How to Keep a Lab Notebook

You are keeping a lab notebook so that you:
1. can refer back to your experiments at a later date, if you want to use the procedure in another context.
2. will learn to keep detailed, clear written notes that others can understand and use (such as for a research or medical position) for a career in the future.

Make sure all writing is clear and all objectives, procedures, results and discussion can be read and understood by someone who is not taking this class.

Table of Contents - Save several pages at the beginning of the notebook for the TOC and update it weekly with the title of each experiment, a date, and a page number. **Number all notebook pages now.**

For Each Lab Experiment:
1. **Title of Each Experiment** - Be concise but descriptive.

2. **Date and Page Number** – Number all pages before you begin, include the year.

3. **Objectives** - In two or three sentences summarize the scientific purpose(s) of the exercise. Do not copy the objectives stated in your manual.

4. **Hypotheses** - Concisely state any hypotheses you have before beginning the lab. This is different and separate from the objectives.

5. **Pre-lab Notes** - Notes taken in lab about the experiments and any background introduction about the labs. The section is optional.

6. **Material & Methods** - Include here all experimental procedures followed and any calculations done in reference to the methods.

7. **Data/Results** - All data generated should be recorded during the lab. Include all tables, graphs, formulas, calculations, and photographs. Summarize in a short (one or two sentences) narrative the data obtained.

8. **Questions** - If there are any associated questions with the lab exercise, they should be answered in this section.

9. **Discussion/Conclusions** - What did you do? Why did you do this (e.g. Why did the instructor think it was so important to do this lab)? What did you learn? How did doing the lab help you learn this? Describe the next experiment you would do to study this topic? What questions do you still have about this topic?

If an experiment will take several weeks, skip pages in the notebook to keep the experimental write-up in the notebook together. Your notebook will not be graded down if there are empty pages in it, but will be graded down for poor organization.
All procedures must be described in detail so that another investigator can follow them at a later time. Write the notebook in such a way that you could come back in two years and be able to repeat the experiments and answer the questions again.

It is important that you keep a timely and accurate notebook of your activities in the laboratory. Use the following guidelines for record keeping:

1. Be honest and concise; keep the record factual; do not editorialize.
2. Before starting an investigation/procedure summarize the scientific purpose of the exercise and any hypotheses you have in two or three sentences.
3. Before starting an investigation/procedure summarize the protocols to be used and reference the location of the complete protocol.
4. Enter data as it is collected (do not rely on loose sheets or memory for making entries at a later time).
5. Date each entry and initial each page as it is completed.
6. Do not erase. If an erroneous entry is made, mark it as such, initial it, and then make the correct entry.
7. Attach support records (photographs, instrument printouts, etc.).
8. Label all tables and graphs to indicate what they represent.
9. Choose scales for graph axes that are easy to work with (i.e. 1, 2, 3; not 1.2, 2.4, 3.6, etc.).
10. Show all calculations (such as solutions made or chi-square analysis).
11. All data generated should be recorded during the exercise. Include any tables, graphs, formulas, calculations, and photographs. Summarize in a short (one or two sentences) narrative the data obtained.
12. At the conclusion of each major part of an investigation (and at the conclusion of the entire investigation) outline your interpretations and conclusions about the data obtained. Also make any suggestions for modifications and/or improvements to the process as well as for resolving any problems that may have arisen during the investigation.
13. In this course, you are not being graded on results. Therefore, an accurate representation of those results with an intelligent explanation of them is far more important than that you obtained any specifically desired outcome.

How to Write a Lab Report

Communication in the sciences is critical, and the most prevalent way of communicating results is via the scientific research paper published in a scientific journal. You will follow standard format outlined below to write your lab reports. **Your lab report should be able to be read by another biology student who has not done the study, and it should be clear to him or her as to what was done, why it was done, how it was done, and what the results are. They should be able to take your report and repeat your experiments and your calculations without any further information.**

1. **TITLE AND AUTHORS** - This includes the title that indicates what the study is about and the names of all of the report authors.
2. **ABSTRACT** - The results should be summarized. Background is stated in one or two sentences. There is a clear statement of the questions addressed. Methods are summarized (no more than three or four sentences). The major findings are reported briefly. A concluding statement is made that relates to the statement of the questions asked. It is written as a single paragraph of no more than 250 words. See next section for more.
3. **INTRODUCTION** - This includes background leading to experiments and a logical argument showing how these questions are addressed. All statements of fact and opinion that are not your own are supported by a reference to the source. The introduction should give important information that is necessary for a lay scientist to understand your lab report. The introduction should contain bibliographic references. This is usually two to four pages long.
4. **MATERIALS & METHODS** - The description (in paragraph form) of the methods contains a brief, concise narrative of important details and does not contain extraneous, unimportant details. **These are presented in enough detail that another scientist could pick up your paper and repeat your experimental protocol.** The design of the study (or experiment) is clear and complete. The rationale for each step is self-evident or clearly indicated. Each factor mentioned is likely to have influenced the outcome of the study (if it does not influence the outcome do not mention it). All factors that are likely to have influenced the outcome of the study are mentioned. When the same procedure is used several times, the procedure need only be summarized one time. Subsequently, just refer to that procedure and indicate any modifications made to it. Also include a brief description of how the data is analyzed (calculations made, statistical analyses, etc.). This is one to five pages long.
5. **RESULTS** - This includes a narrative describing what happened as well as all pertinent tables, figures, photographs, gels, etc. All general statements are supported with reference to the data. Major results are explained in the text, with supporting tables, graphs, and/or figures also included. Implications of the results are not discussed. Data are presented in either tabular or graphical form but not both. Each figure or table makes an important and unique contribution to the report. Each figure or table has an informative caption or legend. Each figure or table is self-sufficient; readers can tell what question is being asked, major aspects of how the question was addressed, and what the most important results are without having to refer to the rest of the paper. The sample size and number of samples are clearly indicated in the graph, table, caption, or legend. All calculations are shown in the text (for example statistical analysis of the data) and are explained fully. This is usually three to five pages long.

6. **DISCUSSION** - What does it mean? Are the hypotheses supported? Why did it happen? What (if any) problems occurred? How were those problems resolved? The data obtained are clearly related to the expectations raised in the introduction and to the question(s) asked and/or hypotheses tested. Facts are clearly distinguished from speculation and/or opinion. Unusual, or unexpected, findings are discussed logically, based on biology rather than apology. All statements of fact and/or opinion that are not your own are referenced as to the source. Your own opinions are based on the results and are referenced by an example from those results. A discussion of future directions, additional questions to be addressed, and ways in which the study could be modified are included. **This section does not include how you felt about doing the experiment or a personal note to the instructor.** This section may also contain bibliographic references. This is usually two to five pages long.
7. **RESULTS/DISCUSSION** COMBINED (PREFFERED METHOD FOR GENETICS LAB)- Alternatively, you may combine results and discussion. The results description is the same as 5 and then the discussion in 6 is truncated focusing on each experiment and answering these questions: What does it mean? Are the hypotheses supported? Why did it happen? What (if any) problems occurred? How were those problems resolved? The data obtained are clearly related to the expectations raised in the introduction and to the question(s) asked and/or hypotheses tested. Then you usually wrap up with a future direction paragraph. This is usually three to six pages long.
8. **LITERATURE CITED** - Citations are provided for every reference in the body of the report. Citations are in the correct format according to the type of reference material. No references are included that are not cited in the body of the report. This is usually one page long. When you take a piece of data or an idea from another, you need to give them credit. This is done by placing a note in the text that you received that information from another and then you cite the whole reference at the end. When you cite material, you **paraphrase** what they said. **Do not use their exact words.** This is plagiarism. If you are not sure how to cite references, see your instructor!

