ZEBRA STRIPED
TB-4L
86 gl4 GLOSSY
107 Tu TUNICATE
112 j2 JAPONICA
118 gl3 GLOSSY
123 c2 COLORLESS ALEURONE
143 dp DISTAL PALE


5
(0) am AMEIOTIC
(9) lu LUTESCENT SEEDLING
14 gl17 GLOSSY LEAF
15 a2 ANTHOCYANINLESS
18 vp2 VIVIPAROUS
19 ps PINK SCUTELLUM
21 bm BROWN MIDRIB
22 bt BRITTLE ENDOSPERM
25 v3 VIRESCENT SEEDLING
TB-5a
27 bv BREVIS
(35) ga2 GAMETOPHYTE FACTOR
(37) ae AMYLOSE EXTENDER
46 pr RED ALEURONE
lw2 LEMON WHITE
55 ys YELLOW STRIPE
eg EXPANDED GLUMES
87 v2 VIRESCENT SEEDLING
6
TB-6a
(0) rgd RAGGED SEEDLING
4 po POLYMITOTIC
TB-6c
Y YELLOW ENDOSPERM
17 ms MALE STERILE
pb PIEBALD
(19) st SILKY
37 pg11 PALE GREEN
(43) Dt2 DOTTED
48 Pl PURPLE PLANT
49 Bh BLOTCHED ALEURONE
(57) su2 SUGARY ENDOSPERM
58 sm SALMON SILK
(59) Pr TB-6b POLYTYPIC EAR
68 py PIGMY
7
0 Hs HAIRY SHEATH
16 o2 OPAQUE ENDOSPERM
18 y8 LEMON YELLOW ENDOSPERM
20 in INTENSIFIER
24 v5 VIRESCENT SEEDLING
(25) vp9 VIVIPAROUS
TB-7b
32 ra RAMOSA
36 gl GLOSSY LEAF
46 Tp TEOPOD
50 sl SLASHED LEAF
52 lj LOJAP
71 Bn BROWN ALEURONE
109 bd BRANCHED SILKLESS
(112) Pn PAPYRESCENT GLUMES
8
TB-8a
0 v16 VIRESCENT
14 ms8 MALE STERILE
28 I JAPONICA



( ) Indicates probable position, based on insufficient data

O Indicates centromere position

● Indicates organizer (NOR)

TB Indicates translocation involving A and B chromosomes, with A break point at broken line or in direction indicated. TB-2S, TB-2L and TB-4L are short designations for TB-2S,3L(6270), TB-2L,1S(4464) and TB-4L,9S(6222) from Rakha and Robertson (1970). TB-10(19) and TB-10(26) are as designated by Lin (1974).

* Lin (1974), Beckett (Personal communication)

After Coe and Neuffer (1977)

HONOR PLEDGE

- **I pledge that this work is entirely my own.**
- **I understand that plagiarism is copying words or original thoughts without proper acknowledgement of the source.**
- **I understand that this includes copying straight from the lab manual, a partner or other student, other lab reports, the internet, texts, etc.**
- **I understand that plagiarism will result in a zero for the report, and that other consequences may follow.**

Print your name: _____

Sign: _____

Date: _____

Lab 3: *Drosophila* as an Experimental Organism

OBJECTIVE: To become familiar with the life cycle of *Drosophila*; to become facile at manipulating experimental organisms; to learn to distinguish the sex of flies and identify wild-type and mutant traits.

BACKGROUND: *Drosophila melanogaster*, a common fruit fly, has been widely used in basic genetic research for almost a century. *Drosophila* are small and fecund, are easily reared in the laboratory, have short life cycles, and many other features you will learn about that make them ideal organisms for studying eukaryotic genetics.

Male and female flies can be distinguished by a number of sexually dimorphic features (Fig.1). The female is larger than the male and has a more pointed abdomen. The male has a wide, dark stripe on the end of the abdomen on the ventral side. However, these characteristics may be hard to distinguish in recently emerged flies. There are two other sex differences that can always be used to tell the sex of a fly. The genital organs are located on the ventral posterior end of the abdomen. The ovipositor of the female is pointed, the claspers of the male are dark and surrounded by heavy, dark bristles that are not present in females. The male also has a distinctive row of dark bristles, called the "sex comb," on the first pair of forelegs. This characteristic is quite distinct even in recently emerged flies.

Figure 1. Male and female *Drosophila*, ventral view, 40x

The life cycle of *Drosophila* is characterized by 4 distinct stages: egg, larva, pupa, and adult (Fig. 2). A single adult female may lay as many as 500 eggs over a 10-day period. The larva hatches the day after the egg is laid. Like other insects, a fruit fly grows by periodically shedding its cuticular exoskeleton. Each successive molt is called an instar. There are 3 larval instars.

The cuticle of the 3rd larval instar hardens and darkens to become the pupal case. Within the immobile pupa, the maggot is remodeled to the adult fly. When metamorphosis is complete, the adult emerges (ecloses) from the puparium.

At first the fly is soft and light in color, the wings are folded, and the abdomen is enlarged. In a few hours, the fly pumps its appendages up with fluid from the abdomen, the wings expand, and its color darkens. A virgin female can be fertilized about 8 - 12 hours after eclosion. Female *Drosophila* can store and use sperm from a single insemination throughout their life. Therefore, when setting up crosses, females must be virgin to insure that fertilization occurs from the sperm of the intended males. Two days after fertilization, she starts laying eggs. Adult *Drosophila* remain fertile as long as they live, which may be several weeks.

The rate of development from egg to sexually mature adult depends on temperature:

DURATION OF STAGES

	LARVAL	PUPAL	ADULTS APPEAR AT
20°C (68°F)	8 days	6 days	15+ days
25°C (77°F)	5 days	4 days	10+ days

We incubate the Drosophila cultures at 21°C for a life cycle of about 14 days.

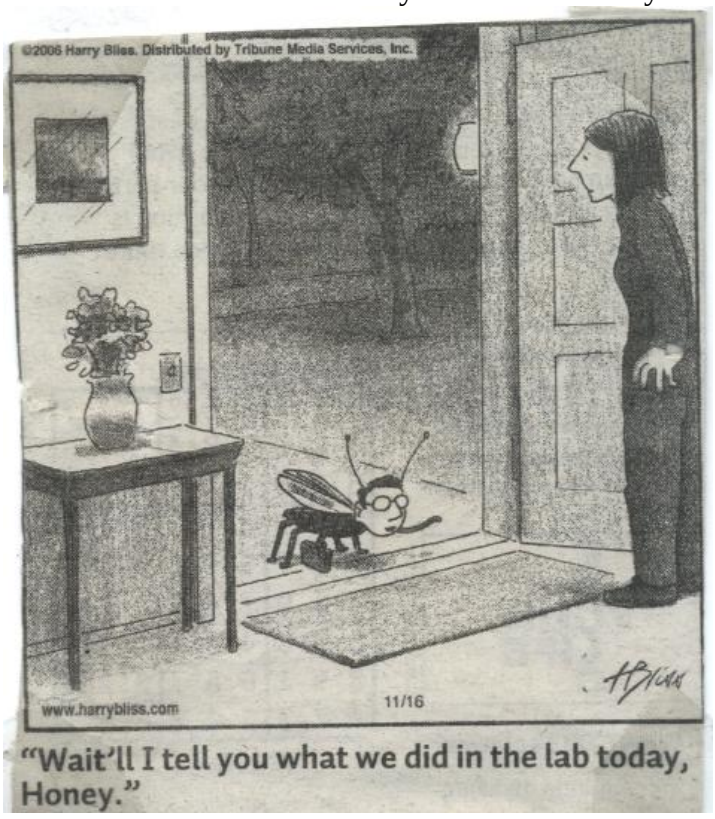
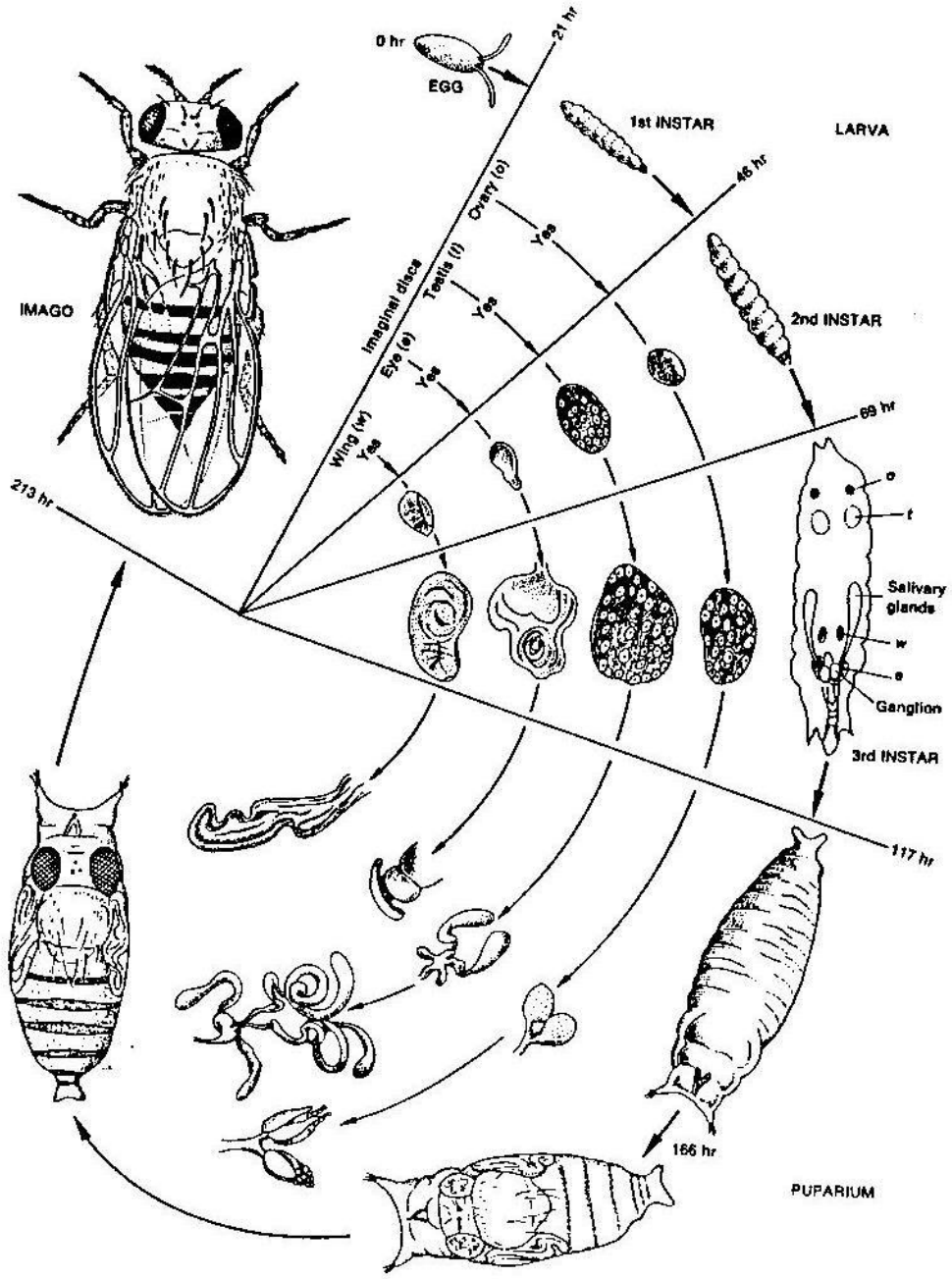


Figure 2. Life cycle of *Drosophila melanogaster*.



A. Mutants of Drosophila

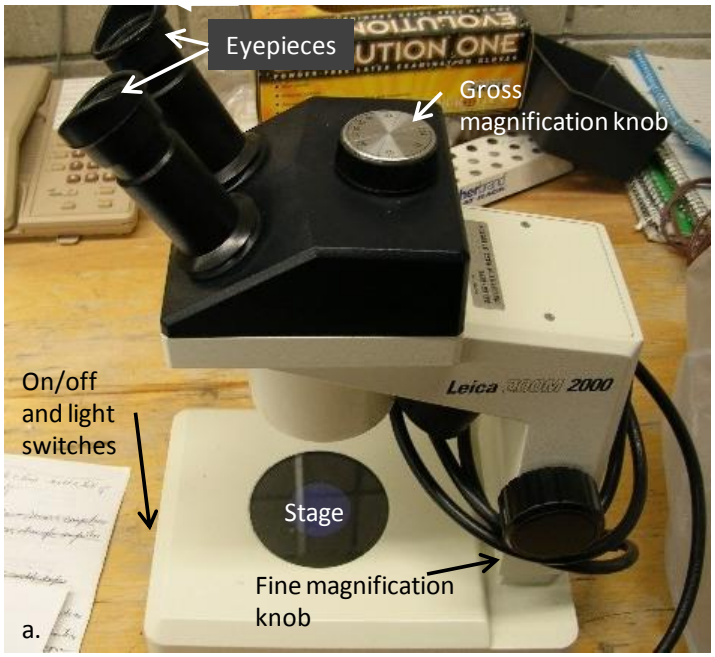
The characteristics of *Drosophila* that are most commonly found in natural populations of flies are called "wild-type" features. A very large number of "mutant" flies with characteristics that differ from the wild-type have been discovered during the many years that *Drosophila* has been used as an experimental organism. These mutants are maintained as particular strains that can be purchased from suppliers or obtained from research laboratories. The white eye mutant is one of the earliest *Drosophila* mutants that was isolated in the laboratory of T.H. Morgan. Look at the flies under a dissecting microscope. The wild-type eye color of *Drosophila* is brick red; the white eye flies have pure white (really colorless) eyes.

There are 3 different pure-breeding strains of flies for you to look at today in vials numbered 1, 2 and 3. Each group must determine the genotype of the flies in each vial by observing whether the flies have the mutant or the wild-type phenotype of each trait. The symbols for these traits are:

TRAIT	MUTATION	SYMBOL	WILD-TYPE	SYMBOL
Eye color	white	w	red	w+
Bristles	scalloped	sd	normal	sd+
Wing length	miniature	m	normal length	m+

Therefore, the genotype of one strain may be "w m+ sd" if it has white eyes, normal length wings and scalloped wings. These strains are pure-breeding, that is, flies of a particular strain are homozygous and therefore genetically identical for the characteristic(s) in question.

All flies in the same numbered vials have the same genotype and all vials contain both male and female flies. Each student should identify the phenotypes of 3-4 flies from each vial and the group should decide together the genotypes of vials 1, 2 and 3. A vial of flies may have 0, 1, 2 or 3 of the mutant traits. The order you list the genes at this point is arbitrary; in the gene mapping experiment you will determine the correct order of these genes.



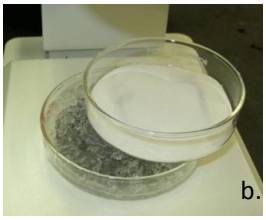


Figure 3.

a. major parts of the dissecting microscope.

b. Petri dish setup.

c. morgue

B. Handling *Drosophila:*

1. Turn on your microscopes. Those that have two lights on their microscopes want only the top light on; the bottom light will not help you see the flies and will melt your ice. Put something on the stage of the microscope to view and bring the magnification up high. The gross magnification knob is on the upper side or top of the microscope and the fine magnification knob is on the lower side of the microscope. Move these two knobs to become familiar with how to focus the microscope.

2. The flies are in vials on ice to immobilize them so you can work with them. They must be kept on ice. Chilled flies will quickly recover when returned to room temperature.

3. Take your glass Petri dish and half fill the larger dish with crushed ice. Set the smaller dish onto the bottom one and push it gently to get a tight connection between the upper dish and the ice . Place a piece of filter paper in the top dish and place this whole setup on the microscope stage on top of a paper towel. As you look at your flies, the ice in your Petri dish will melt. When it does, take off the top dish, dump the water into the sink and refill with ice as needed.

4. Take one vial of flies, remove the stopper, and tap the vial gently to get 3-4 flies onto your filter paper. If you get too many flies, share them with other groups. Don't put flies back into vials once they have been taken out because of the chance of mixing them up. Put the stopper on and put the vial back on ice so that the flies won't wake up.

5. Use the paint brush to move the flies from one place to another and to manipulate the flies under the microscope to see the flies from different perspectives. Examine the flies for the 3 traits we are studying and draw them in your notebooks. Put the mutations next to the wild-type traits to compare the differences side by side. Distinguish between the males and females; separate them into two piles and have the TA check that you can tell the difference between the sexes.

6. When the flies start to wake up and walk around, it's time for them to go. All flies that you look at today are going into the "morgue". The morgue is the white plastic can containing mineral oil that is on your tray. Pick up the fly with your paint brush and put it into the morgue; replace the cover. Do not return any flies to vials. Try to avoid letting flies escape into the lab (and hall and offices).

7. When you are finished looking at the flies, wipe off the stage of the microscope with a paper towel, unplug it, wrap the cord loosely around the microscope and place it back on the cart or in the cabinet. Empty the water and ice from your Petri dish, rinse the dishes in water and place them on the tray next to the sink. Rinse your paint brush in water.

You should be able to identify and distinguish the three mutations and their corresponding wild-type phenotypes in the flies you are looking at today, as well as identifying males and female flies. These skills will be used in the following Gene Mapping Lab.

Lab 4: Gene Mapping—Trihybrid Cross in *Drosophila*

OBJECTIVE: To determine the map distance of three X-linked genes in *Drosophila* by generating an F2 population, and scoring their number and phenotypes.

BACKGROUND: The main emphasis of the first 50 years of genetics, and one that continues to be central today, was to determine the location of genes on chromosomes. The site of a gene is called its locus (plural, loci). Since the site of a gene is important in defining it, the term "locus" can be used as a synonym for "gene". The process of determining the order of loci along a chromosome, and their distances from each other, is called "mapping". That is what you will do in this exercise.

You will be working with 3 of the following 8 sex-linked genes and their mutant phenotypes.

B: *Bar*, narrow eye in male and homozygous female; kidney shaped eye in heterozygous female (partial dominant mutant allele)

cv: *cross-veinless*, wings lack cross veins (recessive mutant allele)

f: *forked bristles*, body bristles stubby with "split ends" (recessive mutant allele)

lz: *lozenge*, eyes smaller, almond shaped, glossy (recessive mutant allele)

m *miniature* wing (recessive mutant allele)

sd: *scalloped*, wing margin scalloped (recessive mutant allele)

v *vermilion* eyed; bright red (recessive mutant allele)

w: *white* eyed (recessive mutant allele)

sn. *singed bristles;* body bristles short and curled (recessive mutant allele)

You will be making crosses between two strains that will segregate for the three genes. For example, one strain may be *y cv f* and the other would be wild-type (y^+ cv^+ f^+) for these three genes; or one strain may be *w* (and wild-type for the other two genes, say y^+ B^+) and the other strain would be *y B* (and wild-type w^+). As you can see, a wild-type locus is designated +.

You will be assigned one of the following crosses involving three genes:

	Parent 1 (P1)		P2
a)	y cv f	x	wild
b)	w m	x	sd
c)	cv f	x	sd
d)	cv f	x	w
e)	y B	x	w
f)	w m	x	lz
g)	w m	x	cv

Note these crosses may differ from year to year, so consult your lab instructor for details.

Sex-linked traits can be determined by their differential outcome in reciprocal crosses. In the table above, the crosses could be made so that P1 (parent 1) was female and P2 was male or the reverse. These are called reciprocal crosses.

In order to get this experiment going on time, you will be setting up the P1 crosses without fully understanding their rationale. You should think through this experiment during the next few weeks in light of what you will be learning in lecture. The worksheet that follows will guide you in this process.

During the coming weeks, you will be continuing the experiment through the F1 to the F2 generations. You will need to score a relatively large number of F2 male flies – 100 /student (300 for a group of 3, 200 for a group of 2). Keep track of what is happening to your cultures. If your flies die or the F1 results indicate that a P1 female was not virgin, you should note this in your lab notebook and you will need to consult your TA.

You will be told when the F2 flies start to emerge. From then on, you will need to go in every few days and score flies until you have collected data on 100 male flies.

Fly schedule	**Task or Event**	**Day**
week 1	Set up P1 cross	0
week 2	Dump P1s	7
week 3	F1s start to emerge	14
week 4	Set up F1 cross	21
week 5	Transfer F1 to backup vials	28
week 6	Dump F1s	35
	First F2s start to emerge	36-46 (scoring days)
week 7	Backup F2s start to emerge	43-53 (scoring days)

As you perform the tasks for this lab in the coming weeks, return to reread the manual for the specific procedures and to review the overall purpose of the lab. You will understand the lab more clearly each time you read it.

Specific Procedures

Procedure- Week 1 – Setting up the P1 cross.

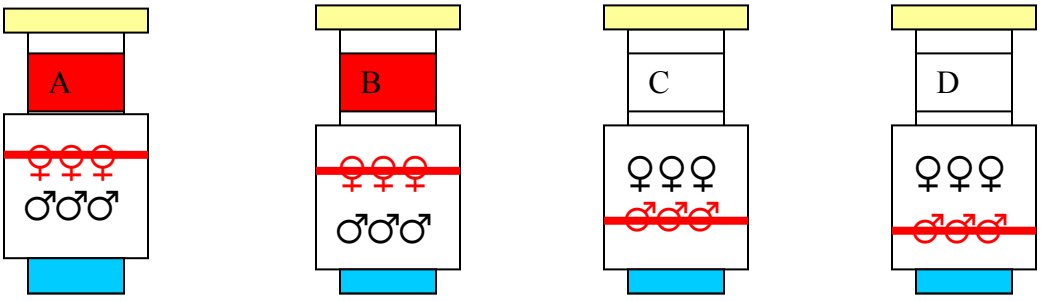


Figure 1. The P1 generation setup: vials “A” and “B” contain red eyed females and white eyed males; vials “C” and “D” contain white eyed females and red eyed males. “A” and “B” are duplicates; “C” and “D” are duplicates. “A” and “B” are reciprocals of “C” and “D”.

All live flies were cleared from these vials 12 hours ago to insure that the female flies that emerge are virgin. Why is it important that they are virgin? Female *Drosophila* can store and use sperm from a single insemination throughout their reproductive life. To insure that our female flies use the sperm of the male flies that we set up, the females must not have previously mated and we insure this by their age. Newly emerged female *Drosophila* will not mate until they are about 12 hours old. Some of these newly emergent flies will have little color, wings that have not fully unfolded and abdomens that are full of fluid. Be sure to look for the sex combs on the forelegs of the males for positive sexual identification because the other characteristics used for identification may be indistinguishable.

1. Label 2 clean vials containing media with **red** tape vials "**A**" and "**B**", and your group name. You set up 2 vials for each cross so that you have a back-up vial in case the first vial has a problem. The red tape signifies the red-eyed female fly used in this cross.
2. Label 2 clean vials containing media with **white** tape vials "**C**" and "**D**", and your group name. The white tape signifies the white-eyed female fly used in this cross.
3. Take a vial of ONE STRAIN of flies from the ice bucket on your table. The flies have been on ice and are immobilized. Take off the plug and tap the vial so that 4-5 flies come out onto a glass top Petri dish lined with filter paper and sitting in the bottom Petri dish filled with ice chips. Replug the vial and place it back into the ice bucket. It is important to keep the flies on ice when they are not being used.
4. Using the dissecting microscope to determine the sex of the fly, put 3-4 **red eyed females** and 3-4 **white eyed males** in vials "**A**" and "**B**". Vials "**A**" and "**B**" are duplicates. Put 3-4 **white eyed females** and 3-4 **red eyed males** in vials "**C**" and "**D**". Vials "**C**" and "**D**" are duplicates. Keep track of which flies you have put into each vial. While you are setting up your P1 vials, lay the P1 vials horizontally in the ice bucket. This will keep the flies groggy while you fill the vials and it will reduce the chance of the flies getting stuck in the blue media in the vial. Keep the vials stoppered while you add the flies. The stoppers can be moved to one side with a finger to add a fly on a paint brush.
5. Don't use any flies that look dried out, are missing parts or appear dead. Put these flies into the morgue. After the flies have been taken out of their original vials, DO NOT put any extra flies back into the original vial. We don't want to take any chance of mixing up the flies. This is why we work with only a few flies at a time. Share extra flies with other groups that may need them. CAUTION: any group accepting a fly from others should always check the sex and genotype of the fly before putting it in their vial. Make sure your flies have woken up and are moving around before you put them in the incubator.
6. Place the four vials of flies into a black box labeled on the outside with your group name and place it in the incubator on the shelf marked with the day your lab meets. This is the P1 generation. These flies will mate and lay eggs for the next 7 days. Next week you will dump the P1's into the morgue, leaving only the F1 in the vials in the form of eggs and larvae. This is known as "clearing" the vials.
7. Put away your microscope and rinse out your dishes and brush.
8. Fill out the P1 section of the Genotype Chart in the lab manual.

Procedure – Week 2 – Dumping the P1 flies

1. Take your flies from the incubator. Have a morgue ready beside you to dump the flies into. Holding the fly vial in one hand and the foam stopper in the other, with a downward motion pull out the stopper over the morgue and the force should dump most of the flies into the morgue. The TA or the lab tech will demonstrate this process for you. Cover the morgue. Replug the vial

and check to see that all living flies came out. If there are still some in the vial, repeat the process. Single flies may be picked up with a paint brush and put into the morgue. Why is it necessary to remove the P1 flies from the vials before the F1s emerge? There is a possibility that the media could fall out of the vial into the morgue and taking with it most of your F1's so don't shake the vial too hard. If this does happen to you, save the vial with the media that's left in it. There will be some eggs and larvae in the vial.

2. Return the vials to the incubator. Your F1 population will start to emerge next week.

Procedure – Week 3 – F1s Start to Emerge
Procedure – Week 4 – Set Up the F1 Crosses

1. Lay your P1 vials on ice to slow the flies down. Place a piece of tape on an empty vial and label it "**A**" or "**B**", which ever vial you're checking. Put it 2/3 into the ice and let it cool.
2. Label two fresh vials containing media with **red** tape, "**a**", "**b**", and your group name.
3. Label two fresh vials containing media with **white** tape, "**c**", "**d**", and your group name.
4. Take the "**A**" or "**B**" vial containing your F1 flies from the ice. Tap the stopper to knock off any flies, quickly remove the stopper and turn it upside down over the empty vial chilling in the ice. Holding both vials together at the tops, tap the vials into the ice knocking some of the flies into the empty vial. Put stoppers on both vials. Let these flies chill on the ice for a few minutes. Check the phenotypes of both males and females to be sure that they have the phenotype that you predicted for that cross. If you find **any** fly that does not show the correct phenotype, the whole vial is **unusable** and you must see your TA for assistance. What are some possible reasons that the F1s have the wrong phenotype? Why is the whole vial unusable if only one fly has the incorrect phenotype? You can use the flies in one vial to set up both vials of the same F1 cross. For instance, if vial "**A**" flies have the correct phenotype they can be used to set up vials "**a**" and "**b**" because vials "**a**" and "**b**" are duplicates.
5. Place 10 flies of cross 1 into vials "**a**" and "**b**". There is no need to specifically count out 4 or 5 males and 4 or 5 females, there will be some of each.
6. Repeat the process of checking the phenotypes of the flies in your "**C**" vial. Place about 10 flies of cross 2 into vials "**c**" and "**d**".
7. Return box with the 4 lower-case lettered vials in it to the incubator, return microscope and wash out dishes and brush. Place P1 fly vials into the labeled box in the lab; they are no longer needed.

Procedure – Week 5 – Transfer F1 flies to double letter vials for backups.

1. Label two fresh vials containing media with **red** tape, "**aa**", "**bb**" and your group name.
2. Label two fresh vials containing media with **white** tape, "**cc**", "**dd**" and your group name.
3. To make sure that you will have plenty of F2 flies to score, we will transfer the F1 flies from the single letter vials ("**c**") that you set up last week to the double letter ("**cc**") vials that you made today. The F1 flies will create another F2 population in these vials and they will emerge a week later extending the time that flies can be scored. You do not need to chill the flies. Have the fresh vial ready next to you. Gently tap the vial containing the flies on the table a few times to knock all of the flies down to the bottom of the vial. Remove the stoppers from both vials. Quickly place the open end of the vial with the flies over the open end of the empty vial. Hold the 2 vials together and tap them on the table to knock the flies from the top vial to the bottom. Be careful not to knock the media out at the same time. Place the stopper back on both vials and check the old vial to see that you removed all flies from it.

4. Repeat this procedure with all of your lower case vials and return all 8 vials to the incubator.

Procedure – Week 6- Dump F1 flies, first F2 population starts to emerge

1. Dump the F1 flies left in the double letter ("**aa**") vials into the morgue in the same manner as you dumped the P1 flies.
2. F2 flies start to emerge from the single letter vials ("**a**") and may be scored for the next 10 days.

Procedure – Week 7 – Second F2 population starts to emerge

1. F2 flies start to emerge from the double letter vials ("**aa**") and may be scored for the next 10 days.

Scoring the flies – this part of the experiment will be done on your own time, not during lab time. Make sure that you have contact information for each person in your group so that you can exchange data information. However, each student is responsible for scoring 100 male flies total and having this data in their notebooks and in their lab reports regardless of how many flies their lab partners score. You may score more than 100; the more you score, the more statistically reliable your data will be. You may come into the lab anytime there is not another course being held in the lab. If the lab door is not open, you can get access to the lab by:

1. Calling Maureen Kelly at 617-287-6611 or in her office W-2-034 or Maureen.Kelly@umb.edu (usual hours 6 AM to 2:30 PM)
2. Calling Public Safety at 617-287-7799 (24 hour availability) You need to have your student ID with you.

Students should coordinate a fly scoring schedule with their lab partners so that flies are scored every day or every other day rather than 2 or 3 students trying to score flies on the same day. To score your flies, take a clean, empty vial and label it with the letter of the vial you want to score. Place the vial in your ice bucket leaving only 1 inch of the vial out of the ice and let it chill for 5 full minutes. Tap the vial with the flies in it on the table a few times to knock the flies off the stopper. Take off the stopper quickly and invert the vial with the flies over the chilled vial on ice. Holding the two vials together, tap the vials into the ice knocking the live flies into the chilled vial. Replace the stopper on the live fly vial and stopper the chilled vial as well. The flies will be immobilized in about 5 minutes and then you can tap 7-8 out at a time and score them.

You will only score male flies; females go right into the morgue. Making a table of reciprocal classes of phenotypes you expect to get will make data collection all the more efficient. Make a column for each vial ("**a**" and "**aa**" can all be scored as "vial **a**"; they have the same set of parents). Keep track of which vials the flies you score come from. Males are scored by phenotype of the 3 traits we are studying. For example, if your first male fly was white eyed, normal length wings and singed bristles, you would make a row labeled w m+ sn and list a 1 next to it for the vial it came out of. Once a male fly is scored, it goes into the morgue. Take only a few flies out of your chilled vial at a time; you don't want your data flying away before you have time to score it! Females tend to emerge before males so don't be alarmed if your early flies are mostly female.

There will be ice in a cooler and ice buckets on the counter. Set up a microscope; gather your materials and a morgue. When you have finished, please return the microscope to the cabinet, dump out your ice, rinse the Petri dishes and brushes and return your flies to the incubator.