<u>More information:</u>

1. TABLES & FIGURES - Each table should have a title and footnotes that explain any information that is not self-explanatory. Figures should have a legend that clearly identifies what each part of the figure represents.
2. CALCULATIONS - Each calculation is fully explained as to why it is needed and what the results mean.
3. INDIVIDUAL ASSIGNMENTS AND HOMEWORK - are to be done by the individual alone. Thoughts and ideas may be shared between people within groups and among the class, but all written work will be done individually with no sharing of quotations, sentences, mathematical problems, or sections of the report.
4. PLAGARISM AND CHEATING - will result in the student or group getting a zero for the assignment and a failing grade for the class.

Also see the External links in Blackboard.

How to Write an Abstract

Generally, the abstract is a brief description of what you did and what you found. Like a paper (or lab report), an abstract should contain an introduction, methods, results, and conclusion (see below). You may also include a sentence or two about why you did whatever you did as well, if you feel it is important. The abstract must not be a summary of your thought processes for writing the report. Do not write "the experiment was completed, the data was analyzed, and conclusions were drawn." Every scientific paper has an abstract at the beginning to let the reader know what the paper is about and to make an informed decision whether the entire paper is worth reading. Abstracts also are printed on the internet, such as in PubMed, where the whole article does not appear, and are used to decide which articles you need to read. A third use of abstracts is to summarize the work you will be presenting at a meeting, so people will know if they should come to see your complete presentation. Since a potential reader may have to sift through a lot of abstracts, the relevance and quality of your work may be judged on the basis of your abstract alone. **Therefore, the abstract must stand on its own**.

The abstract of a paper should be written last, after you have drawn all of your conclusions. It should be clear, concise, and should hold all of your major conclusions as well as the general methods of your study. Remember, in science, the abstract of a paper is usually all your colleagues will read.

1. What is the general topic you were investigating and why is it important? One to two sentences.
2. What are the specific questions you are addressing with this project? The Abstract should not include your complete methods. Provide a one or two sentence overview.
3. How did you do this experiment? For a single paragraph abstract, one or two sentences are needed. You are not trying to be complete, just give a general idea of how you did it.
4. What did you observe? One to three sentences should be enough: state only your main point(s).
5. What were your conclusions? This can be between one to three sentences.

With the method outlined above, you should be able to produce a good abstract in less than an hour. If you haven't clearly and carefully thought through what you did in the experiment, writing the abstract should help you do so.

Also see the External links in Blackboard.

Probability and Mathematical Formulas in Genetics

(*P*) Probability = $\frac{\text{number of times an event occurs}}{\text{total number of events}}$

OR

$$P = \frac{\text{number of offspring with a given phenotype}}{\text{total number of offspring}}$$

In the cross Tt X Tt

	T	t
T	TT	Tt
t	Tt	tt

We expect 1 TT: 2Tt: 1 tt, where T is tall and t is dwarf.

1. What is the probability of a dwarf plant?
   P = 1 dwarf/4 total = 1/4 dwarf or 25%

2. What is the probability of a tall plant?
   P = 3 tall/4 total = 3/4 or 75%

3. What is the probability of a heterozygous plant?
   P = 2 heterozygous/4 total = 1/2 or 50%

The sum rule states to add probabilities when you are looking for two mutually exclusive events.

1. What is the probability of either a homozygous tall or a heterozygous tall in the above cross?
   P = 1/4 + 2/4 = 3/4 or 75% chance of tall

2. What is the probability of either a homozygous tall or a dwarf in the same cross?
   P = 1/4 + 1/4 = 1/2 or 50%

3. What is the probability of either a homozygous recessive or a heterozygote in the same cross?
   P = 1/4 + 1/2 = 3/4 or 75%

When looking for the probability of a *particular order* of events or offspring, use **the product rule,** multiplying the probabilities of each individual event.

1. What is the probability that the first three seedlings in the above cross will all be dwarf?
   P = 1/4 X 1/4 X 1/4 = 1/64 or 1.6%

2. What is the probability that in the first 4 seedlings we will have a tall then a dwarf than a tall then a dwarf?
   P = 3/4 X 1/4 X 3/4 X 1/4 = 9/256 = 3.5%

Use **the binomial expansion** when looking for the probability of *unordered* events.

$$P = \frac{n!}{x!\,(n-x)!}\, p^x\, q^{n-x}$$

n = the total number of events
x = the number of events in one category
p = the individual probability of x
q = the individual probability of the other category

1. What is the probability that in the above cross in 8 seedlings, we will have 3 dwarf (and 5 tall)?

   n = 8 x = 3
   p = 1/4 q = 3/4

   $$P = \frac{1 \times 2 \times 3 \times 4 \times 5 \times 6 \times 7 \times 8}{(1 \times 2 \times 3)\,\{(8-3)!\}}\,(1/4)^3\,(3/4)^{8-3} =$$

   $$\frac{40320\,(1/64)\;\;(243/1024)}{6 \times (1 \times 2 \times 3 \times 4 \times 5)} = \frac{149.50}{720} = 0.208 = 20.8\%$$

2. What is the probability that in the above cross we would get 4 tall plants out of 5 seedlings?

   n = 5 x = 4
   p = 3/4 q = 1/4

   $$P = \frac{1 \times 2 \times 3 \times 4 \times 5\,(3/4)^4\,(1/4)^{5-4}}{1 \times 2 \times 3 \times 4 \times \{(5-4)!\}} =$$

   $$\frac{120 \times (81/256)\,(1/4)}{24 \times (1!)} = \frac{9.49}{24} = 0.3955 = 40\%$$

Use **chi-square** analysis to test the validity of a genetic hypothesis.

Formulate a hypothesis about your data (linked or unlinked genes, inheritance pattern). Then use your experimental data to determine if the hypothesis is accepted or rejected.

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

O = observed results
E = expected results based upon your hypothesis
Σ means to sum the calculations in each category. If there were four categories, then the formula would be worked out as:

$$\chi^2 = \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2} + \frac{(O_3 - E_3)^2}{E_3} + \frac{(O_4 - E_4)^2}{E_4}$$

Then determine the degrees of freedom, which are n – 1 (n = categories or phenotypes). Here it would be 3 (4 -1 = 3).

1. A true breeding tall plant with white flowers is crossed to a dwarf plant with purple flowers. All of the F_1 were tall with purple flowers. The F_1 were selfed and the resulting 1000 F_2 progeny looked like the following:

tall, purple flowers	568
tall, white flowers	187
dwarf, purple flowers	185
dwarf, white flowers	60

In this case we expect a 9:3:3:1 ratio for unlinked genes or 9/16 tall, purple: 3/16 tall, white: 3/16 dwarf, purple: 1/16 dwarf, white. **Where did I get this ratio?** Our expected numbers are:

tall, purple 563 (9/16 X 1000)
tall, white 188 (3/16 X 1000)
dwarf, purple 187 (3/16 X 1000)
dwarf, white 62 (1/16 X 1000) use whole numbers for all

$$\chi^2 = \frac{(568 - 563)^2}{563} + \frac{(187 - 188)^2}{188} + \frac{(185 - 187)^2}{187} + \frac{(60 - 62)^2}{62} =$$

$$\chi^2 = \frac{25}{563} + \frac{1}{188} + \frac{4}{187} + \frac{4}{62} = 0.13$$

df = 3. *P* is smaller than the critical value, see p. 19. This is within our expected range and so we accept our hypothesis that the two genes are unlinked.