WORKSHEET

Predicting possible outcomes based on a hypothesis is an essential feature of any well-planned experiment in biology. Experiments should test hypotheses. This is particularly true of genetics. (What are the hypotheses you are testing in this experiment?) The following exercise and questions will help to guide you through this mapping experiment, and make predictions about your results. This should be recorded in your lab notebook during class.

1) Diagram your P1 crosses, putting down all the genotypic information you have. Here is an example, using the cross between yellow, cross-veinless females and lozenge males. You know that all the genes involved are sex-linked and that these inbred lines are homozygous.

Female	Male
$\frac{y \;\; cv \;\; lz^+}{y \;\; cv \;\; lz+}$	$\frac{y^+ \;\; cv^+ \;\; lz}{Y}$

Note that this order of loci is arbitrary, and may not be correct. In this experiment, you will determine the true order of the loci (and the distance between them).

2) Predict what your F1 results should be. With the example above, the female parent would make one kind of X chromosome (y cv lz^+) and the males being the heterogametic sex would make two types of gametes, (X chromosome with y^+ cv^+ lz and a Y). The F1 generation would therefore have females with an X chromosome from its mother and an X from its father while the males would have the X from its mother and the Y chromosome from its father.

Females	Males
$\frac{y \;\; cv \;\; lz+}{y+ \;\; cv+ \;\; lz}$	$\frac{y \;\; cv \;\; lz+}{Y}$

Note that the female is a triple heterozygote (y+ y cv+ cv lz+ lz)

3) A. Predict what the F2 genotypes and phenotypes would be if the genes were unlinked (you can do this with a branch diagram). For the above cross, females will segregate for yellow and wild-type body and they will segregate for crossveinless and crossveined, but all will be normal eye (not lozenge) since the paternal X carries that wild-type allele. Males will receive any combination of alleles from the F1 mother and a functionally recessive Y chromosome from its father, so all three traits will segregate in F2 males and 8 different phenotypes (2x2x2) will be present in these F2 males.

B. Predict what the F2 phenotypes and genotypes would be if crossing over did not occur in the F1 females. For the above case, the F2 females would be:

$\frac{y \;\; cv \;\; lz^+}{y \;\; cv \;\; lz+}$	or	$\frac{y^+ \;\; cv^+ \;\; lz}{y \;\; cv \;\; lz+}$
yellow, crossveinless and wild-type eye		wild-type for all traits

Males would be:

$\frac{y \;\; cv \;\; lz^+}{Y}$	or	$\frac{y^+ \;\; cv^+ \;\; lz}{Y}$
yellow, crossveinless and wild-type eye		lozenge eye and wild-type for other traits

Note, crossing over does not occur in *Drosophila* males and the Y chromosome does not carry any of these genes so it is functionally recessive.

4) With the two predictions above, (A. if the genes are unlinked and B. if there is no recombination in the female and the genes are completely linked) you now have the extreme results that could occur with this cross. Your results will likely land somewhere between these two extremes.

5) You should also notice that the male F2 progeny and the female F2 progeny give you very different results (as is often the case with X-linked traits). Since the Y chromosome of the F1 father is functionally recessive, scoring the F2 sons, is basically like scoring a testcross (recall a test cross could be AaBbCc x aabbcc). In our example, the F1 mother is a triple heterozygote and her gamete is being matched with a recessive gamete, the Y, from the father.

The female F2 will not segregate for any gene that was dominant in the F1 father (lozenge in this case) and thus the female F2 progeny are not that useful and you will only need to score the males.

6) You will find it most convenient if you score your data (**F2 male phenotypes**) in terms of the predicted genotypes from step 3A) arranged as reciprocal/complimentary classes (We are not showing the recessive Y chromosome).

For example:

y	cv	lz^+	y^+	cv	lz
y^+	cv^+	lz	y	cv^+	lz^+
y^+	cv	lz^+	y^+	cv^+	lz^+
y	cv^+	lz	y	cv	lz

The pair that should be most common would be the non-crossover category (NCO, the non recombinant chromosomes of the F1 female, identified in 3B above). This is also called the parental class, because these are the original chromosomes contributed to the F1 female from the parental generation. In this example it would be which pair? The first pair, y cv lz^+ , y^+ cv^+ lz, is the NCO category. Of the remaining three pairs, you cannot predict which will be the least frequent (double crossover, DCO) and which two will be intermediate (the two single crossover, SCO) categories without knowing the map order and the orientation of alleles.

7) You will find that arranging your data in this way will help you to organize your calculations of the distances between loci. Then you will be able to work out your map of the X chromosome.

You will write a detailed report on this lab. The instructor will tell you how it should be organized. The report should include a clear presentation of your data, the calculation of the map, and a comparison of your map with the well-established map of the *Drosophila* X-chromosome. To do this final step, you will determine the expected number of progeny in each of the 4 categories (NCO, SCO, SCO, DCO) using the map distances from the book and with the number of male progeny scored by you and your partners. For example if you scored 300 flies and the map for the three genes from the book was:

y 10 cv 20 lz

then, assuming no interference, the expected number in the DCO category would be:

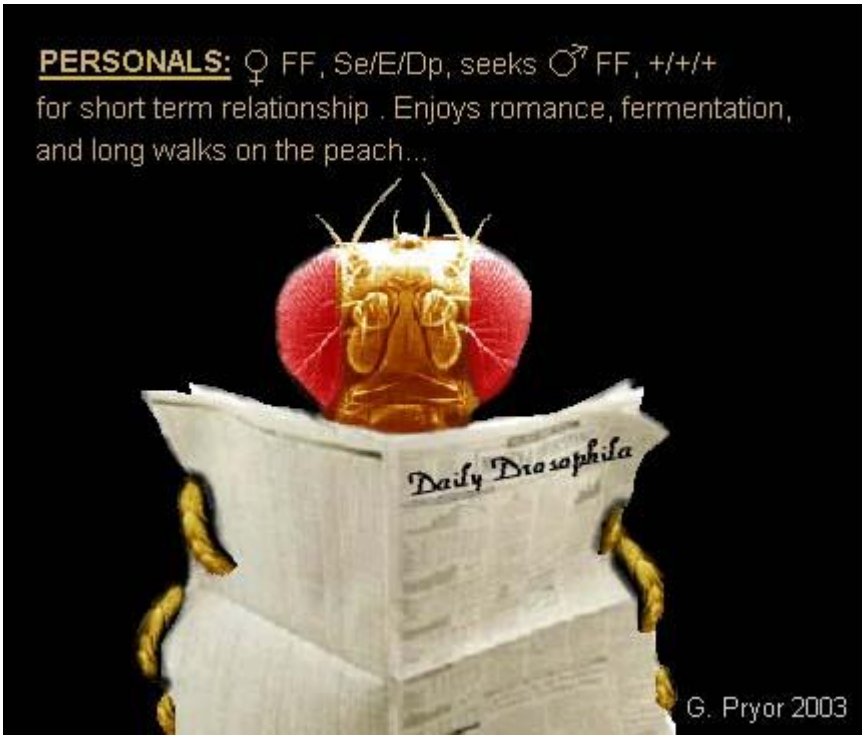
(0.10) (0.20) (300) = 6

Since 10% of the progeny (or 0.10 x 300 = 30 flies) involve recombination between y and cv, and this 30 includes the SCO + DCO, then the expected first SCO category is 30 – 6 = 24. That is, (SCO + DCO)/Total = (24 + 6)/300 = 10%, the map distance between y and cv.

Likewise, 60 (0.20 x 300) flies involve recombination between cv and lz, and the SCO category would be 60 – 6 = 54. The NCO category would be the remainder, 300 – (24 + 54 + 6) = 216.

You could test, with χ^2, your scores for these four categories against these expected values. You of course will need to calculate these expected values for your genes and the proper distances.

The chart on the following page will help you keep track of the genotypes of the male and female flies of each generation of your experiment and the identifying letter of their vials. Fill in the genotypes of the flies as you set up each cross.



Trihybrid *Drosophila* Cross Genotype Chart

		Cross 1- Red Tape		**Cross 2-White Tape** (Reciprocal of Cross 1)	
Vial ID		**A**	**B** (Duplicate of A)	**C**	**D** (Duplicate of C)
P1	♀	_____	_____	_____	_____
	♂	_____ y	_____ y	_____ y	_____ y
Vial ID		**a**	**b** (duplicate of a)	**c**	**d** (duplicate of c)
F1	♀	_____	_____	_____	_____
	♂	_____ y	_____ y	_____ y	_____ y
Vial ID Backups		**aa**	**bb** (duplicate of aa)	**cc**	**dd** (duplicate of cc)
F1 Transferred	♀	_____	_____	_____	_____
	♂	_____ y	_____ y	_____ y	_____ y

HONOR PLEDGE

- **I pledge that this work is entirely my own.**
- **I understand that plagiarism is copying words or original thoughts without proper acknowledgement of the source.**
- **I understand that this includes copying straight from the lab manual, a partner or other student, other lab reports, the internet, texts, etc.**
- **I understand that plagiarism will result in a zero for the report, and that other consequences may follow.**

Print your name: _____

Sign: _____

Date: _____

Lab 5: Gene Interaction in *Drosophila*

OBJECTIVE: To determine the type of gene interaction that affects eye color in *Drosophila*.

BACKGROUND:

In the beginning of this course, we examined simple genetic situations in which a particular trait was controlled by a single gene locus. In most cases, however, phenotypes, especially morphological features, are not controlled simply by one gene. Rather, several gene loci usually contribute to the development of a phenotype. This laboratory exercise focuses on the ways that genes at different loci can interact to influence a phenotype. **This topic is discussed more fully in your text, *Concepts of Genetics*, in the chapter on Extensions of Mendelian Genetics.**

In this laboratory, we will look specifically at the white eye phenotype of *Drosophila*. You already know that white eyes can be the result of a mutation at a single locus (the *white* locus). However, white eyes can also be due to the combined effect of mutations in more than one gene. In this lab, you will consider three cases of gene interactions that result in white eyes. You will first make predictions on the phenotypic distributions of the F2 generation in each case. Then you will consider data from three different crosses and formulate a hypothesis as to their most likely mode of inheritance. Finally, you will test your hypotheses using the chi square test.

A. Genotypes that can yield white eye phenotypes

Possibility 1: Two genes on two different autosomes.

brown, chromosome 2
scarlet, chromosome 3

flies homozygous for both brown and scarlet have white eyes

flies homozygous for brown have brown eyes

flies homozygous for scarlet have bright orange eyes

Possibility 2: Two genes on different chromosomes, one X-linked and one autosomal.

brown, chromosome 2
vermillion, X-linked

flies homozygous for both brown and vermillion have white eyes

flies homozygous for brown have brown eyes

flies homozygous for vermillion have bright orange eyes

Possibility 3: Two genes distantly linked on the same chromosome.

brown, chromosome 2
cinnabar, chromosome 2
brown and cinnabar are separated by about 46 map units

flies homozygous for both brown and cinnabar have white eyes

flies homozygous for brown have brown eyes

flies homozygous for cinnabar have bright orange eyes

B. Experimental Outline

For each experiment, one of three stocks of phenotypically white-eyed *Drosophila* indicated above (in section A) was mated to an inbred red-eyed fly. Reciprocal crosses were made. All three experiments were carried out in the following manner:

	Reciprocal Cross 1			Reciprocal Cross 2		
	Female		Male	Female		Male
Parentals	White	X	Red (wild)	Red (wild)	X	White
↓						
F1		?			?	
↓						

The F1 individuals from a given reciprocal cross were mated to produce F_2 populations.

C. DATA

Below are F2 data from three different experiments (A, B, and C) that use red-eyed (wild-type) and white-eyed *Drosophila*.

Experiment A - F2 Population:

	Reciprocal Cross 1		Reciprocal Cross 2	
	Females	Males	Females	Males
red	82	94	105	79
brown	13	22	21	20
orange	7	18	12	18
white	16	24	15	25

Experiment B - F2 Population:

	Reciprocal Cross 1		Reciprocal Cross 2	
	Female	Male	Female	Male
red	218	224	256	251
brown	88	85	74	84
orange	92	73	81	73
white	26	31	37	25

Experiment C - F2 Population:

	Reciprocal Cross 1		Reciprocal Cross 2	
	Females	Males	Females	Males
red	47	63	121	48
brown	26	17	43	24
orange	57	50	0	65
white	16	21	0	16

D. ANALYSIS

Your task is to determine which of the three possibilities best fits the data for each of the experiments shown above.

1. Write out the expected phenotypes and genotypes for the parentals and the F1 and F2 generation for Possibility 1, 2, and 3. (What are these various types of gene interactions called?) You should also calculate expected ratios of phenotypes for each generation. Note that in possibility 2, which involves an X-linked gene, you must calculate the expected ratios for the reciprocal crosses separately. (Why?) For Possibility 3, you will need to think about the relationship between map units and the frequency of crossing over. Also remember that crossing over does not occur in *Drosophila* males.

2. Examine the data in each experiment (A, B, or C) and, choosing from the three possibilities, suggest a hypothesis that accounts for the white phenotype. The basis for choosing your hypothesis should be explained fully in your lab notebook.

3. Test your hypothesis by analyzing the data provided for each experiment using the chi-square test (see your text). The chi square analysis will enable you to compare the observed data with your expected values and decide whether the observed data support your hypothesis. You should test the goodness of fit of the data to specific null hypotheses (e.g. "the loci are not on the X chromosome" or "the loci are unlinked"). You can reject certain possibilities for each experiment. You can also accept (fail to reject) others. You need not test all models for each experiment, but you will need to logically progress through the study. For example, you will not need to test the data of an experiment for fit to a model of "two loci linked on an autosome at 46 map units" if the data fit a model of unlinked loci. If appropriate for a given experiment, you can combine the male and female data or the data from reciprocal crosses.

Include this laboratory in your lab notebook. For each set of data, be sure to include your hypothesis (i.e. the genetic model that you are testing such as "two independent loci with one on the X chromosome"). Also include your predictions based on your hypothesis and test your hypothesis with chi square.

LAB 6: Plant Molecular Genetics

DNA Extraction, PCR, and Gel Electrophoresis

DNA Extraction [Week 1]

Silene latifolia

OBJECTIVE: To determine the sex of dioecious plants through DNA extraction, PCR and gel electrophoresis analysis and to expose you to several important tools commonly used in molecular biology

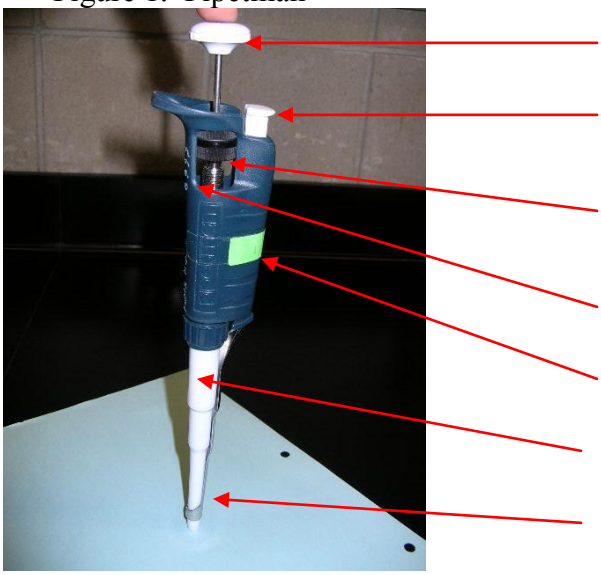
BACKGROUND:

While most plants are hermaphroditic (possessing flowers with both male and female sex organs), about 5% of flowering plants are dioecious (individual plants are either male or female). Some well-known dioecious species are: asparagus, kiwi, *Cannabis sativa*, mulberries and ash trees. *Silene latifolia*, a common herbaceous weed, is also dioecious. The sex determination system in *S. latifolia* is similar to that found in mammals; males are XY and females are XX.

In this lab, each group will collect a small sample of leaf material from two plants of known sex (one male and one female) and also from two plants of unknown sex. You will extract DNA the first week. The second week you will set up a polymerase chain reaction (PCR) to amplify (make millions of copies) a specific small sequence (between 300 and 800 base pairs in size depending on which region is targeted) on the Y chromosome. You will visualize the amplified Y specific DNA as a single band of specific size by agarose gel electrophoresis in the third week. You cannot be certain that any of the steps work until the last week, but there are various controls in place so that you should be able to tell where the experiment failed (if that happens). For your unknown samples, you will see no band on the gel in the last week if any one of the following occurs: no or poor DNA extraction the first week, no or poor amplification by PCR the second week, the gel is made improperly on the third week or both unknowns are female and did not have the Y chromosome sequence to amplify. You should be able to distinguish between these alternatives by examining the results obtained by the various controls. Detailed protocols follow.

Working with Pipetman

Figure 1. Pipetman



We will be using the P1000, the P200 and the P20 Pipetman which are labeled on top of the plunger button. Choose the appropriate Pipetman for the volume you want to transfer; the Pipetman is more accurate at its higher volumes; volumes are in microliters (µl). The P1000 has a red top number in which "1" equals 1000 µl or 1 ml. The P20 has a red bottom number which is 0.1 µl. Do not set the Pipetman to a volume above its limit, such as 105 on the P1000 because this can break the tool. Sample volume indicators are shown for each Pipetman below. On the bottom volume indicator number there are notches that allow you to measure in increments of 2/10. For example, if you moved the volume knob two notches to the left of the 4 on the P200, you would be measuring 74.4 µl. Ask your TA if you have questions about pipetting.

Pipetman Use

1. Tightly attach the correct size pipet tip to the shaft of the Pipetman: clear or blue for the P1000 and yellow for the P200 and the P20.
2. Use a fresh pipet tip for each new measurement except when you are pipetting the same liquid repeatedly and you do not touch the sides of the vessel with the pipet tip.
3. To transfer a liquid, depress the plunger to the first stop. Place tip into the solution and slowly release the plunger drawing up the liquid. Don't let the plunger snap up! Place the tip into the transfer vessel and depress the plunger to the first stop. Wait one to two seconds and depress the plunger fully to the second stop. Depress the tip ejector button to dispose of the tip.
4. Use care in drawing liquid from a container that is full; put the pipet tip in far enough to keep it submerged but not so far that it forces the liquid to spill out of the container.
5. Do not use the Pipetman at a volume below or above its capacity; this can break the Pipetman.
6. Do not lay the Pipetman down horizontally or invert it while it is holding liquid.
7. Draw liquid into the Pipetman using a vertical position or less than a 20° angle.
8. Consistency in your pipetting technique leads to repetition in the volumes you draw.

	P1000	P200	P20
Range:	200 – 1000 µl	20 – 200 µl	2 – 20 µl
Volume setting:	830 µl	74 µl	10.5 µl

Figure 2. Volume indicators on 3 different sized Pipetman.

DNA Extraction [Week 1]

Note: Gloves should be worn throughout this procedure to prevent DNA and enzymes from your hands getting into your extractions.

Note: In step "9" you will *discard* the supernatant; in step "11" you will *discard* the supernatant. In step "14" you will *save* the supernatant and transfer it to a new tube. This solution contains your DNA. Read the procedure carefully so you only have to do the extraction once.

1. **Each lab team will extract DNA from a total of 4 plants:** 1 known male, 1 known female and 2 unknowns (one from each of the unknown groups). Label microcentrifuge tubes for the 4 plants you are going to test including a group symbol.

2. **Sample the leaf tissue.** Take a labeled 1.5 ml microcentrifuge tube to the corresponding plant and put the leaf between the tube and the cover of the tube. Push down on the tube cap to punch out a sample of leaf tissue and let it fall into the tube. Punch out another piece of tissue so you have 2 pieces in each tube. Repeat this procedure for your other 3 samples. Close the tubes and put them on ice.

Figure 3. Sampling leaf tissue using a microcentrifuge tube

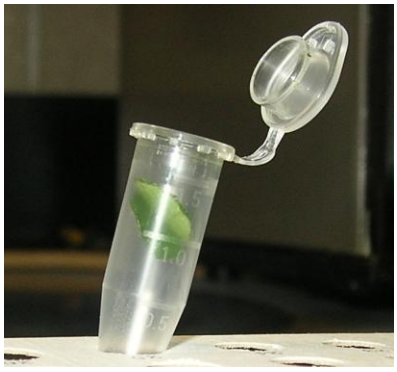


Figure 4. Two pieces of tissue

3. **Clean the 4 blue pellet pestles** by spraying ethanol onto a Kim wipe and wiping each one down. Stand them upside down in a small beaker to dry for one minute before you use them.

4. **Add 500 µl of Cell Lysis Solution (CLS) and 115 µl of PPS (Protein Precipitation Solution) to each of your 4 sample tubes.** Use a new tip for each sample.

5. **Grind the plant tissue with the pellet pestle to break open the leaf cells.** Grind and twist the pestle in the tissue, then release and repeat. Use a different pestle for each sample! The solution should be bright green. In this step you have broken open the cells. The cell lysis solution and the PPS contain buffers and inhibitors to prevent enzymes (including DNAses) from degrading components in the solution.

6. Check that your tubes are closed tightly and c**entrifuge samples for 5 min. at 14,000 rpm to pellet plant debris.** Be sure that the centrifuge is properly balanced before you turn it on.

7. **Transfer about 500 µl of the supernatant of each tube to a new labeled 1.5 ml microcentrifuge tube and add 500 µl of Binding Matrix (BM).** Shake the tube of Binding Matrix very well until it is totally dissolved ***before*** you use it ***and*** shake it between each use.

8. **Incubate the tubes at room temperature with gentle agitation for 5 minutes.** Slowly invert each tube back and forth by hand about every 30 seconds for 5 minutes. Using a vortex at this step could shear the DNA. The DNA has bound to the binding matrix.

9. **Centrifuge at 14,000 rpm for one minute to pellet Binding Matrix. Discard supernatant by pouring it off** into your waste solution flask**.**

10. **Add 500 µl Ethanol Wash (EW) and gently resuspend the pellet using the force of the liquid from the pipet tip.** Continue drawing up the liquid and pipetting it over the pellet until the pellet is completely resuspended. This will take a few minutes.

11. **Centrifuge at 14,000 rpm for 1 minute and discard the supernatant by pouring it off.**

12. **Centrifuge again at 14,000 rpm for 1 minute and remove residual liquid with a small pipet tip.**

13. **Elute the DNA by gently resuspending the Binding Matrix in 100 µl of DNAse-free water (DES).** You may do this by hand or use a vortex ON A SLOW SPEED (vortex speed #3). This will take a few minutes. When the binding matrix has been completely resuspended, incubate the tubes for 5 minutes at 55ºC in a heat block.

14. **Centrifuge at 14,000 rpm for 1 minute and transfer eluted DNA (in the supernatant) to a clean labeled 0.6 ml microcentrifuge tube.** Avoid transferring particles of binding matrix with the eluted DNA. Bring your DNA to your TA to store until next week. Make sure your tubes are labeled with your group symbol and closed tightly.

Clean up:
Used tubes, Pipetman tips and plastic pipets can be thrown in the regular trash. Waste solution must be collected; it cannot go down the drain. Pour it into the large jar labeled "waste solution

with ethanol" on the counter. Return all unused materials to your tray. Ice can be dumped in the sink.

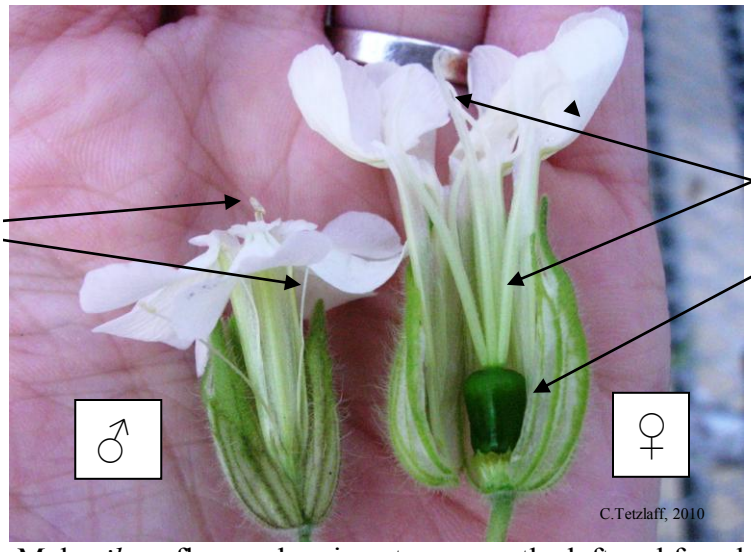
Thought Question:

1. If enzyme inhibitors were not added with the Cell Lysis Solution, what would be the effect?

Glossary:

Centrifuge – v. - to rotate a solution at high speed in order to separate it by density using centrifugal force
Elute – v. - to extract one material from another, usually with a solvent
Incubate – v. – to maintain an organism or a biochemical system at specific conditions for its growth or reaction
Pellet – n. – a small rounded or spherical mass of solid material precipitated by centrifugation
v. – to produce a small rounded or spherical mass of solid precipitated material by centrifugation
Supernatant – n. - the liquid overlying sediment or precipitate from centrifugation
Vortex – v. – to spin the contents of a tube at varying speeds
n. – a machine that spins the contents of a tube at varying speeds

Figure 3. Centrifuge, left and below; vortex, right



Male *silene* flower showing stamen on the left and female *silene* flower showing pistils and ovary on the right.

LAB 7: Plant Molecular Genetics II

Polymerase Chain Reaction (PCR) [Week 2]

BACKGROUND: This week you will use PCR to increase the number of copies of a specific fragment of DNA that is known to reside on the Y chromosome. Your extractions from last week contain a small sample of DNA from the full genome (about 6 billion base pairs in this diploid) of the plants you chose. There are not however, very many copies of any one gene or sequence. If you make millions of copies of one specific sequence and not amplify the remainder of the genome, you should be able to visualize (on a gel next week) that DNA as a distinct band that is the size of the sequence that you amplified. This is what PCR does. It is the exact process that is now used routinely in forensic studies where a small amount of DNA is available.

PCR uses a special enzyme, *Taq* polymerase. This is a DNA polymerase just like others that you have studied except that it comes from the bacteria ***T****hermus* ***aq****uaticus* that grow in hot springs. The DNA polymerase of this bacterium is heat stable. In PCR, DNA is first denatured (becomes single stranded) by heating to near boiling (94 ºC) [recall that *in vivo*, DNA during replication becomes single stranded as **Helicases** swing into action]. The reaction is then rapidly cooled, allowing the primers (short sequences of DNA or RNA) to anneal to the complementary region of the DNA [recall again, that *in vivo* **Primase** makes RNA primers during replication, here we are supplying a specific set of pre-made primers that flank the target gene]. Lastly, the *Taq* DNA polymerase synthesizes a new single strand of DNA starting with the primer and using the original denatured single strands as template (you should know how this works). We have synthesized specific primers (remember that DNA polymerase needs a primer) that are complementary to sequences that flank a gene on the Y chromosome. The primers flank that sequence so that we are amplifying our target sequence on both denatured single strands of DNA. One primer will bind to one of the two strands of DNA on the "left" side of the target gene and allow synthesis 5' to 3' through the target gene and the other primer binds to the other strand on the opposite, "right" side of the target region and allows the synthesis (again 5' to 3') of the target. This sequence of events (heating to denature the DNA and cooling to replicate it) is repeated many times to amplify exponentially (copies are doubled with each cycle) our specific gene so that we can visualize it on a gel.

PROTOCOL:

1. **Dilute the extracted DNA according to your TA's instructions into 4 clean labeled 0.5 ml tubes.** Put your original extraction tubes aside; you'll be using your dilution tubes now. Too much DNA can make it more difficult for the primers to find and bind to the target DNA in the one minute of time they have, so we dilute the DNA.

2. **Label five 0.5 ml tubes with a marker, no tape**; the four plant DNA samples plus one negative control with water instead of DNA. The positive control containing diluted

male *Silene* DNA is already aliquoted in a 0.5 ml tube labeled "+". **Keep all 6 tubes on ice**.

3. **Add 5 µl of diluted DNA to its appropriate tube using a new tip for each sample.** Close tubes and leave on ice.

4. **Prepare a single cocktail of the components listed below in the tube labeled "C" which already contains both primers.** Make enough cocktail for 7 tubes (one more than the number of samples so that you do not run out of the mixture). Use the volumes listed in the "x 7" column. Each group will make one cocktail. Once made, keep your cocktail on ice until ready to use. The TA will add the TAQ to your cocktail last. **NOTE:** The enzyme, *Taq polymerase*, is viscous and tends to sink to the bottom of the tube, so mix the cocktail well but gently by inverting repeatedly; don't vortex.

Cocktail	1 reaction	**x 7**
sterile dH_20	6.8 µl	**47.6 µl**
5X PCR green flexi buffer	5 µl	**35 µl**
dNTPs (2.5mM)	2.5 µl	**17.5 µl**
$MgCl_2$ (25mM)	2.5 µl	**17.5 µl**
primer 1- forward (10 µM)	1.5 µl	**(10.5 µl) – already in tube "C"**
primer 2 - reverse (10 µM)	1.5 µl	**(10.5 µl) – already in tube "C"**
Taq polymerase (5 units / µl)	0.2 µl	1**.4 µl - TA adds this to your tube**
	20.0 µl	140 µl

5. **Pipette 20 µl of the cocktail into each of the 6 sample tubes containing 5 µl of diluted DNA and mix.** You made extra so you should have a little left over. Centrifuge your tubes for 10 seconds to make sure that all liquid is in the bottom of the tubes.

6. **Put one drop of mineral oil on top of the sample** (the oil prevents the small volume of liquid from evaporating through the many heating steps). Your instructor will place them into the thermocycler. Each cycle of steps 2, 3 and 4 will double the number of copies of your target DNA until you have enough that you can easily see it on a gel (next week). The program will take 3 hours so we will continue next week. See cycles below:

PCR cycles:

Step 1: 94ºC - 4 minutes

Step 2: 94ºC - 1 minute – denature the DNA

Step 3: 60ºC - 1 minute – primers anneal to the DNA template

Step 4: 72ºC - 1 minute – DNA polymerization

Step 5: Return 35X to step 2

Step 6: 72ºC - 5 minutes

Step 7: Hold at 10ºC

Clean up:

Pipetman tips and tubes used for dilutions can be thrown in the trash. Ice can be emptied in the sink.

Thought Questions:

1. What is your annealing temperature? _____°C
2. What might happen in a PCR if the annealing temperature is too high or is too low?
3. Draw what happens to a single double stranded piece of DNA through two cycles of PCR.
4. If you assume that you start with a single piece of DNA for your target region (say you extracted the DNA from just one cell), how many copies of your target would you have after 30 cycles of PCR? If you were a forensic scientist (investigator of crime scenes) does this help explain why a single drop of blood at a crime scene provides enough material to look at an individual's genes and "fingerprint" the individual?

Glossary:

Aliquot – v. – to divide a solution into equal volumes

Thermocycler - n. - a machine that can be programmed to cycle through various lengths of time at certain temperatures in order to allow polymerase chain reaction (PCR) to take place on DNA samples.

Figure 1. Thermocycler

LAB 8: Plant Molecular Genetics III

Agarose Gel Electrophoresis [Week 3]

BACKGROUND:

Recall that last week you amplified by PCR a specific sequence of DNA that is found on the Y chromosome. We now want to see the millions of copies of that sequence in those samples (males) that had a Y chromosome. Agarose gel electrophoresis is a method to separate DNA molecules according to size. The agarose forms a matrix or weave of strands with holes through which DNA migrates; long pieces of DNA travel slowly while short pieces travel fast. Higher concentrations of agarose produce a thicker weave with smaller holes and only small molecules of DNA can pass through. Lower concentrations of agarose have bigger holes allowing long molecules to pass. Refer to your text for a description of this process. After separation, DNA molecules are visualized by staining with ethidium bromide (EtBr), which inserts itself between the nitrogenous bases of DNA, and excitation of the EtBr molecules with UV light.