2. If we have two Manx cats that are heterozygous (Tt) for the dominant tailless trait, and we cross them, we get this result. In four litters, we obtained:

tailless	19
tailed	13

Our hypothesis is that this trait follows simple Mendelian inheritance. We expect a 1TT: 2Tt: 1tt result. **Where did I get this ratio?**

Our expected numbers are :

tailless	24
tailed	8

$$\chi^2 = \frac{(19 - 24)^2}{24} + \frac{(13 - 8)^2}{8} = \frac{25}{24} + \frac{25}{8} = 4.166 \text{ and df} = 1$$

Our chi-square value is larger than the critical value, so we must reject our hypothesis. The inheritance of this trait does not follow simple Mendelian inheritance. Upon further characterization of these kitties, it is revealed that the homozygous dominant TT is lethal in the embryo stage. So, our Punnett Square would give us 2/3 tailless and 1/3 tailed when figuring the

embryonic lethality of the TT genotype. Throw out the TT offspring and use 3 as the denominator.

So our revised expected numbers are: tailless 21
tailed 11

$\chi^2 = \frac{(19-21)^2}{21} + \frac{(13-11)^2}{11} = \frac{4}{21} + \frac{4}{11} = 0.55$ and df =1

This chi-square value falls within $P = 0.5$ and $P = 0.2$, so we will accept our hypothesis of homozygous dominant embryonic lethal.

A few points about probability (modified from Laurie Caslake, Ph.D.)
Probability is the number of times an event happens divided by the total number of times it could have happened. Probability is also explained in your book.

Sum Rule: The combined probability of two (or more) mutually exclusive events occurring is the sum of their individual probabilities.

'Or' statements indicate the need to add: the probability of a black or brown colt
= P(black colt) "or" P(brown colt)
= P(black colt) + P(brown colt)

'At Least' statements indicate the need to add: the probability of at least three out of five
= P(3 out of 5) "or" P(4 out of 5) "or" P(5 out of 5)
= P(3 out of 5) + P(4 out of 5) + P(5 out of 5)

Product Rule: The probability of the order of independent events can be determined using the product rule.

Statements that indicate the probability of independent events occurring in a certain order indicate the need to multiply: the probability of first a black colt, then a brown colt, then a black colt will be born
= P(black colt) then P(brown colt) then P(black colt)
= P(black colt) x P(brown colt) x P(black colt)

Product Rule: The joint probability of both of two independent events occurring is the product of their individual probabilities.

'And' statements indicate the need to multiply: the probability of a black, female colt
= P(black colt) "and" P(female colt)
= P(black colt) x P(female colt)

Binomial Expansion: The probability of an unordered combination of events can be determined using binomial expansion.

Statements using wording like "the probability of three out of seven" having one trait indicate the need to use the binomial expansion: if two heterozygous individuals have eight children, what is the probability of exactly two being homozygous recessive?
In this case, n = the total number of events = 8

p= the probability of one kind of event (or x) = 0.25 or 1/4 (homozygous recessive)
q= the probability of the alternative = 0.75 or 3/4 (not homozygous recessive)
x= events of the first type (homozygous recessive) = 2

$$P = \frac{n!}{x!\,(n-x)!}\,(p)^x\,(q)^{n-x}$$

Practice Problem:
This problem will not be graded and is not a part of your notebook check. It may show up on the lab final, however. It's for more practice if you need it. Also perform the problems at the end of the chapters.

You cross a true-breeding pea plant with red flowers to a true-breeding pea plant with white flowers. All of your offspring have red flowers. Which gene is dominant? Why? What is the genotype of your offspring?

You then cross the offspring to each other. What ratio do you expect? Why?

You count 1000 plants and look at their flowers. Your results are as follows:
740 red
260 white

Does this follow a simple Mendelian inheritance pattern? Why or why not?

Lab One: Central Dogma of Molecular Biology

Objective: You will utilize models to understand the Central Dogma of Molecular Biology, focusing on the functions of the DNA coding strand, using the codon table, and exploring how proteins fold. You will also analyze the consequences of DNA mutations on the final gene product.

Special thanks to Craig Kohn, Waterford, WI for the use of this lab (used with permission), modified by PAM.

The function of a protein is determined by its shape, and the shape of the protein is determined by its amino acids. Because proteins are smaller than microscopic, we would have a pretty hard time doing a hands-on lab on this topic. However, we can explore proteins in an indirect way through modeling.

Many things in science are explored with models – the scientific method itself is about modeling complex ideas into simpler formats so that we can better understand them. Scientific models may also help us to do things that would otherwise be impossible. For example, there is no way that we could have sequenced the 6 billion bases in the human genome without prior experience with simpler organisms like nematodes with genomes smaller than our own.

A model is a substitute for the actual thing we are studying, but it is also similar to what it represents. It tends to follow the same rules as the actual object, and it provides us with a simpler idea of a more complex process so that we can better understand it.

In this case, you will be using pipe cleaners, beads, and cut up straws to model how proteins fold, and how mutations affect the shape of proteins. **Each group should make at least one normal and one mutated protein.**

<u>There are 3 basic laws of protein folding:</u>

1. **Hydrophobicity** – hydrophobic (*water hating*) amino acids will always try to get to the inside of a protein.
    a. Because our bodies are mostly water, hydrophobic amino acids basically try to 'hide' in this kind of environment; hence proteins generally have a hydrophobic core with the hydrophobic residues buried in the protein.
    b. On the other hand, hydrophilic (*water loving*) amino acids need to be exposed to an aequous environment. Hydrophilic amino acids will try to move as far away from the center of the protein as they can.

2. **Charge** – amino acids can have one of three charges – positive, negative, or neutral.
    a. Like opposite sides of a magnet, positively and negatively charged amino acids try to move toward each other,
    b. Like the same pole of a magnet, amino acids with similar charges (positive and positive, or negative and negative) will try to move as far apart from each other as they can.
    c. Neutral amino acids remain largely unaffected.

3. **Cysteine Bonds** Cysteine amino acid pairs will move toward each other and form disulfide bonds whenever they can.

To model amino acids in a protein, we will use colored beads. Read the hints and tips below before getting started.

1. Hydrophobicity – yellow beads will represent hydrophobic amino acids; orange beads will represent hydrophilic amino acids. As such, all yellow beads should be as far inside the protein as they can, and orange beads should be on the outside whenever possible.

2. Charge – blue beads will be a positive charge and pink beads will be the negatively charged amino acids (for your model, neutral amino acids will not be used). Pink and blue beads should be near each other (if they can); conversely, blues should be as far apart as possible (and similarly for pinks).

3. Cysteine bonds – we'll use green to represent the amino acids cysteine; green beads should form pairs whenever they can.

4. Some amino acids may have multiple beads! For example, Arginine is both positively charged (blue) and hydrophilic (orange). As such, you would use both a blue and orange bead together to represent this amino acid.

Amino Acid	Code		Charge	Hydrophobicity
Alanine	Ala	A	Neutral	Hydrophobic
Arginine	Arg	R	Positive	Hydrophilic
Asparagine	Asn	N	Neutral	Hydrophilic
Aspartic acid	Asp	D	Negative	Hydrophilic
Cysteine	Cys	C	Neutral	Hydrophilic
Glutamine	Glu	Q	Positive	Hydrophilic
Glutamic acid	Gln	E	Negative	Hydrophilic
Glycine	Gly	G	Neutral	Hydrophobic
Histidine	His	H	Positive	Hydrophilic
Isoleucine	Ile	I	Neutral	Hydrophobic
Leucine	Leu	L	Neutral	Hydrophobic
Lysine	Lys	K	Positive	Hydrophilic
Methionine	Met	M	Neutral	Hydrophobic
Phenylalanine	Phe	F	Neutral	Hydrophobic
Proline	Pro	P	Neutral	Hydrophobic
Serine	Ser	S	Neutral	Hydrophilic
Threonine	Thr	T	Neutral	Hydrophilic
Tryptophan	Trp	W	Neutral	Hydrophobic
Tyrosine	Tyr	Y	Neutral	Hydrophobic
Valine	Val	V	Neutral	Hydrophobic

5. Be sure to have a cut up straw (one inch pieces work well) in between each amino acid so that you know where one ends and the next begins! You should use multiple pipe cleaners to fit all of your amino acids!

6. You will begin by creating the mRNA strand (transcription) corresponding to the gene shown in the box below. Remember that for every…
    a. G in DNA you would add a C in mRNA
    b. C in DNA you would add a G in mRNA
    c. T in DNA you would add a A in mRNA
    d. A in DNA you would add a U in mRNA
    e. 5' in the DNA template strand, you would add a 3' in mRNA, and vice versa

7. From your mRNA strand, you will need to create codons; remember that a codon is a group of 3 bases that codes for a specific amino acid. Your codons are read in the 5' → 3' direction, and the frame is determined by the start codon.

8. 8. You will then need to use your codons to determine the amino acid sequence (in the 5' → 3' direction) of your polypeptide (translation).

9. Once you have your order of amino acids, you will need to find your respective beads and assemble them onto a pipe cleaner. It's easier to manipulate if you attach several pipe cleaners together, rather than use one. Again, be sure to separate each bead by a cut up straw! (TERM (STOP) is not an amino acid but a command; it will not have a bead).

10. Finally, you will need to fold your protein. Start by moving your orange to the outside and your yellow to the inside. Then connect your opposite charges and cysteine amino acids (wrap them around each other using the pipe cleaner). Your finished protein should have an 'outer shell' of hydrophilic and charged amino acids with an inner center of hydrophobic amino acids.

11. When you are done, you will also have to create a second & third protein from the same gene after it has been mutated.

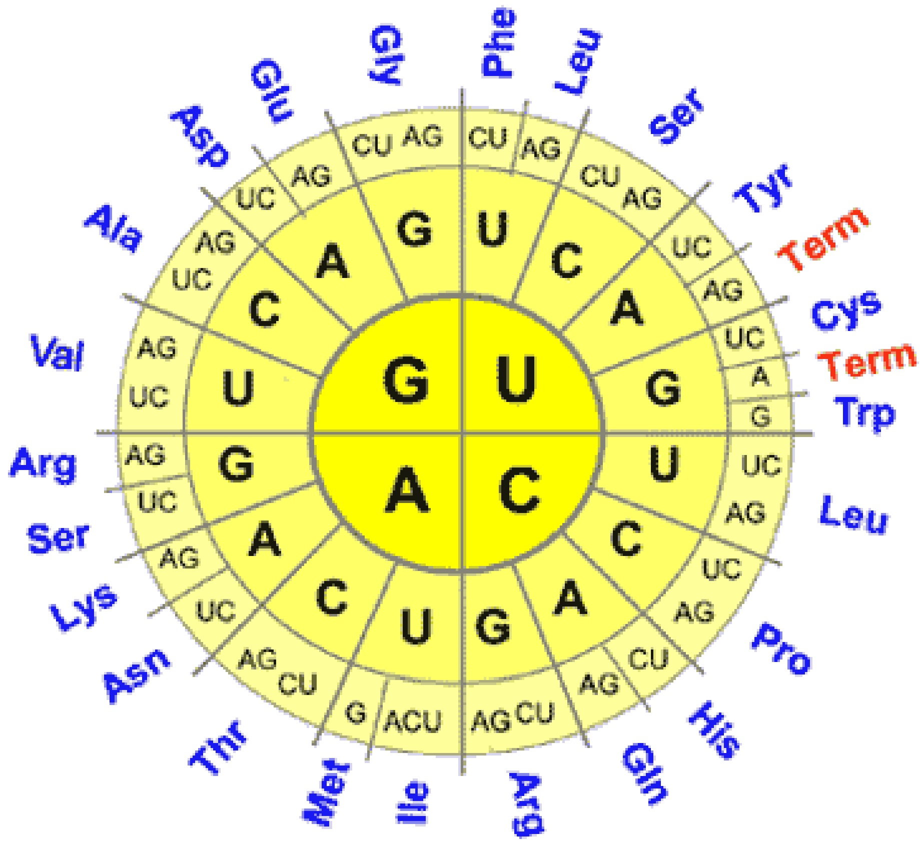
    DNA Template Strand:

3'TACTTACGATGGTACACGCAATCTATACTCAAATATAGGACCTTGACGTCGAATCT
CCACTGTACCTTGAACCTGACT5'

What is the mRNA strand (written in codons, i.e. 3 X 3)?
5'-AUG-AAU- ???

What is the DNA coding strand sequence? How does it relate to the mRNA sequence and to the template sequence?

What is the Amino Acid sequence of the polypeptide? Don't forget NH2 and COOH.



Once you have finished your protein, compare yours with other groups' proteins. They should look similar. Take a picture of your protein with your phone/tablet/etc. You will need to paste in a picture (black and white is fine) of your protein into your notebook.

DNA Template Strand:

3'TACTTACGATGGTACACGCAATCTATACTCAAATATAGGACCTTGACGTCGAATCT
CCACTGTACCTTGAACCTGACT5'

Now create a second, mutated version of your gene by either adding or deleting a base. Write your mutated gene, mRNA, and amino acid sequence below. Then create your mutated protein.

What is the template strand of your mutated DNA?

What is the sequence of your mutated mRNA transcript? What is the coding strand sequence of your mutated DNA?

What is the amino acid sequence of your mutated protein?

Now perform the same translation and folding with the new polypeptide. Take a picture of this mutated protein and place a copy in the notebook.

Finally, you will change one nucleotide in the sequence and perform the same actions. This change must result in a **missense** mutation.

In your notebook you will have template strands, 3 coding strands, 3 mRNA sequences, 3 amino acid sequences, and 3 pictures of folded polypeptides.

Now inspect one other group's frameshift and missense mutation polypeptides. What were their amino acid sequences? COMPARE AND CONTRAST your mutants with theirs. You are encouraged to use images (i.e. pictures) to do this analysis. Why are your polypeptides similar or different in structure?

Questions to be answered in your notebook:

1. Scientists often refer to a polypeptide or protein as a "gene product". Why do you think this is the case?
2. How did each mutation change the structure of your polypeptide? What amino acid/acids changed and how did the resulting polypeptide change?
3. What are mutations "good" for?
4. How do mutations occur naturally?
5. What is an allele?
6. How do mutations and alleles relate in a genetic sense?
7. How does protein folding affect protein function?
8. How does amino acid sequence affect protein function?
9. How does amino acid sequence (primary sequence) dictate 3D protein structure?
10. Can a single nucleotide change lead to a null (nonfunctional) mutant?
11. How can a single nucleotide change in a coding strand lead to no change in the protein structure or function?
12. Why are there two methionines in your original polypeptide?
13. What type of bond do cystines form? Draw this bond.
14. What happens when you fry an egg?

Lab Two: Genetics of Maize

Objectives:
1. Through this laboratory exercise, you will become comfortable testing genetic questions with progeny analysis.
2. You will be able to propose hypotheses.
3. You will be able to test your hypotheses with chi-square analysis.
4. You will become familiar with the implications of epistasis.

Genetic experiments typically span several generations of the organism, which, in the case of maize, would take several years to carry out. In this lab you will be analyzing the results of crosses designed to study the inheritance of several genes that influence kernel color and morphology. You will be receiving the progeny ears of crosses that have been ongoing for several generations.