Procedure

1. **Each group makes a 1.5 % agarose mini gel.**
    a. Add 0.6 g agarose to small flask with 40 ml 1X TBE (Tris-Boric Acid-EDTA) buffer
    b. Heat in microwave until boiling (about 1 min.; specific directions for each microwave will be given). Make sure the agarose is dissolved.
    c. Cool the flask for about 10 minutes until you are able to comfortably hold it in your hand and pour the solution into a gel tray with an 8-10-well comb. Let the gel sit for ~ 20 minutes.
    d. When the gel is solidified and opaque, remove the black baffles on either side of the gel and gently remove the comb leaving wells in which your samples will be loaded.

2. The instructor will fill the electrophoresis chambers with 1X TBE buffer.

3. **Load 20 µl of each of your samples to the wells of the gel using a new Pipetman tip for each sample.** Put your pipette tip almost to the bottom of the tube and take only the sample and not the mineral oil on top of the sample. Draw up the solution very slowly; the green buffer is very viscous and air bubbles will occur if you try to draw it up too quickly. You should have a little of your sample left over. Load the samples in this order: 1 – positive male control, 2 – known male, 3 – known female, 4 – unknown 1, 5 – unknown 2, 6 – negative control, 7 – ladder. If you have more than 7 wells, leave the outside ones empty; record the sample order in your notebook. Be careful not to puncture the bottom of the well with your pipette tip. Practice loading gels with the materials provided before you load your own samples.

4. **To well # 7 (or the last well of your series), add 10 µl of 100 base pair ladder (it has blue dye in it).** This is a mixture of DNA of standard sizes (100 bp, 200bp, 300bp etc) and can be used to determine the size of your amplified DNA and to show that the gel has run properly.

5. Check with your instructor about the voltage and time needed to run your gel. DNA is negatively charged so you want it to run to the positively charged electrode. **Your gel has finished running when the yellow dye reaches ¾ the length of the gel.** Unplug your gel from the power supply. Lift the cover off the gel by putting your thumbs on the white posts and pulling the cover up with your fingers. Don't pull it off by the black and red electrical leads!

6. When the gel has finished running, **stain the gel in the prep room**. Dump some of the buffer from your gel rig into the sink. Using a spatula or your gloved hand, lift the gel from the tray and gently place it in the plastic container of ethidium bromide for 15 min. Using the spatula lift the gel out of the EtBr and place it in the container of water for 10 min. to destain. The containers are numbered so you will know which one your gel is in. ETHIDIUM BROMIDE IS MUTAGENIC SO WEAR GLOVES, DO NOT SPLASH AND CLEAN-UP AFTER YOURSELF.

7. **View gel on the ultraviolet light transilluminator in the prep room. Wear gloves and safety glasses to protect your eyes**. **Ultraviolet light can burn your cornea.** After the lab, your TA will take a picture of your gel and email it to you to use in your lab report.

Clean up
The 1X TBE buffer can be disposed of into the sink. Wash the flask you used to make the gel and wash the gel rig with all its parts. Turn off and unplug power supply. Leave all used tubes on your tray. Throw away used pipet tips. Dump ice in sinks.

Thought Questions:
1. What molecular structure of DNA makes it negatively charged?
2. If you do not see a band for one or both of your unknowns, what evidence do you have that this is not because the gel was made improperly, or that the PCR of week 2 failed or that your DNA extractions were poor? In other words, how can you be sure that one or both of the unknowns are female?
3. Suppose that you found a species *S. ambivala* that is closely related to *S. latifolia,* such that they share the same Y chromosome marker. However, *S. ambivala* is hermaphroditic. What would you expect to see on your gel, if you had taken twenty randomly chosen *S. ambivala* plants, and performed the same experiment?

Glossary:
Buffer solution – n. – a solution that is capable of neutralizing both acids and bases to keep the pH of a system constant.
UV transilluminator – n. – a light box in which DNA fragments in a gel, stained with ethidium bromide will be visible.

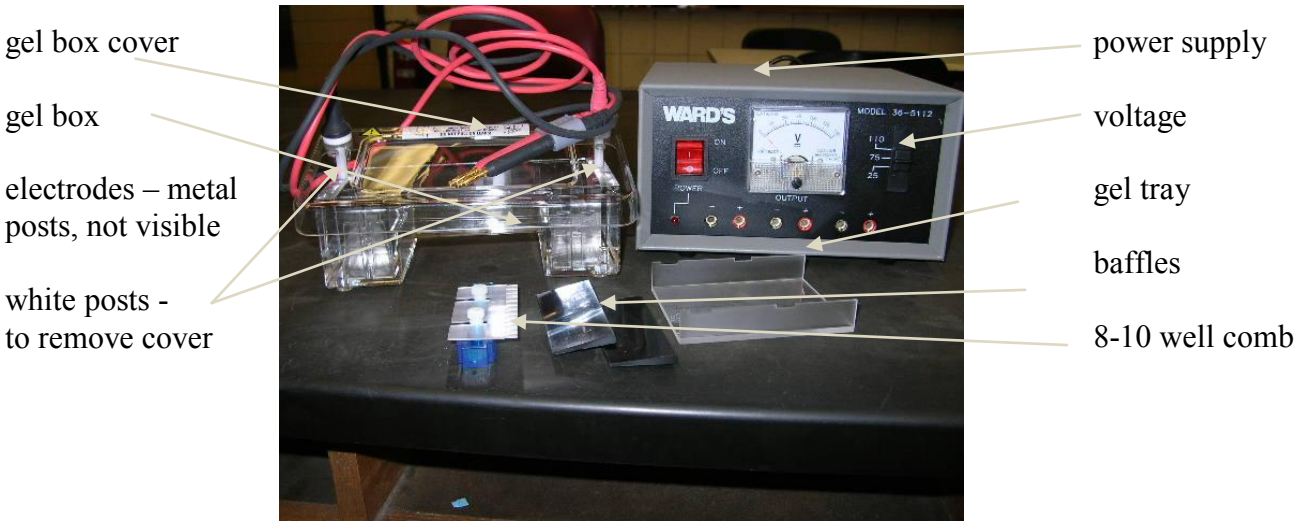


Figure 1. electrophoresis gel rig and power supply

HONOR PLEDGE

- **I pledge that this work is entirely my own.**
- **I understand that plagiarism is copying words or original thoughts without proper acknowledgement of the source.**
- **I understand that this includes copying straight from the lab manual, a partner or other student, other lab reports, the internet, texts, etc.**
- **I understand that plagiarism will result in a zero for the report, and that other consequences may follow.**

Print your name: _____

Sign: _____

Date: _____

LAB 9: Bacterial Mutation I
Ultraviolet Light Mutagenesis of *E. coli* Cells

Mutagenizing Cells, Serial Dilutions and Plating of Cells [Week 1]

BACKGROUND:

Ultraviolet (UV) light can be used to induce genetic changes (mutations) in bacteria cells. To accomplish this, one must first determine the sensitivity of a bacterial strain to UV light. In this laboratory, this sensitivity will be established quantitatively by treating bacterial cells with UV light for different lengths of time and calculating the proportion of cells that have been killed by each treatment. This experiment will demonstrate the killing effects of UV light, the quantitative relationship between UV dose and response, and the proper dose of UV light that would be required in order to generate mutants. The experiment will also provide experience in handling bacteria, performing serial dilutions and viable cell counts, and graphing and interpreting data on a semi logarithmic scale.

Relevant sections from textbook (Klug and Cummings, *Concepts of Genetics*): Chapter on DNA: Mutation, Repair, and Transposable Elements

INTRODUCTION:

A gene mutation is a heritable change in the gene's nucleotide sequence. Such genetic changes are a major source of genetic variability within populations. In addition, mutations are important tools for geneticists. By studying organisms bearing mutations, geneticists can identify the genes that control particular phenotypes, can study the transmission of these genes, and can analyze the cellular processes that produce these phenotypes.

Mutations can be either **spontaneous** or **induced**. Spontaneous mutations are those that arise "naturally," without intervention of any artificial mutagenic agents. Induced mutations, on the other hand, result from either chemical or physical mutagenic treatments. Because spontaneous mutations occur at very low frequencies, geneticists commonly induce mutations in order to obtain an ample supply to use for experimental purposes. Mutations can be induced by a broad range of chemical agents. Some of these chemical **mutagens** are described in the Mutation chapter of your textbook. In addition, mutations can be induced by physical factors such as ultraviolet light, gamma rays, and X-rays. All of these agents have characteristic mechanisms for inducing mutations and thus induce particular types of mutations.

In this laboratory, we will examine the mutagenic effects of UV light. This form of radiation is a common tool used by microbial geneticists for inducing mutations experimentally. While ultraviolet radiation has lower energy content than ionizing radiations such as gamma rays and X-rays, it can produce a mutagenic or lethal effect in cells exposed to wavelengths of 210 to 300 nanometers. Within the cell, UV light is absorbed primarily by the nucleic acids; in particular, UV light damages the pyrimidine bases within DNA. On exposure to UV light, adjacent pyrimidine (especially thymine) molecules can bond covalently to one another, forming **pyrimidine dimers**. This dimer formation distorts the DNA structure, interfering with proper DNA replication and transcription.

Besides exploring the mutagenic effects of UV light, this lab will give you experience working with the bacterium *E. coli*. *E. coli* cells offer many advantages for inducing and analyzing mutations. For example, they are small and easy to grow in very simple medium; in addition, they have short generation times (about 30 minutes) and thus can be grown in large numbers in short periods of time. (A typical "saturated" culture can have as many as 10^9 bacterial cells per milliliter.) Furthermore, individual cells give rise to visible colonies on solid medium, which allows many phenotypes to be visualized easily. Finally, a wealth of information has accumulated about the genetics and physiology of *E. coli*; as a result, highly sophisticated genetic techniques have been developed for studying this organism.

In this lab, we will treat cells with UV light in order to cause random mutations. Some of these random mutations will occur in genes that are essential for survival. Cells that undergo mutations in essential genes will be killed. In order to determine the sensitivity of our strain of *E. coli* to UV light, we will treat the cells with various doses of UV light. As the UV dose is increased, the mutation rate will rise, as will the death rate of the cells. We will then determine the percentage of cells that have survived and the percentage that have been killed at these different UV doses. When graphed, this information produces a **killing curve**, which shows the relationship between UV dose and its lethal effects. Biologists often use the information from a killing curve to establish optimal conditions for inducing mutations in subsequent experiments.

Materials Needed:

Glass beads 4 mm for spreading *E. coli* on plates
Test tubes (in racks) for dilutions (filled with 10 or 1 ml 0.1M $MgSO_4$)
Vortex mixer
P200 Pipetman and tips
Petri plates (empty), 5 for each group

LB plates, 19 for each group
Black markers
Safety glasses
Disposable gloves
Timers
sterile 5 ml pipettes
pi-pumps for 5 ml pipettes
UV lamps
E. coli cells resuspended in 0.1 M $MgSO_4$
E. coli waste jars
Jar for used beads with funnel
Empty trays to carry plates of bacteria to UV light in prep room

Safety Considerations: UV is a mutagen as well as an eye-damaging agent. Never look directly at the UV lamp! Always wear safety glasses when using UV.

Although your skin is not highly sensitive to small doses of UV, you should also wear plastic gloves to minimize exposure to your hands.

Procedure:

1. Prior to class, your instructor will grow *E. coli* cells in 500 ml of nutrient medium overnight at 37C. This gives a concentration of approximately 10^9 cells per milliliter. Just prior to class, the bacteria will be diluted 1/5 in a solution of 0.1 M $MgSO_4$. You will receive a sample of these cells in a sterile test tube. All manipulations in this lab should be performed using sterile technique. Your instructor will demonstrate these methods and describe serial dilutions to you. You will label your test tubes with tape for these serial dilutions.

2. **Label 5 empty, sterile Petri dishes on the bottom: B - 3 sec, C - 6 sec, D - 15 sec, E - 30 sec, and F - 50 sec.** (A – 0 sec, are the control cells and will receive no treatment.) Vortex your sample of cells and use a 5 ml pipette and a green "pi-pump" to **pipette 5 ml of the cell suspension into each of these dishes**. These cells will be used for UV treatment for the times marked.

3. **Mutagenize your cells**. When your dishes of cells are ready, place them on a tray singly, not stacked, and take them into the prep room where UV light source is set up. When you are working in this room, remember to wear safety glasses and plastic gloves. Your instructor will demonstrate how the cells will be mutagenized. The important points are as follows: UV does not penetrate plastic, so the lid of the Petri dish must be removed during UV treatment. The uncovered plate will be slid into the UV apparatus and shaken gently on a tray to minimize UV exposure to your hands. One student should move the plates in and out, while another student in the group acts as timekeeper. After the cells have been mutagenized for the designated numbers of seconds, the plates should be covered. Dilutions and plating of the cells will then be carried out in the main lab.

4. In order to calculate the percentage of cells that have survived each treatment, it is necessary to count the number of viable cells in all the treated cultures, as well as in the untreated control (that is, the cells from the original test tube.) Such **viable cell counts** can be made by diluting cells for each condition, then spreading the cells on agar plates. During an incubation period, each cell on the plate will divide and eventually produce a **colony** of cells visible to the naked eye. During the next lab period, these colonies can then be counted. After taking into account the dilution factors that were used, the number of viable cells per milliliter in the various cultures can then be calculated.

By calculating:

$$\frac{\text{\# viable cells per ml in treated culture}}{\text{\# viable cells per ml in untreated control}} \times 100,$$

we can determine the percentage of cells that survived that particular treatment.

5. In order to count the number of separate bacterial colonies on an agar plate, it is necessary to dilute the cell suspensions so that a countable number of colonies (a few hundred at the most) grow on each plate. If we keep track of our dilution factors, we can calculate later the number of cells we started with. In order to dilute the cells to this extent, **serial dilutions must be carried out**. In this method, an initial dilution is made, then this dilution is diluted a second time, this second dilution diluted a third time, etc. What are the advantages of this serial method of dilution?

6. Since we do not know ahead of time which dilutions will yield the appropriate numbers of colonies, several dilutions will be made and spread on plates. You will be provided with test tubes filled with either 10 or 1 milliliter (ml) of 0.1 M $MgSO_4$ for making dilutions. To dispense

0.1 ml (=100 μl), use the P200 Pipetman provided. After each step of the serial dilution, be sure to vortex the solution to evenly distribute the cells in the tube. Your instructor will demonstrate the use of these items. See the following page for a guide to making the appropriate dilutions. Label your test tubes with treatment, for ex. "B" and dilution, for ex. "$1/10^4$" and line up the test tubes needed to make the dilutions for each treatment.

7. Once appropriate dilutions are made, **0.1 ml of these dilutions (vortex again) should be spread on an agar plate**. Label each agar plate on the bottom with the letter denoting the UV exposure, the dilution factor and your group name. To spread the *E. coli* cells, take a tube of sterilized glass beads and pour them into one agar plate, lifting the lid only high enough to add the beads. Add the beads slowly because they bounce and roll easily. Close the lid. Use a Pipetman to transfer 0.1 ml of the appropriate, diluted cells to the surface of the agar. Close the lid. Holding the lid, move the plate back and forth on the bench sending the beads criss-crossing the plate and spreading the cells on it. Turn the plate once in a while and shake back and forth again to make sure you have all of the surfaces covered evenly. Shake each plate for one full minute. The plates should be spread until all the liquid soaks in. When you are finished shaking your plate, lift the lid up to dump the used glass beads into the wash jar with the funnel on top. Your instructor will demonstrate this technique. Plate all of the treatments and dilutions asked for.