Methods:
You will have to analyze ears of corn for number and phenotype of progeny.
1. Count the number and type of kernels for each cross. When you count ears of corn, close your eyes and turn the ear of corn over in your hand, choose a row of kernels with your thumb, and open your eyes. Count the number of each type of kernels in this row. Now count the number of seeds of each type in the adjacent four rows (two on each side of the chosen row) and tabulate the entire five rows. This will ensure enough kernels are counted for statistical analysis. **DO NOT WRITE ON THE EARS OF CORN!**
2. Propose a hypothesis based upon the information given in this lab exercise (below and on the next page). This is a proposed ratio of offspring with genotype and phenotype based upon a Punnett Square you perform.
3. Use Chi-square analysis to accept or reject your hypothesis.

The phenotype of maize kernels is governed by many different loci. You will be studying the inheritance of several in this exercise:

R = color in aleurone
rr = no color in aleurone but can see color in endosperm

Su = starchy {smooth looking} (dominant)
susu = sweet {shriveled} (recessive)

C'= color inhibitor (dominant over C) in aleurone
C = required for color production in the aleurone (dominant)
cc = no color production in the aleurone (recessive)

Y = yellow color in endosperm
yy = white endosperm

Epistasis is the interaction between two or more genes to control a single phenotype. If two genes are involved in a specific pathway and functional products from both are required for expression, then one recessive allele in either allelic pair will give the mutant phenotype. For example, in plants:

Gene	*C*		*P*	
Precursor	à	Intermediate	à	Anthocyanin (pigment)

If a pure line pea plant with colored flowers (genotype = CCPP) is crossed to pure line, homozygous recessive pea plant with white flowers, the F_1 plant will have colored flowers and a CcPp genotype. The normal ratio from selfing this dihybrid is 9:3:3:1, but epistatic interactions of the C and P genes will give a modified 9:7 ratio. The following table describes the interactions for each genotype and how the ratio occurs. See the part on epistasis in your book.

Genotype	**Flower Color**	**Enzyme Activities**
9 C_P_	flowers colored; anthocyanin produced	functional enzymes from both genes
3 C_pp	flowers white; no anthocyanin produced	p enzyme nonfunctional
3 ccP_	flowers white; no anthocyanin produced	c enzyme nonfunctional
1 ccpp	flowers white; no anthocyanin produced	c and p enzymes nonfunctional

<u>Monohybrid Cross</u>

Box 1&2. A true-breeding colored aleurone (the outer layer of endosperm), in this case purple, maize line was crossed to a true-breeding yellow aleurone maize line. The resulting ears contained kernels with only colored aleurone. The gene governing this inheritance pattern has been named *R*. *R* is colored and *r* is not colored (yellow). The F_1 colored kernels were grown and the plants were crossed to each other. You have been given the results of this cross. What is your proposed phenotypic and genotypic ratio for the offspring (kernels) of this cross? Perform a Chi-square to prove or disprove this ratio.

Box 10. A true-breeding colored aleurone (the outer layer of endosperm), in this case purple, maize line was crossed to a true-breeding yellow aleurone maize line. The resulting ears contained kernels with only colored aleurone. The gene governing this inheritance pattern has been named *R*. *R* is colored and *r* is not colored (yellow). The F_1 colored kernels were grown and the plants were test-crossed (crossed to a homozygous recessive plant, which thus has yellow aleurone). You have been given the results of this cross. What is your proposed phenotypic and genotypic ratio for the offspring (kernels) of this cross? Perform a Chi-square to prove or disprove this ratio.

Dihybrid Crosses

Box 3&4. Many different genes influence the appearance of the mature dry kernel of maize. Ears of corn containing plump kernels are controlled by a gene called sugary, *Su*. The *Su* gene codes for an enzyme known as starch debranching enzyme. When this gene is in the homozygous recessive condition, less sugars are polymerized into starch resulting in sweeter tasting corn. In addition, this low starch kernel loses more moisture during the drying period and therefore shrivels more than the high starch kernel produced when a dominant allele of *Su* is present. A true-breeding colored aleurone, starchy endosperm maize line was crossed to a true-breeding yellow aleurone, sugary endosperm maize line. The resulting ears contained kernels with only colored aleurone and starchy endosperm. The F_1 kernels were grown and the plants were crossed to each other. You have been given the results of this cross. What is your proposed phenotypic and genotypic ratio for the offspring (kernels) of this cross? Perform a Chi-square to prove or disprove this ratio.

Box 5. A true-breeding colored aleurone, starchy endosperm maize line was crossed to a true-breeding yellow aleurone, sugary endosperm maize line. The resulting ears contained kernels with only colored aleurone and starchy endosperm. The F_1 kernels were grown and the plants were **test-crossed** (that is bred to a true-breeding plant with yellow aleurone and sugary endosperm). You have been given the results of this cross. What is your proposed phenotypic and genotypic ratio for the offspring (kernels) of this cross? Perform a Chi-square to prove or disprove this ratio.

Box 6. The C gene comes in multiple alleles. C is colored and allows the aleurone to be colored in the kernels (if the R allele is in the genotype). The c allele is uncolored and does not allow the aleurone to have a color (even in the presence of the R allele), so the color of the endosperm shows through (yellow or white). The C' allele is a color inhibitor that also inhibits the color of the aleurone (even in the presence of the R allele). C' is dominant over C. C is dominant over c. A true-breeding strain with the color inhibitor (yellow) and with starchy kernels was crossed to a true breeding strain colored (purple) strain with sweet kernels. The F_1 were yellow and starchy. They were selfed (crossed to themselves) and you have been given the results of this cross. What is your proposed phenotypic and genotypic ratio for the offspring (kernels) of this cross? Perform a Chi-square to prove or disprove this ratio.

Box 7. The endosperm of maize can either be yellow or white. This endosperm color is governed by a gene with two alleles, Y has yellow endosperm and y has white endosperm. However, this endosperm color is masked when the aleurone is colored purple. A true-breeding maize line with the color inhibitor and yellow endosperm (C'C'YY) (so they were yellow) was crossed to a true-breeding line with purple aleurone (and white endosperm)(CCyy). The F_1 were crossed and you have been given the results of this cross. What is your proposed phenotypic and genotypic ratio for the offspring (kernels) of this cross? Perform a Chi-square to prove or disprove this ratio.

Box 8. A true-breeding maize line with the color inhibitor gene and the gene for colored aleurone (C'C'RR), which thus had uncolored aleurone and yellow endosperm, was crossed to a true-breeding strain with the color gene, which was recessive for the r allele (CCrr) so the aleurone of this strain was also uncolored allowing the yellow endosperm to show through. The F_1 had yellow kernels as well. These were selfed and you have the results of this cross. What is your proposed phenotypic and genotypic ratio for the offspring (kernels) of this cross? Perform a Chi-square to prove or disprove this ratio.

Box 9. A true-breeding maize line with the genotype CCrr, which thus had yellow endosperm, was crossed to a true-breeding strain with the genotype ccRR, also having yellow kernels. The F_1 had colored kernels. These were selfed and you have the results of this cross. What is your proposed phenotypic and genotypic ratio for the offspring (kernels) of this cross? Perform a Chi-square to prove or disprove this ratio.

For EACH box, you should have

1) a hypothesis
2) a cross
3) a Punnett square
4) data
5) Chi square analysis of your data

Question:
One of the overarching themes of this lab and accompanying class is the Central Dogma of Molecular Biology. What is the Central Dogma and how does this lab fit into the Central Dogma?

How to Use Chi-Square Analysis

With chi-square analysis as applied to genetic problems, the **only hypotheses you can test** are as follows:

1. **Testing "goodness of fit."**
   The ratio of offspring will follow simple Mendelian inheritance rules, such as the results of the cross Tt X Tt will yield 1 TT: 2 Tt: 1 tt.
2. **Testing gene linkage.**
   If two genes are being studied, then **we will always hypothesize that the genes are NOT linked**.

Use the following steps to test your hypothesis:

1. State the hypothesis in detail, specifying the genotypes and phenotypes of the parents and possible progeny by working out a Punnett Square for the cross.
2. Use the rules of probability to make explicit predictions about the types and proportions of progeny that should be observed if the genetic hypothesis is true.
3. Convert the proportions to numbers of progeny. You do this by multiplying the proportions by the total number of progeny.
4. Use the chi-square formula to determine a χ^2 for the data.
5. Determine degrees of freedom for your cross (n - 1).
6. Interpret whether the χ^2 value for the data allows you to accept or reject your hypothesis. If your Chi square value is more than the table value, reject your hypothesis.

χ^2 TABLE

P =	0.05
df	
1	3.841
2	5.991
3	7.815
4	9.488

df = degrees of freedom
P = probability

X-Linked Traits (modified from Laurie Caslake, Ph.D.)

For a X-linked (sex-linked) trait, when you do a Punnett Square, you will need to keep track of the sex chromosome and the allele at the same time. Here's an example:

A woman with normal vision (but a carrier for color blindness) marries a man with normal vision. The cross: X^BX^b x X^BY. What is the genotype of the parental generation and the genotype and phenotype of the F_1 generation?

there are three possible genotypes for females	and two for males
X^BX^B = normal vision	X^BY = normal vision
X^BX^b = normal vision, carrier for color blindness	X^bY = color blind
X^bX^b = color blind	

F_1	phenotype	genotype
	1/4 female normal vision	X^BX^B
	1/4 female carrier	X^BX^b
	1/4 male normal vision	X^BY
	1/4 male color blind	X^bY

Practice Problems:
These problems will not be graded and are not a part of your notebook check. They may show up in the final. They are for more practice if you need it. Also perform the problems at the end of the chapters.

1. What would the proportions be if a colorblind man had children with a homozygous normal woman? What would the proportions be if a colorblind woman had children with a normal man?

2. If a father and a son both have defective color vision, is it likely that the son inherited the trait from his father? Why or why not?

3. In the mosquito *Anopheles culicifacies*, golden body (*go*) is a recessive X-linked mutation, and brown eyes (*bw*) is a recessive autosomal mutation. A homozygous XX female with a golden body (normal eye color) is mated to a homozygous XY male with brown eyes (normal body color). Predict the phenotypes of their F_1 offspring. If the F_1 progeny are intercrossed, what phenotypes and in what proportions will appear in the F_2?

4. A golden bodied male (with normal eyes) was crossed to a brown eyed female (with a normal body color). Predict the phenotypes of their F_1 offspring. If the F_1 progeny are intercrossed, what phenotypes and in what proportions will appear in the F_2? Are they the same as for the above cross?

Mapping Linked Genes

These practice problems will not be graded and are not a part of your notebook check. They are for more practice if you need it. Also perform the problems at the end of the chapters.

1. If genes are linked on the same chromosome, we can calculate how far apart they are.

In diploid eukaryotes using a test cross

$$\text{map distance} = \frac{\text{number of recombinant offspring}}{\text{total number of offspring}} \times 100$$

A. To determine the map units apart two genes are on a chromosome, cross two flies, female heterozygous for aristaless and dumpy wings to a male homozygous for aristaless and dumpy wings.

$$\frac{al\ dp}{+} \times \frac{al\ dp}{al\ dp}$$

Why do we use the female as the heterozygous? How do we know this is on an autosome?

Our experimental results are as follows:

aristaless, dumpy wings	557
normal arista, normal wings	560
aristaless, normal wings	84
normal arista, dumpy wings	83

First we propose a hypothesis that these two traits are unlinked. **Why do we use unlinked?**

Our expected data are as follows: 321 of each phenotype combination. **Where did I get this number?**

$$\chi^2 = \frac{(557-321)^2}{321} + \frac{(560-321)^2}{321} + \frac{(84-321)^2}{321} + \frac{(83-321)^2}{321}$$

$$\chi^2 = \frac{(236)^2}{321} + \frac{(239)^2}{321} + \frac{(-237)^2}{321} + \frac{(-238)^2}{321} = 702.9$$

So we must reject our hypothesis of unlinked genes.

These genes are therefore linked, and I can find out how far apart they lie on the chromosome.

$$\text{map distance} = \frac{\text{number of recombinant offspring}}{\text{total number of offspring}} \times 100 = \frac{84+83}{1284} \times 100 = 13.0\ \text{cM}$$

B. Are the genes bent wings and shaven bristles linked? If so, how far apart are they?

Test cross a heterozygous female: $\frac{bt\ sv}{+}$ x $\frac{bt\ sv}{bt\ sv}$

Our experimental results are as follows:

bent wings, shaven bristles	592
normal wings, normal bristles	589
bent wings, normal bristles	9
normal wings, bent bristles	10

I hypothesize that these two traits are unlinked.
My expected data are: 300 of each phenotype.

$$\chi^2 = \frac{(592-300)^2}{300} + \frac{(589-300)^2}{300} + \frac{(9-300)^2}{300} + \frac{(10-300)^2}{300} = 1125.2$$

I reject my hypothesis; the genes must be linked. Map units = $\frac{19}{1200}$ X 100 = 1.6 cM

Practice problem:
Are the genes black and purple linked? If so how far apart are they?

A homozygous wild type fly is crossed with a homozygous black (black bodies) purple fly (purple eye color). The F_1 females are test crossed to a black, purple fly. The following data were obtained.

black	32
purple	28
black, purple	485
wild type	512

Three Point Crosses

You can determine if three genes are linked on the same chromosome. Here is one method for doing so. Your book has a variation on this. The results will be the same.

1. Cross two flies, true breeding for three different traits.

2. Test cross the F_1 females.

3. Group offspring according to phenotypes in the F_2. First group in threes, then in twos.
   The parental class will be the most frequent.
   Then the single crossovers.
   The double crossovers will be the least frequent.

4. Use the double crossover to determine the gene in the middle. In the double crossovers, the least frequent phenotype, only one gene will differ from the parental. This becomes the gene in the middle. Write out the three possible orders that you could get with the genes. Determine which order would give you the combination of phenotypes you observe in the double crossover data. There will only be one possible order of genes that give you that phenotype.

5. Now write down the gene order you have determined and calculate the distance between pairs of genes.

6. Draw the map.

Example:

1. I cross a D wild type to a G that has scarlet eyes, ebony body, and spineless bristles.

   $\underline{st^+ \ e^+ \ ss^+}$ x $\underline{st \ e \ ss}$

   $st^+ \ e^+ \ ss^+$ $\quad$ st e ss

All of the progeny will be heterozygous for each allele.

2. I cross D heterozygote from the F_1 to a G that has scarlet eyes, ebony body, and spineless bristles.

   $\underline{st^+ \ e^+ \ ss^+}$ x $\underline{st \ e \ ss}$

   st e ss $\quad$ st e ss

3. The F_2 looked like this:

wild type	347
ebony	68
ebony, scarlet	10
ebony, spineless	67
ebony, scarlet, spineless	368
scarlet	54
scarlet, spineless	78
spineless	8

Reassort phenotypes:

Parental: parentals are the parental phenotypes and are the most if the three genes are linked.

red eyes, tan body, normal bristles	347
scarlet eyes, ebony body, spineless bristles	368

Single crossovers: are the second most numerous in three gene linkage.

red eyes, ebony body, normal bristles	68
red eyes, ebony body, spineless bristles	67
scarlet eyes, tan body, spineless bristles	78
scarlet eyes, tan body, normal bristles	54

Double crossovers: will be the least numerous.

red eyes, tan body, spineless bristles	8
scarlet eyes, ebony body, normal bristles	10

Reassort genotypes again:

red tan	347 + 8 = 355
red ebony	68 + 67 = 135
scarlet tan	78 + 54 = 132
scarlet ebony	368 + 10 = 378
	Total 1000

red normal bristles	347 + 68 = 415
red spineless	67 + 8 = 75
scarlet normal	54 + 10 = 64
scarlet spineless	368 + 78 = 446
	Total 1000

tan normal	347 + 54 = 401
tan spineless	78 + 8 = 86
ebony normal	68 + 10 = 78
ebony spineless	368 + 67 = 435
	Total 1000

4. scarlet spineless ebony
   spineless ebony scarlet
   ebony scarlet spineless

$\underline{st^+ \quad ss^+ \quad e^+}$	$\underline{ss^+ \quad e^+ \quad st^+}$	$\underline{e^+ \quad st^+ \quad ss^+}$
st ss e	ss e st	e st ss

What are the phenotype and genotype of the double crossovers?
red eyes, tan body, spineless bristles AND scarlet eyes, ebony body, normal bristles

What gene is in the middle? Spineless is in the middle. These two double crossovers have two of the parental phenotypes each and then there is the "odd man out." This odd man out is the gene in the middle.