8. **The spread plates should then be stacked upside down in two piles, taped together and placed in a 37°C incubator.** After the colonies have grown, your instructor will refrigerate the plates until the next lab period.

Serial Dilutions:

Note: Be sure to label all tubes with tape before making dilutions. All volumes are in ml. After adding the appropriate number of cells to the $MgSO_4$ solution in each dilution tube, be sure to mix the solution well using a vortex mixer. After dilutions are made, <u>spread 0.1 ml samples</u> on labeled agar plates.

A. Control Cells (No UV treatment):

$1/10^2$ = 0.1 ml control cells + 10 ml $MgSO_4$

$1/10^4$ = 0.1 ml of $1/10^2$ + 10 ml $MgSO_4$

$1/10^5$ = 0.1 ml of $1/10^4$ + 1 ml $MgSO_4$

$1/10^6$ = 0.1 ml of $1/10^5$ + 1 ml $MgSO_4$

$1/10^7$ = 0.1 ml of $1/10^6$ + 1 ml $MgSO_4$

Of these control dilutions, plate $1/10^4$, $1/10^5$, $1/10^6$, $1/10^7$.

B. 3 Second UV:

$1/10^2$ = 0.1 ml cells + 10 ml $MgSO_4$

$1/10^4$ = 0.1 ml of $1/10^2$ + 10 ml $MgSO_4$

$1/10^5$ = 0.1 ml of $1/10^4$ + 1 ml $MgSO_4$

$1/10^6$ = 0.1 ml of $1/10^5$ + 1 ml $MgSO_4$

Of these dilutions, plate $1/10^4$, $1/10^5$, $1/10^6$.

C. 6 Second UV:

$1/10^2$ = 0.1 ml cells + 10 ml $MgSO_4$

$1/10^4$ = 0.1 ml of $1/10^2$ + 10 ml $MgSO_4$

$1/10^5$ = 0.1 ml of $1/10^4$ + 1 ml $MgSO_4$

$1/10^6$ = 0.1 ml of $1/10^5$ + 1 ml $MgSO_4$

Of these dilutions, plate $1/10^4$, $1/10^5$, $1/10^6$.

D. 15 Second UV:

$1/10^2$ = 0.1 ml cells + 10 ml $MgSO_4$

$1/10^3$ = 0.1 ml of $1/10^2$ + 1 ml $MgSO_4$

$1/10^4$ = 0.1 ml of $1/10^3$ + 1 ml $MgSO_4$

Plate all dilutions.

E. 30 Second UV:

1/10 = 0.1 ml cells + 1 ml $MgSO_4$

$1/10^2$ = 0.1 ml of 1/10 + 1 ml $MgSO_4$

$1/10^3$ = 0.1 ml of $1/10^2$ + 1 ml $MgSO_4$

Plate all dilutions.

F. 50 Second UV:

1/1 (Plate directly)

1/10 = 0.1 ml cells + 1 ml $MgSO_4$

$1/10^2$ = 0.1 ml of 1/10 + 1 ml $MgSO_4$

Plate all dilutions.

Clean up: Empty all liquid from tubes and original Petri dishes into the *E.coli* waste jars. Used tips, Petri plates, paper towels, gloves and plastic tubes go into the orange autoclave bags and not into the regular trash. Place all used empty test tubes into the large tub of water to soak; **remove all tape on the tubes or caps first.**

Lab 10: Bacterial Mutation II
Ultraviolet Light Mutagenesis of *E. coli* Cells II

Worksheet and Questions [Week 2]

During this second lab period, you will examine the plates that were spread with cells during the last lab. If certain plates have fewer than about 500 colonies, then you should count the colonies. Your instructor will demonstrate some tricks for counting bacterial colonies, such as marking off each colony as it is counted or dividing the plate into quadrants and counting only one quadrant. From these counts you should theoretically be able to calculate the number of viable cells per milliliter (ml) in the original cell suspensions.

Don't worry if your plates show unexpected results or if the colonies did not grow. In case this is true, we are also providing you with sample data which were generated by your instructor. You can use these data to compute the numbers of viable cells per ml that were in each sample. As part of this exercise, you should think carefully about the dilutions that were made and about how that information must be used in your calculations. Also remember to figure in that you plated only 0.1 ml rather than 1.0 ml. You can use the worksheet provided to record the numbers of colonies and organize your calculations.

Even if you don't use your own data, you should examine your own plates carefully. Are the colonies evenly spread? Are the numbers of colonies within a single dilution series consistent with each other? Think about possible sources of error in the procedures that were used.

Once the **cell titers** of the original untreated and UV-treated samples have been calculated, you can use this information to determine the percentage of cells that survived a particular UV treatment. Specifically, the percent survival is calculated as follows:

$$\frac{\text{\# viable cells per ml in treated culture}}{\text{\# viable cells per ml in untreated control}} \times 100$$

We are interested in estimating what dosage of UV light results in 99% killing of the culture. To make this estimate, you should graph % survival vs. time of UV treatment. This type of graph is called a "killing curve." For this graph, it is necessary to use semi-log graph paper, which will be provided. You should examine this paper carefully, noting that the numbers along the Y-axis increase geometrically instead of arithmetically. You should plot % survival on the Y-axis and seconds of UV treatment on the X-axis. What do you expect to be the relationship between these values? Your instructor will explain further how to graph your data on semi-log paper. Think about why this type of graph paper is useful for this purpose.

Clean up:

All plates, gloves and paper towels should be put into the orange autoclave bags taped to the counter.

You should include your worksheet as well as answers to the following questions in your lab notebook:

1. What is the relationship between UV dose and cell killing?
2. What do you suppose is the relationship between killing rate and mutation rate?
3. Why is it useful to estimate the UV dose that will result in 1% survival?
4. What are the advantages of the serial dilution method for diluting cells?
5. What are the possible sources of error when generating viable cell counts and killing curves?
6. Suppose you are working in a genetics laboratory and want to select bacterial mutants of a certain type. What are the advantages and disadvantages of using UV light as your mutagenic agent? (You may want to consult your textbook.)
7. Many procedures suggest that when mutagenizing bacterial cells with UV light, the dish of cells should be shaken gently during the actual mutagenesis. Can you speculate on the reason for this?
8. When geneticists mutagenize cells with UV light in order to select mutants, they often keep the cells in the dark after the mutagenesis step. Can you speculate on the reason for this? You will need to consult your textbook.

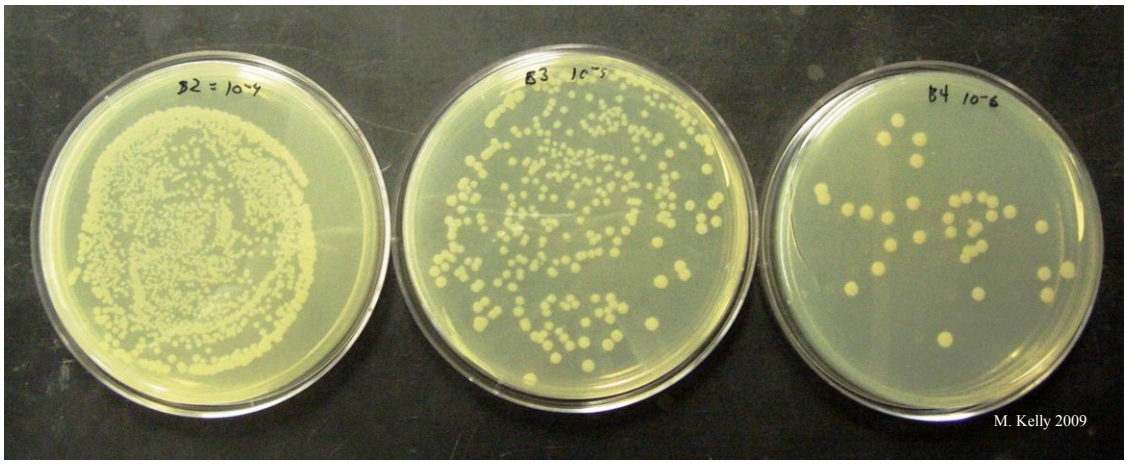


Figure 1. A series of *E. coli* plates with "B" treatment.

Ultraviolet Mutagenesis of Bacterial Cells Worksheet

Key: TMTC = Too many to count; ND = No data; C = Contaminated plate

UV Exposure (sec)	Dilution	# Colonies from 0.1 ml	Overall dilution factor	# Viable cells / ml after mutagenesis	% Survival	Mean % survival of each treatment
0	$1/10^4$				100	100
0	$1/10^5$				100	
0	$1/10^6$				100	
0	$1/10^7$				100	
3						
3						
3						
6						
6						
6						
15						
15						
15						
30						
30						
30						
50						
50						
50						

LAB 11: GENE REGULATION: the *lac* operon

OBJECTIVE: To understand the control of the *lac* operon by monitoring the response of *E. coli* upon induction with lactose in the presence and absence of glucose.

INTRODUCTION

Genes are transcribed into messenger RNA, which is then translated into protein. While most *E. coli* genes exist in only one copy, the amounts of proteins produced from different genes are highly variable. These amounts range from a few molecules per cell to tens of thousands of molecules per cell. Furthermore, the rate of production of certain proteins is constant throughout the life cycle of *E. coli*, whereas the rate of production of other proteins varies enormously, depending on the environment and the cell's needs. These variations in amounts of proteins produced from different genes result from differential gene expression. The specialized cell types in higher organisms show even more dramatic examples of differential gene expression. For example, all the various cell types in a given mammal contain the same set of genes, but different cell types select different subsets of these genes to synthesize different subsets of proteins. Thus, large amounts of digestive enzymes are synthesized in pancreatic acinar cells but not in muscle cells. Conversely, large amounts of contractile proteins are synthesized in muscle cells but not in pancreatic acinar cells.

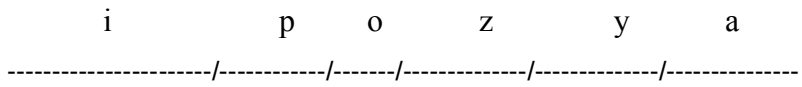
The lactose operon of *E. coli* is one of the best understood bacterial systems in which the expression of genes is controlled by the chemical environment. The lactose operon is an inducible system in which three proteins are synthesized when lactose is present, but not when lactose is absent. Thus their synthesis is "induced" by lactose. Lactose is a disaccharide sugar which is used as a carbon source for energy and for synthesis of various cellular molecules. The first step in the metabolism of lactose is breakdown into two monosaccharides, galactose and glucose, by the enzyme beta-galactosidase (ß -galactosidase). The levels of ß -galactosidase are almost nondetectable in *E. coli* cells that are grown without lactose. However, when lactose is added to the growth medium, the levels of ß -galactosidase increase about a thousand-fold within a few minutes. The induction of ß -galactosidase is coordinated with two other proteins that function in lactose utilization: a permease which transports lactose through the cell membrane, and a transacetylase which probably inactivates toxic compounds similar to lactose. Also note that ß -galactosidase has a second minor enzymatic activity, which catalyzes the conversion of lactose to a closely related disaccharide, allolactose.

When lactose is not available in the environment of *E. coli*, then cellular production of proteins involved in utilizing lactose would waste cell resources. Thus it makes perfect biological sense that ß -galactosidase, permease, and transacetylase are synthesized only when lactose is present. Furthermore, the lac operon has a second level of control that also helps the cell conserve resources: if glucose is present, then ß -galactosidase, permease and transacetylase cannot be induced by lactose. This also makes biological sense since glucose is metabolized more easily than lactose; thus it is inefficient to utilize lactose when glucose is available as a carbon source. The suppression by glucose of the induction of ß -galactosidase is called catabolite repression.

In theory, the enzymes of the lactose operon could be regulated at any of the steps that influence the activity of gene products: the transcription of the gene by RNA polymerase, the translation of mRNA, the degradation of mRNA, activation of the proteins after translation, or inactivation or decay of the proteins. However, in the lactose operon, induction of proteins is controlled exclusively by varying the rate at which RNA polymerase transcribes the mRNA.

The mechanisms by which lactose induces the coordinate synthesis of these three proteins were elucidated by Jacob and Monod in their famous operon model. Detailed discussion of the lactose operon can be found in your genetics textbook.

Briefly, the lactose operon includes 6 components, which are found in the following positions on the *E. coli* chromosome.



i = lactose repressor

p = promoter (binding site for RNA polymerase)
o = operator (binding site for the repressor)
z = ß -galactosidase
y = permease
a = transacetylase

I. The structural genes encoding ß -galactosidase, permease, and transacetylase are adjacent to each other on the *E. coli* chromosome. In this diagram, transcription occurs from left to right. The expression of all three genes is controlled by a single promoter (p), and a single polycistronic mRNA encoding all three proteins. The polycistronic mRNA ensures that levels of ß -galactosidase, permease, and transacetylase are regulated coordinately.

II. The transcription of the polycistronic mRNA is regulated by interactions of three components: the inducer (lactose), the lactose repressor, and the operator. The repressor is an allosteric protein with two binding sites: one site binds specifically to the operator DNA sequence; the other site binds allolactose, the derivative of lactose mentioned above. When lactose is absent from the medium, the lactose repressor binds to the operator. Note that the operator is located between the promoter (the site of binding of the RNA polymerase), and the transcription start site of the genes controlled by that promoter. Thus, the lactose repressor binds to the operator in a position which obstructs transcription of the polycistronic mRNA. When lactose is present, allolactose binds to the repressor, causing the repressor to change shape. The repressor then releases the operator, the RNA polymerase transcribes the polycistronic mRNA, the mRNA is translated, and the levels of ß -galactosidase, permease and transacetylase increase. The lactose repressor is considered to exert negative control over the lactose operon because it inactivates the operon when it binds to the operator DNA sequence.

III. As noted above, the induction of ß -galactosidase, permease, and transacetylase by lactose is suppressed if glucose is present in the *E. coli* environment. This suppression involves a second mechanism of regulation superimposed on the repressor-operator. Briefly, glucose must be absent for RNA polymerase to bind to the promoter. The mechanism of catabolite repression is described further in your genetics text.

In this lab, we will treat cells with lactose and measure induction of ß -galactosidase. In addition, we will test the effect of glucose on the induction of ß -galactosidase. To measure ß -

galactosidase activity in cells, we add to cells a colorless compound, o-nitrophenylgalactoside (ONPG). When ß -galactosidase is present, this enzyme breaks down ONPG, yielding o-nitrophenol (ONP), which has a yellow color. The amount of yellow color (from ONP) can be measured by testing the absorbance (optical density, O.D.) of the solution at 420 nanometers (wavelength) in a spectrophotometer. The amount of yellow color is proportional to the level of the ONP product, which in turn is proportional to the level of ß -galactosidase activity.

Materials: (for each group of students)

1. Approximately 50 ml of a log-phase culture of *E. coli B*, grown in a chemically defined medium with glycerol (M9-glycerol).
2. Approximately 50 ml of a log-phase culture of *E. coli* that has been grown in a chemically defined medium with glycerol (M9-glycerol), then 0.2% glucose for 30 minutes. This culture will be shared by the entire class.
3. 2.5 ml of 5% lactose
4. 4 ml of o-nitrophenylgalactoside (ONPG) at 4 mg/ml
5. 1 ml toluene (This is kept in the hood.)
6. 9 ml 1 M Na_2CO_3
7. 2 ml pipettes and pi-pumps
8. 10 test tubes for Bausch and Lomb spectrophotometer, one filled ¾ with deionized water
9. Bausch and Lomb visible light spectrophotometer
10. Shaking water bath set at 37^{o}C with brackets to hold 125 ml Erlenmeyer flasks.
11. Markers for labeling tubes
12. Ice buckets
13. Water bath set at 37^{o}C
14. Test tube racks to hold spectrophotometer tubes
15. Pipetman and tips, 1000 µl
16. Timer

Safety Precautions:

Note that toluene is volatile and toxic. Keep the stock bottles of toluene in the fume hood, and avoid breathing the fumes.