So, the gene order is scarlet spineless ebony, and the genotype of the F_1 female is

$\underline{st^+ \quad ss^+ \quad e^+}$
st ss e

5. The gene order is scarlet spineless ebony.
Map distance between scarlet and spineless is $\frac{75 + 64}{1000} \times 100 = 13.9$ cM

Map distance between spineless and ebony is $\frac{86 + 78}{1000} \times 100 = 16.4$ cM

Map distance between scarlet and ebony is approximately $\frac{135 + 132}{1000} \times 100 = 26.7$ cM

6. Map:

st 13.9cM ss 16.4cM e
| | |
st 30.3cM e

Practice Problem:
This problem will not be graded and is not a part of your notebook. It's for more practice if you need it. Also perform the problems at the end of the chapters.

This is the cross: Cc Ss Bb x cc ss bb
Two flies were crossed, one heterozygous for the **dominant traits** curly wing, short bristles, and blue body and the other **homozygous recessive** for straight wings, long bristles, and tan body. These three genes are linked. The progeny were as follows:

c S B	normal, short, and blue	13
c s b	normal, long, and tan	473
C S b	curly, short, and tan	9
C s B	curly, long, and blue	1
C s b	curly, long, and tan	15
c s B	normal, long, and blue	9
CSB	curly, short and blue	479
c S b	normal, short, tan	1

Which are the parentals? Which are the single crossovers? Which two are the double crossovers?

How far apart is wing shape and bristle length?

How far apart is wing shape and body color?

How far apart is bristle length and body color?

Map the three genes in relation to each other.

Lab Three: Genetic Experiments with *Drosophila*

Modified from Carolina Biological Supply Company: *Carolina Drosophila Manual*

Objectives:

You will be issued three different stocks of *Drosophila* of "unknown" genetic constitution for the purpose of making controlled matings to:

1. determine the inheritance of the traits found in the stocks. It will be necessary that you ascertain the genetic variation for wing type and eye color in the original stocks by describing the variation found (use drawings if needed).
2. determine the inheritance of the different forms of each trait (dominant or recessive, X-linked or autosomal).
3. describe the genes responsible for the traits. You will describe linkage relationships between these genes and/or linkage on the sex chromosomes.

Theoretical *Drosophila* Assignment

Using information obtained from your book, lecture notes, Mapping Linked Genes, and this lab, perform the following assignments in your lab notebook before coming to class. This assignment will help you to get oriented as to what we are doing in lab and why! I helped you out on the first two.

1. Given two vials of flies, one with all wild type characteristics and one with crimson eyes (crimson) and no wings (wingless), design a series of experiments to determine:

   A. if crimson and/or wingless are dominant alleles.

   Answer: cross the flies with crimson eyes and no wings to wild type and look in the F_1 generation. What are the phenotypes in the F_1? The dominant traits show up in the F_1.

   B. if crimson and/or wingless are X-linked alleles.

   Answer: perform reciprocal crosses (i.e. male wild type crossed to female crimson, wingless and male crimson, wingless crossed to female wild type) and see if you get the same results in the F_1 in both crosses or if the results are different. If the results are different, then the gene or genes are X-linked.

   C. if crimson and wingless are linked genes or if they assort independently.

   D. the map units between crimson and wingless, if crimson and wingless are linked.

2. Given three vials of flies, one with all wild type characteristics, one with sepia eyes (brown in color) and vestigial wings (stumpy wings), and one with an ebony body (black in color) and sepia eyes, design a series of experiments to determine:

   A. which are the dominant alleles.

   B. if any alleles are X-linked alleles.

   C. if any of the genes are linked genes or if they assort independently.

   D. the map units between linked genes, if you find any genes are linked.

3. Given three vials of flies, one with sepia eyes, one with wrinkled wings, and one with apterous (no) wings, design a series of experiments to determine:

   A. which are the dominant alleles.

   B. if any alleles are X-linked alleles.

   C. if any of the genes are linked genes or if they assort independently.

   D. the map units between linked genes, if you find any genes are linked.

Fly Experiment Timeline/Outline and Goals for Each Step

This lab will take several weeks and it can get confusing as to what we are doing and why. This is an outline/timeline of each step and the goals for each step. Hopefully this will keep you on track as well as help you stay organized so that you may easily and effectively write your lab report.

Ultimate goals:

1. Determine if the mutants genes are
    A. dominant or recessive
    B. autosomal or X-linked
    C. either gene combination is linked
2. If genes are linked, determine how far apart they are.

How do you do this?

We will cross wild type flies with mutant flies, mutant for two phenotypes. This will produce F_1s. The F_1s will be analyzed to determine which genes are dominant and which are recessive and if the genes are autosomal or X-linked. Then the F_1s will be allowed to sib-mate (in new vials) to produce F_2s. The F_2s will be sorted by phenotype and counted to determine if the genes are linked.

Fly Experiment Week One

1. Make 2 vials of media (per group).
2. Observe phenotypes and sexes of flies.
2. Place in each vial parental flies for one cross.
    Female virgin wild type X male sepia vestigial
    Female virgin wild type X male black vestigial

Goals

1. Learn basic fly techniques, like making media, fly manipulation, and fly husbandry.
2. Set up parental crosses to produce heterozygous offspring.

Week Two

1. Anesthetize parents.
2. Double check phenotypes and sexes.
3. Place in morgue.

Goals

1. Ensure flies placed in vials were what you wanted.
2. Remove parents so that they are not mistaken for F_1 flies when the F_1s emerge, nor will they mate with the F_1s.

Week Three

1. Make 6 vials of new media (per group).
2. Clear all vials of adult flies and anesthetize F_1 flies.
3. Check for phenotypes of F_1.
4. Transfer F_1 flies to a new vial with fresh media. Make one vial for each to ensure you will have enough F_2 flies for counting.
5. Save old vials in case of experimentor error.

Goals
1. Determine if mutant genes are dominant or wild type.
2. F_1s are transferred so that they can lay eggs for you to harvest F_2s.

Week Four
1. Remove all flies from the second set of vials.

Goals
1. Remove adult flies so you will not confuse them with the F_2s.

Week Five
1. Clear all vials of flies.
2. Anesthetize, sort, and count them, by phenotypes.
3. Analyze data for X-linkage and gene linkage.

Goals
1. Use the data from the F_2s to determine chromosomal loci (X-linked or autosomal) and gene linkage.
2. If linked, determine how far apart the genes are. Use Z value.