Experimental Procedures

(Note: Before starting, read the entire lab handout to be sure you understand the reasons for each experimental step.)

In this experiment, you will measure the induction of ß -galactosidase in cells after the cells are provided with lactose. The levels of ß -galactosidase will be measured at exactly the point that lactose is added (time zero), and at 5 minute intervals up to 30 min. For this experiment, you will use cells grown with glycerol as a carbon source. In addition, you will use cells grown in glycerol plus glucose to test whether glucose inhibits the ability of lactose to induce ß -galactosidase.

These experiments have two stages. First, lactose will be added to cells to induce ß -galactosidase. Second, we will measure the levels of ß -galactosidase in the cells.

Part I. Induction of ß -galactosidase:

Summary: You will be given a 50 ml log-phase culture of *E. coli* that was grown with glycerol as the only carbon source. You will be adding lactose to induce ß -galactosidase, then removing samples at regular intervals to check for enzyme activity. You will also use a culture grown with glycerol plus glucose, and will remove a sample 30 min after the addition of lactose.

1) For the cells grown in glycerol, label the top of the spectrophotometer tubes with a marker as follows: 0, 5, 10, 15, 20, 25 and 30. For the glucose experiment, label one tube, G-30. Add two drops of toluene to each of the 8 tubes, and place the tubes in an ice bucket. These tubes will receive samples of *E.coli* cells after different induction times. Be sure you understand the plan of the experiment. What is the purpose of the toluene?

2) When you are ready to start, choose which numbered flask you will use (1-9) and write your group name on the sign-up sheet for that flask. The 5% lactose has been measured exactly (2.5 ml) so you can dump the entire contents of that tube into your *E. coli* culture and mix well. Immediately start your timer (this is time zero), and immediately transfer 2 ml with a sterile pipette and a blue pi-pump to the chilled tube marked "0". Mix gently and return the tube to the ice bucket. Put the remaining *E. coli* culture in the shaking water bath at 37°C so that induction can occur.

3) At 5, 10, 15, 20, 25, and 30 min after the addition of lactose to the glycerol culture, transfer 2 ml of this culture to the appropriately labeled tube with a sterile pipette. Swirl gently to mix the cells with toluene, and place in the ice bucket. Swirl the culture in the flask immediately before you take each sample as the cells tend to settle to the bottom when the flask is not moving. Used pipettes can be stored in the *E. coli* waste jars until the end of the lab.

4) During the sampling time, the TA will add 5 ml of 5% lactose to the 50 ml culture of *E. coli* grown with **glucose**. This culture will be shared by the entire class to test whether a culture grown with glucose will induce β-galactosidase when lactose is added.

5) 30 min after the TA added the lactose to the glucose culture, transfer 2 ml of this culture to the tube labeled "G-30". Again mix cells gently with the toluene, and put the tube on ice.

Part II. Measurement of ß -galactosidase:

6) After all the bacterial samples have been mixed with toluene in the tubes, again swirl each tube gently. To prepare these tubes for enzyme measurement, set the tubes in a test tube rack, in a 37°C water bath for approximately 10 min to increase their temperature.

7) We are now ready to test the levels of ß -galactosidase in these induced cell samples. Add 0.4 ml ONPG to each tube as precisely as possible using the 1000 µl Pipetman. The ONPG can be added to the tubes while they are in the water bath. Mix gently but thoroughly; then incubate at 37°C for 5-15 minutes. The point at which ONPG is added is time zero, since you are timing the reaction from that point. Be sure to start your timer then. Periodically examine your tubes in the water bath. When any of the tubes show a slight yellow color, note the time and let the tubes incubate for 5 more minutes. After the 5 minutes, take the rack out of the water bath and bring it to your bench. Add 1.0 ml of 1 M Na_2CO_3 to all eight spectrophotometer tubes and mix gently. Stop your timer; this is the time that you will use in your calculations. The first tube to show color will be the one with the highest level of ß -galactosidase. Can you predict which tube this will be?

8) Blank the spectrophotometer for 420 nm using the tube of M9-glycerol medium. Measure the absorbance of all of your experimental tubes at 420 nm. Next, blank the spectrophotometer for 550 nm with the tube of M9-glycerol medium and measure the absorbance of all of your experimental tubes at 550 nm. Wipe down each test tube with a

Kim wipe before inserting it into the spectrophotometer to make sure it is clean. The procedure for using the spectrophotometer is described at the end of this lab.

There are two reasons for reading the optical density of each sample at both 420 and 550 nm. First, the optical density reading at 420 nm is actually due to a combination of absorbance by the ONP and optical density due to light scattering by *E. coli* cell debris. It is possible to correct for the light scattering of cellular debris at 420 nm by the following formula:

$$OD_{420} = 1.75 \times OD_{550}$$

Second, we wish to measure the amount of ß – galactosidase / cell. Since the light scattering (turbidity) at 550 nm is proportional to cell density, it provides an estimate of the number of *E. coli* cells in each sample.

9) The amount of enzyme activity per cell in each sample can be calculated from the formula below:

$$\text{Enzyme Units} = 100 \times \frac{OD_{420} - (1.75 \times OD_{550})}{t \times OD_{550}}$$

where t is the length of incubation with ONPG (in minutes).

10) When you have finished analyzing your data, the kinetics of induction of ß -galactosidase by lactose should be graphed, with the number of enzyme units per cell on the Y-axis vs. time of incubation with lactose on the X-axis. Plot the enzyme units per cell of the glucose sample (G30) as a single point and don't connect it to the line of the rest of the data.

Clean up

Please pour your *E. coli* samples from your test tubes into the ***E. coli* WITH toluene** waste jar on the counter. This *E. coli* must be treated as hazardous waste. Empty your *E. coli* culture flask into the ***E. coli* WITHOUT toluene** waste jar on the counter. Place your empty test tubes and flask into the container to soak. The test tubes containing M9-glycerol medium and colored solutions can be reused; leave them in your rack. Place all used pipettes in the orange autoclave bag. Dispose of tips, gloves and paper towels in the regular trash. Turn off the spectrophotometers, unplug and leave on the bench.

Glossary:

Spectrophotometer - spec·tro·pho·tom·e·ter (spĕk'trō-fō-tŏm'ĭ-tər) n.
An instrument for measuring the intensity of light of a definite wavelength transmitted (or absorbed) by a substance or a solution, thus providing a measure of the amount of material in the solution absorbing the light.

Dictionary.com. *Merriam-Webster's Medical Dictionary*. Merriam-Webster, Inc. http://dictionary.reference.com/browse/spectrophotometer (accessed: August 09, 2011).

Notes on Procedures

1. The bacteria are grown on chemically defined medium containing glycerol as the only carbon source. Obviously, if the growth medium contained glucose, it would suppress induction of the operon by lactose.
2. Either lactose or isopropylthiogalactoside (IPTG) can be used to induce the lactose operon. IPTG is a "gratuitous inducer" of the lactose operon. It can mimic allolactose and interact with the *lac* repressor to induce the lactose operon, but it cannot be metabolized or used as a carbon source by *E. coli*.
3. The o-nitrophenol solution is yellow. Therefore, it transmits yellow light and absorbs violet light. The spectrophotometer is used to measure the amount of light that is absorbed as it passes through the solution of ONP. The absorption of light by a solution is described by the Beer-Lambert Law

$$\text{Absorbance} = \text{O.D. (optical density)} = \log_{10} [I_O/I] = E\, l\, c$$

where I_O = the intensity of incident light, I = intensity of transmitted light, c = concentration of the absorbing substance, l = length of light path through the solution, E = extinction coefficient. In these measurements, l is constant since we always use the same diameter tubes, and E is constant for any given substance. Thus, the absorbance or O.D. of the solution is directly proportional to the concentration of absorbing molecules.

4. In measuring ß -galactosidase activity, a sample of *E. coli* is first mixed with a few drops of toluene. The toluene permeabilizes (makes holes in) the *E. coli* cell membrane. This kills the bacteria and prevents further changes in ß -galactosidase levels. The holes in the cell membrane also enable the enzyme to react with the ONPG. If the cell membranes were not permeabilized, beta-galactosidase would remain inside the cells, ONPG would remain outside, and the enzyme could not break down the ONPG.
5. The formula above shows that the amount of ONP produced is proportional to the number of cells and the time of incubation. Thus, both time and cell number must be measured in each enzyme measurement. After allowing the reaction between ONPG and ß -galactosidase to proceed, the reaction is terminated by the addition of Na_2CO_3. The Na_2CO_3 not only develops the color, but it also stops the reaction by changing the pH of the reaction mixture to pH = 11 where the enzyme is not active. This allows the experimenter to precisely control the length of the enzyme reaction, and then measure the absorbance in the spectrophotometer at his/her convenience.

Thought Questions:

Record the answers to the following questions in your notebook.

1. Review Jacob and Monod's operon model. Then list all the metabolic changes which lead to synthesis of ß -galactosidase when lactose is added to *E. coli* cells growing in M9-glycerol medium.
2. It is possible to estimate how long the cells took to synthesize ß -galactosidase after adding the inducer by extrapolating the linear, increasing portion of the curve to the time-axis. This interval is determined by the rate of addition of bases by the RNA polymerase and the rate of polypeptide chain elongation. How well do your observations agree with calculations that RNA polymerase adds bases at a rate of about 30 per sec, amino acids are added at a rate of about 6 per sec in *E. coli* cells at 37^{O}C, and ß -galactosidase is 1170 amino acids long?

Figure 1. GENESYS 20 Spectrophotometer

Starting up the instrument

When you turn on your GENESYS 20 spectrophotometer, it performs its power-on sequence. This sequence includes checking the software revision, initializing the filter wheel and the monochromator. The power-up sequence takes about 2 minutes to complete. Allow the instrument to warm up for 30 minutes before using it.

Note: *Be sure that the cell holder is empty before turning on the instrument.*

Absorbance and % Transmittance measurements

1. Press **nm ▲** or **nm ▼** to select the wavelength (in nanometers).
   Note: *Holding either key will cause the wavelength to change more quickly*.

2. Insert your blank into the cell holder and close the sample door.

3. Press **0 ABS/100%T** to set the blank to 0A or 100%T.

4. Remove your blank and insert a sample into the cell holder. The sample measurement appears on the LCD display. Do not hesitate to ask for more instruction if you are not sure you are using the machine properly.

5. Fingerprints on the test tubes can alter the optical density readings so wipe down your tubes each time before you put them in the spectrophotometer with a Kim wipe.

Using Micropipetors

Learning Goal: To familiarize students with the correct use of micropipetors.

Introduction: Frequently in genetics, molecular biology, or cell biology labs, we need to measure or transfer very small volumes of liquids. Micropipetors are precise measuring devices used for this purpose. There are many different brands of micropipetors, the most common being the Pipetman produced by the company Gilson. Although the details of various models differ, the basic components of micropipetors are similar; these are depicted in **Figure 1**.

The tiny volumes we typically measure often range from less than 1 to 1,000 microliters (written as μL). Recall that 1 microliter (μL) is a millionth of a liter (L) and one thousandth of a milliliter (mL). Table 1 shows the three Pipetman models that we will be using and their recommended ranges of volumes.

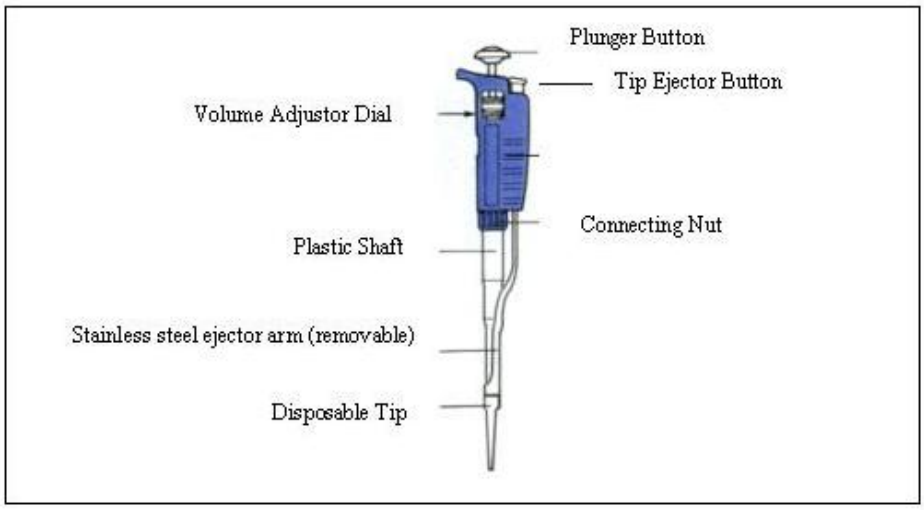


Figure 1 Parts of the Micropipetor

Model of Micropipetor	**Recommended Range of Volume**
P20	2 to 20 μL
P200	20 to 200 μL
P1000	200 to 1000 μL

Table 1. Pipetor models used in this lab. Many more models are available.

Prior to using a micropipetor, it is critical to know what model you are using, how to set the volume, how to read the volume, and how to attach a tip. Micropipetors usually contain a label at the top of the device indicating the model (**Figure 2)**.

Figure 2. Top images of the P20, P200, and P1000:

HOW TO READ THE VOLUME:

Figure 3. Volume Indicator Dials: The volume indicators are shown for a P20, P200, and P1000.

In **Figure 3** above, the P20, which has a range of 2 to 20 µL, is set to measure 6.86 µL. The dial contains 3 slots for numbers, with the bottom slot in red. The first slot, which is set to zero in **Figure 3**, sets the tens place. The second slot, which is set to 6 in **Figure 3**, sets the ones place. The red slot indicates the tenths (whole number) and hundredths place (notches). Each red notch measures 0.02 µL.

In **Figure 3** above, the P200, which has a range of 20 to 200 µL, is set to measure 132.4 µL. The dial contains 3 slots for numbers. The first slot, which is set to 1 in **Figure 3**, sets the hundreds place. The second slot, which is set to 3 in **Figure 3**, sets the tens place. The third slot sets the ones (whole numbers) and tenths place (notches). Each notch measures 0.2 µL.

In **Figure 3** above, the P1000, which has a range of 200 to 1000 µL, is set to measure 262 µL. The dial contains 3 slots for numbers, with the top slot in red. The red slot, which is set to zero in **Figure 3**, sets the thousands place. This slot should never be set to a number other than 0 or 1! The second slot, which is set to 2 in Figure 3, sets the hundreds place. The third slot sets the tens place (whole number) and the ones place (notches). Each notch measures 2 µL.

HOW TO ATTACH A TIP PROPERLY:

i. Find the correct tips. Different models of the micropipetor require different sizes and/or types of tips. If you use the wrong size tip you may not measure the volume accurately. The P20 and P200 use small yellow tips, and the P1000 uses large blue tips.
ii. Leave the tips in their tip boxes.
iii. Firmly attach the tip to the shaft, without touching either the shaft or the tip, as depicted below in **Figure 4**.

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

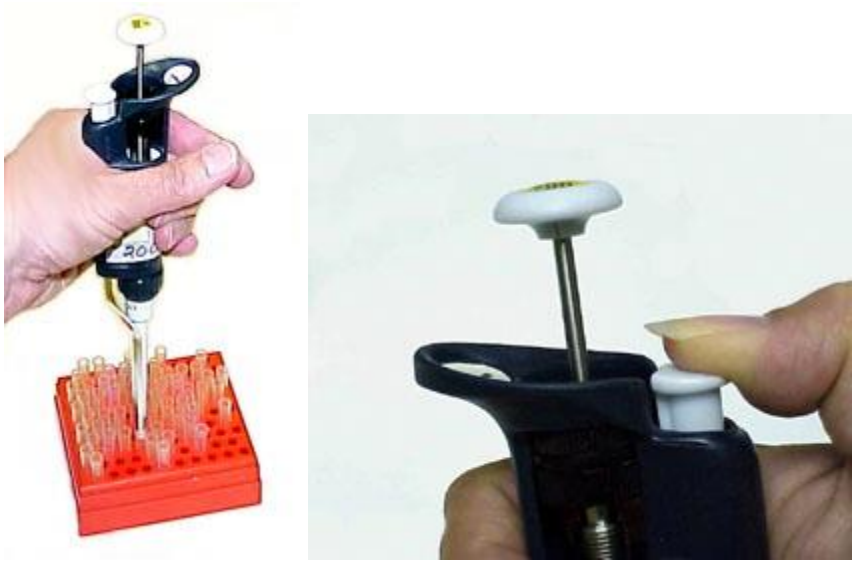


Figure 4 Attaching and removing tips: Left, the proper method for attaching a tip from a tip box. Right, the proper method for using the tip ejector button.

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

- Be consistent with speed and smoothness when using the plunger
- Hold consistent pressure on the plunger at the first stop
- Maintain vertical positioning of the micropippetor
- Avoid air bubbles
- Change tips in order to prevent contamination.
- **NEVER** drop a micropippetor.
- **NEVER** rotate the volume adjuster either below or above the range of the instrument.

- **NEVER** lay a filled micropippetor on its side. (This will contaminate the shaft.)
- **NEVER** immerse the barrel of a micropippetor in a liquid above the tip.
- **NEVER** allow the plunger to snap up when liquid is being drawn into the tip.

Procedure

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

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

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

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

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

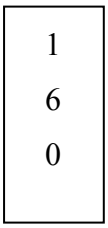
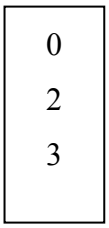
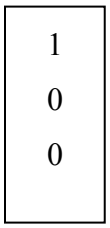
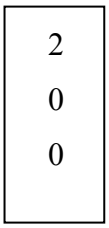
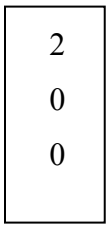
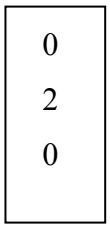
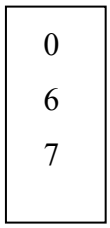
Figure 5. Using the Plunger: Left, the plunger is not depressed. Middle, the plunger is at the first stop. Right, the plunger is at the second stop.

References:

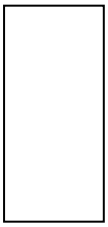
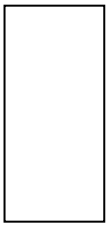
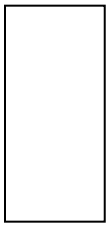
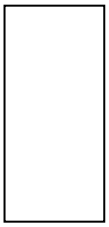
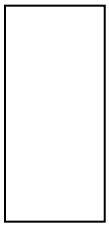
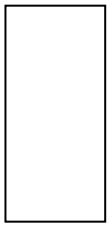
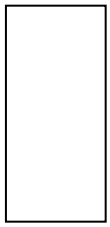
This handout is based on materials from the University of Michigan-Dearborn (http://www.umd.umich.edu/casl/natsci/slc/slconline/MICRPIP/index.html) and from STL Biochimie Genie Biologique (http://stlbgb.apinc.org/spip.php?article8).

PROBLEMS

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

p20	p200	p1000	p20	p200	p1000	p1000
						
_____	_____	_____	_____	_____	_____	_____

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

p20	p200	p1000	p20	p200	p1000	p1000
						
13.6 µL µL	122.6 µL	236 µL	3.7 µL	34.3 µL	689 µL	200
_____	_____	_____	_____	_____	_____	_____

3. Each student should pipet the following volumes of liquid into separate tiny test tubes (microfuge tubes) provided. Select the proper Pipetman, set the volume, and transfer the proper volume into a test tube. Use the smaller tubes, 0.7 mL for the first 5 volumes and the larger tube, 1.5 mL for the last volume.

3.5 µL

15.4 µL

46.5 µL

153.8 µL

522 µL

878 µL

The Use of and Power of Semi-Log Graph Paper

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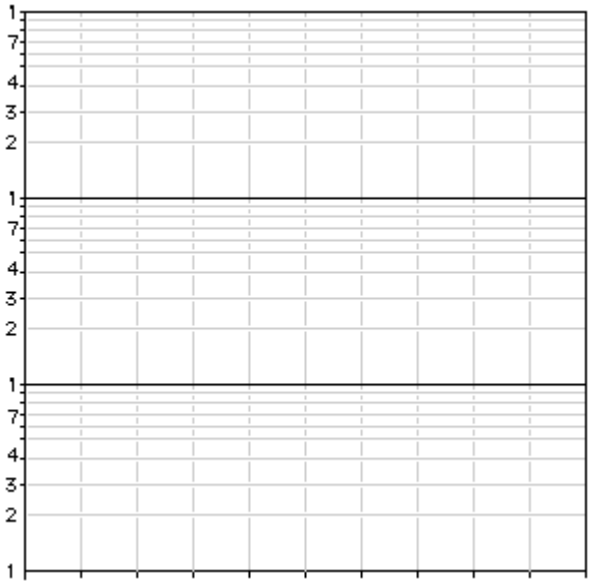
This kind of graph paper allows you to graph exponential data without having to translate your data into logarithms. The paper does it for you! At stationery and university bookstores, you can buy semilog graph paper with anywhere from 1 to 5 or 7 cycles. "Cycles" will be explained in the next paragraph. For those of you who are likely to use many different kinds of graph paper, including semi-log and log-log graph papers, you might be interested in purchasing a book, from which you can photocopy the specific type you need at the moment.

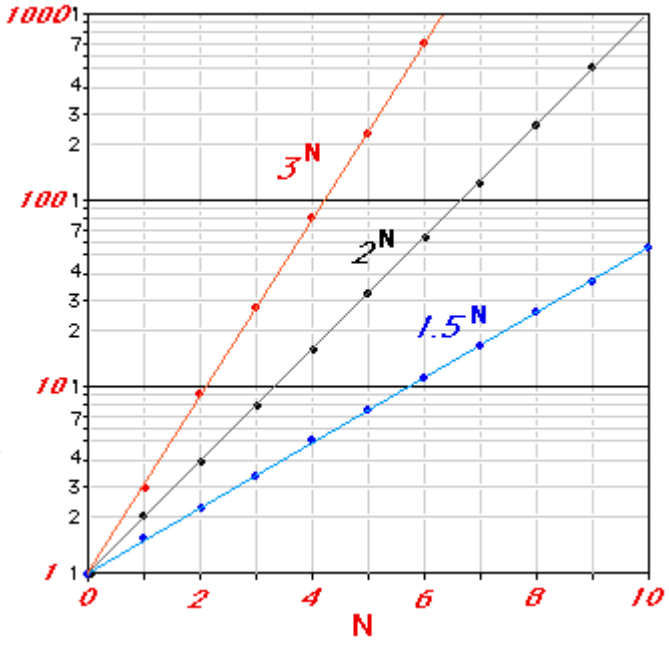
Shown here is what is known as 3-cycle semilog graph paper. You will notice that the vertical axis is very peculiar as the numbers only go up from 1 to 9 and start all over with 1 again, over and over. This is because the distances indicate logarithmic distances. And you will remember that there is no log of zero! (If you want to print out a full-page copy of the graph, click on the graph to the right.)



As shown in the next figure, you MIGHT consider the line across the bottom as equal to one, and the next horizontal line labeled as 1 should be ten, and the next 1 is 100 and the top line is 1000. The lines' basic numeric value may not be changed. The only thing allowed is the placement of the decimal point - and they always differ by only one decimal place per cycle. Thus you might have 0.001, 0.01, 0.1 and 1.0, or you might have 10^5, 10^6, 10^7 and 10^8. So you can see that semilog graph paper can plot both very small numbers as well as astronomical ones.

Let's see how this works and what the supposed power of the semilog graph is all about. In brief it is terrific for plotting anything that is exponential - such as compound interest.

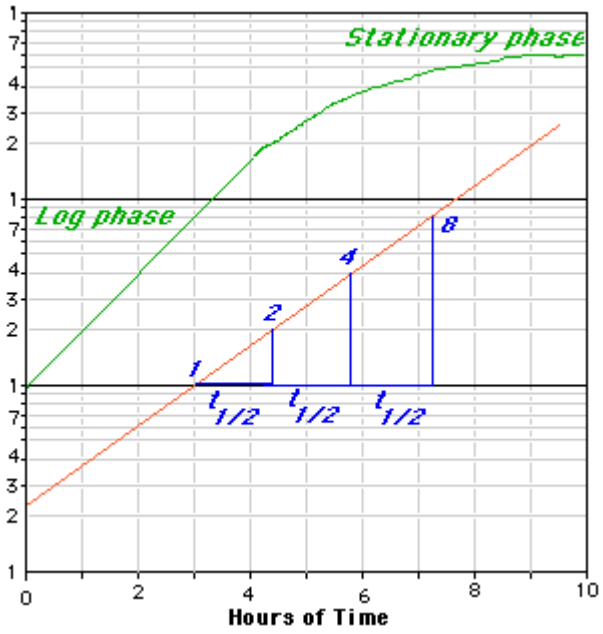
Suppose that you make a series of numbers such that each one is double the one before it (black). (Or you might make each one 3 times the value of the previous one (red); or, for fun, 1.5-times the previous one (blue).) Starting with the series that is of the powers of two (the black line), we find 2^0 (=1) and mark it at the lower left corner; then move to 2^1 (=2), and plot that on the second major vertical line; then move to 2^2 (=4) and plot that on the next major vertical line, and so on



with 2^3 (=8), 2^4 (=16), and so on. And - AMAZINGLY - we get a straight line. Scientists love straight lines! If something gives a straight line on semilog graph paper, we call it an exponential function.

You will see that the 3^x and the 1.5^x also give straight lines. Wonderful! Any type of exponential function can be plotted to give a straight line!

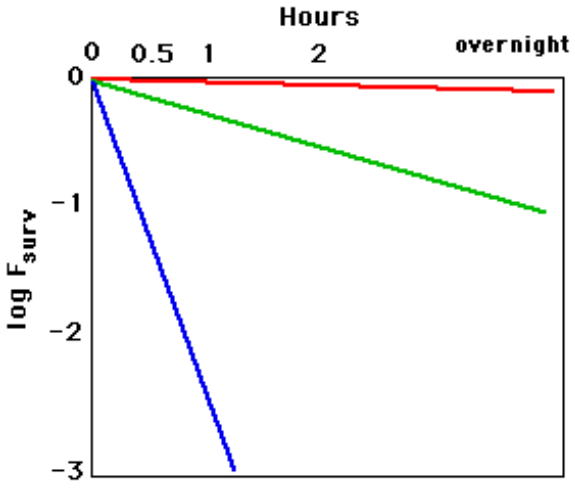
For any values that increase exponentially with time, one can use these graphs to easily determine doubling times (the blue figures associated with the red line). All you have to do is take any point on the red line, and then go move upwards to a point that is double that value and see how much horizontal distance was gained. That is called the doubling time, or $t_{1/2}$. Of course, for convenience, you wouldn't choose a value like 22.86 and have to find twice that. Rather you would choose 1.00 and easily find 2.00, as was done in this diagram. Also shown are some successive doublings to 4 and then to 8. You will notice that the horizontal distances remain the same for each of the sections.



In reality, you are more likely to get data that plot like the green line. This is called a growth curve in microbiology. For a while, in the "log phase" the microbes are growing exponentially (see the straight portion of the line?), but then they begin to slow down because on any number of reasons or combination of reasons - buildup of waste products, exhaustion of food, etc. Soon growth ceases and the line runs horizontally in "stationary phase." Quiz: what is the doubling time for this microbe during exponential growth? (Answer: it doubles about once per hour.) Another name for doubling time is "generation time" or just plain "g".

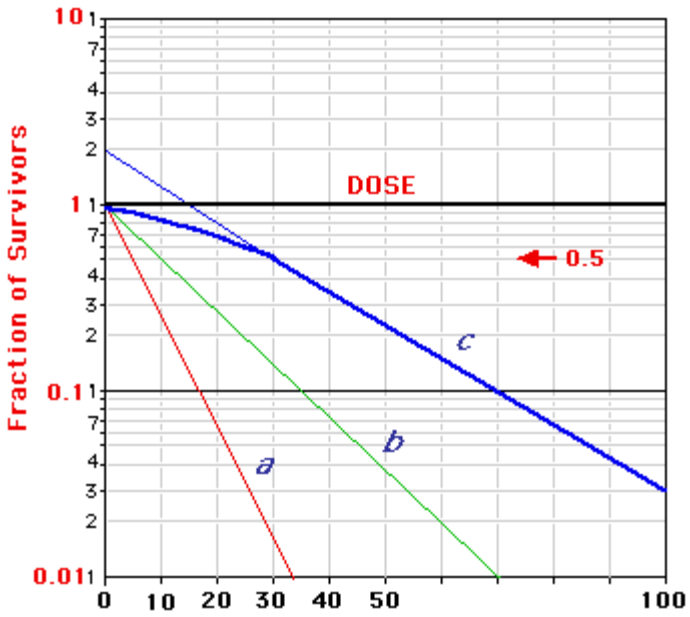
SURVIVAL and Radioactive DECAY CURVES

The difference between growth curves as shown above and survival curves is from which corners the line or lines radiate. Above they have come from or near the bottom left and gone upwards. Survival curves come from the upper left and go downwards. That corner is defined as "1" or 100%. (And remember that the log of 1 = zero!) If you are using three cycle semilog paper, that means the upper cycle goes from 100% to 10%, the middle



cycle is from 10% to 1%, and the lower cycle from 1% to 0.1%.

SURVIVAL CURVES and MINIMUM LETHAL HITS



This collection of "dose plots" or "survival curves" starts with it origin at the red "1". From it arise three plots, a, b, and c. Plot "a" is of the most sensitive critter, while that of "c" is of the least sensitive critter.

Game theorists have proven that if you extend asymptotes from the straight portions of the curves back to the vertical axis, where that line crosses indicates the minimum number of lethal hits.

Let's discuss the meaning of that for a moment. It has been shown in battle scenarios that the average soldier is usually shot several times and still doesn't die - although it is plausible that one bullet in just the right place - brain, heart, can kill. Thus the minimum number of hits to be lethal for a soldier is one. On the other hand, if we talk not about bullets but gamma rays, then the minimum number of lethal hits in a human must be much larger for immediate death. Perhaps there are a critical 1,000 cells in the body such that if ALL of them are knocked out - each by a single gamma ray, then the minimum number of lethal hits is 1,000. Thus if you were to irradiate a large population of people with gamma rays and make your dose plot, you would find the extrapolated asymptote crossing the vertical axis at 1,000. (Again, remember that most people will be hit with millions of gamma rays - but most of them would be in inconsequential places.)

Thus when you look at this figure, you see that both "a" and "b" are critters for which the minimum number of lethal hits is one. But for "c" the line extrapolates to "2", and so that critter requires at least two hits to kill it.

Another consideration that you can derive from these plots is the LD_{50} ("lethal dose for 50%"; the dose that kills 50% of the critters). For "a" the LD_{50} is about 5. You find this by moving down the red line to where it crosses the horizontal 0.5 line and at that point look down at the bottom to see how many hits that indicates.

For "b" LD_{50} is about 10, but you run into a problem with "c" because its curve doesn't start declining immediately. So, for "c", you must go to a straight portion and then determine how many hits it takes to decrease the number to a half. So go down the blue line where it is straight to some convenient place and then measure off how many more hits it takes to drop in half. You should get something like 16 hits.