The *Drosophila* Life Cycle

The fruit fly (*Drosophila melanogaster*), which you will use for these experiments, is a typical insect. It goes through four stages in its life cycle: egg, larva, pupa, and adult. The complete reproductive cycle from egg to hatching adults is short; the average period is about 13 days with a range of approximately 10 to 20 days depending upon the culture temperature. Pure-breeding mutant stocks for many traits are available for experimental genetic studies. This fact, combined with a short reproductive cycle, the ease of handling, and low cost, makes them an excellent laboratory subject for experimental genetics. The same laws of heredity that are demonstrated in the fruit fly also apply to larger, more complex organisms.

Drosophila flies are ready for mating within about ten hours after emerging from the pupa case. So, the male can fertilize a female anytime after she is ten hours old. After a short courtship in which the male circles the female while vibrating his wings, the female spreads her wings laterally and insemination takes place. Sperm is received in seminal receptacles within the female and is used to fertilize eggs laid by the female for her entire life. As sperm from the first mating is still present at the second mating, it is not possible to cross the same female to different males and get accurate results.

The eggs are usually fertilized at the time of laying, and early embryonic development takes place within the egg case. The female will not lay eggs in any quantity until about 24 hours after a mating. Thereafter, she will lay from 50 to 100 eggs per day. You can sometimes see the eggs on the surface of the food and especially around the edge where the food joins the vial. The eggs are usually present within a day after the flies have been placed in the vial. They are only about 0.5 mm long and bear 2 filaments at the anterior end. These prevent eggs from sinking into the soft food. The eggs must have contact with air and will drown if immersed for long periods.

Within 2 days after the eggs have been laid, very small larvae will hatch out and crawl in the media. They are so tiny that you may have difficulty seeing them except with a

stereomicroscope. This larva represents the first instar. They will eat almost all the time and double their size about every 24 hours. After its first molt (shedding of its outer skin), the larva will form the second instar. They will eat, grow, and molt again to form the third instar, which will be about 5 mm in length if grown on food rich in yeast. The larval stages feed in the media for about 6 days and then crawl up on a dry spot to pupate.

The third instar will pupate after about 6 or 7 days after hatching under ideal growing conditions. The larvae crawl up out of the food and adhere to a relatively dry surface, which may be the side of the vial or a piece of netting inserted into the food. The soft larval skin dries and gradually develops a brown pigmentation. It is now known as a pupa case, and within this, the metamorphosis from the larval to the adult form takes place. It is easy to tell when the time for emergence is near. The pupa case is a rather dark brown and the folded wings can be seen as two elongated dark areas within. Also, the pigment of the eyes will be developed and can be seen as two bright red spots (in the case of the wild type flies). Emergence is to be expected within 24 hours when such characteristics can be seen. The adult emerges by forming its way through the anterior end of the case. The newly emerged fly will be very long, relatively unpigmented (light color), and with folded wings (the adult will be able to fly about 2 hours after hatching). It will darken rapidly, however, the wings soon unfold, and the body becomes more compact.

Methods:

Records

The success of any experiment depends upon a complete, concise and workable system of records. All original records should be kept in your notebook. No records are to be kept on loose sheets of paper. In case of a mistake in the notebook, cross it out; do not erase. This lab will take several weeks to finish. Skip several pages for this in your notebook in order to keep all of the fly work together. This makes it easier for you to keep your notebook organized.

It is necessary to make observations only on those characteristics to be studied in your experiment. In this experiment, three pairs of traits will be followed: eye color, body color, and wing type. A suggested chart format for these records is included. Be sure that you are certain of the differences involved in your flies and that you classify all flies completely. Before you start your experiment, plan a system of records for your experiment and also devise a crossing procedure.

Each controlled mating that you make should be assigned a mating code. For each mating, record the dates of mating, transferring and counting in your notebook. The minimum you will need to do to complete this experiment is:

1. describe the phenotype of the male and female flies of the original stock cultures;
2. make controlled matings between each stock culture and the wild type flies;
3. describe the F_1 generation from these matings, tabulated by sex and phenotypes;
4. sib mate F_1 generation flies from the same controlled mating;
5. describe the F_2 generation from the sib matings, tabulated by sex and phenotypes;
6. determine if any of the genes is linked;
7. and if so map them by using F_2 data to calculate a Z value.

Preparing Media
You will be given *Drosophila* vials. We will use an instant culture media. The medium, which has a white, flaky appearance, is dehydrated and already contains a mold inhibitor and does not need cooking or sterilization. We will use the media with an additive that turns the medium blue when dissolved in water; this allows easier observation of some of the developmental stages of *Drosophila*.

To prepare a culture, obtain a clean plastic culture vial from the bin labeled food vials. Place 10 - 15 ml fly media in the vial. **Add ONLY 3 grains of yeast. Too much will produce carbon dioxide and kill the flies.** Next, add the same amount of water to the culture vial. You may need to lightly tap or gently swirl the culture vial so the medium becomes rehydrated. The medium will be quite fluid at first, but within a few minutes it will become more solidified and will be ready to use. **Your media should be the consistency of gooey instant mashed potatoes.** Add a piece of netting and a foam stopper.

Handling *Drosophila*
As you first look at the tiny *Drosophila* given to you for study, you may think that handling such tiny creatures will be very difficult. With a little practice in the proper techniques; however, you should find it relatively simple. Certainly, you can tell little about them with the naked eye, so some means of magnification must be used. You will use a stereomicroscope with a built in light. The flies show best against a white background, so you will use a white card upon which to place the flies. The microscope lights get hot, and this can kill the flies, so use the lowest light setting and be very judicious with your use of the microscope light.

1. Anesthetizing Procedure
Anesthetizing is necessary to quiet the flies so they can be studied and transferred. We will use FlyNap®. With relatively short exposure times, the flies remain anesthetized for 30 to 45 minutes. Young flies may remain anesthetized for several hours. Thus, the flies should not begin to recover while they are being examined, which virtually eliminates the need to reanesthetize them.

Transfer the flies to an empty culture vial. These are in the bin labeled FlyNap vials. To get flies in an empty culture vial, invert the culture vial. Since the flies are negatively geotropic, they tend to crawl up and away from the stopper. Flies that remain near the stopper can be shaken away by turning the vial upright and tapping the vial down on a soft surface, **though not so hard as to lodge the flies into the medium**. When the stopper is removed, the flies can be easily shaken down into the anesthetizing vial. Immediately plug both vials.

Dip an anesthetic wand into the FlyNap ® bottle and allow any excess liquid on the wand to drip back into the bottle. Then place the wand into the vial with the flies you want to anesthetize. The FlyNap® will evaporate into the atmosphere of the vial. Within a few minutes (about one minutes in an empty vial) the flies will be anesthetized and can be manipulated. Pour the flies out of the vial onto a white card and place under a microscope. **Do not overanesthetize your flies because they will die!**

2. Techniques for Microscopic Study

Use a soft brush to brush the flies up into a narrow column across your white card. This card can be moved back and forth under the microscope to examine the different flies in the column. To manipulate the flies while viewing them, use the brush. Practice moving the flies about with the brush. Turn them over and become familiar with every part of the body. Note the color of the eyes, the wing type, and the body color. If you have any flies in your original stock cultures that appear to have a much lighter body shade that the other flies, it probably means they are newly emerged. It requires about 2 hours before they attain their full pigmentation. Also, if you have one fly that has just emerged its wings may still be folded tightly. You might think that you have found some strange new mutation, but within a short while the wings will be expanded. If you see some flies with the wings folded together over the back and the abdomen curved downward, throw them away, they have been overanesthetized and are dead.

3. Distinguishing Sex

It is very important you learn how to identify the two sexes of *Drosophila*. If, because of mistaken identification, you allow a male to go into a vial with a group of virgin females that are later used in a genetic cross, the results will not be accurate. As you move a male and female side by side into view, note the distinctions. From the dorsal surface, the most noticeable sex difference is the pigmentation at the posterior part of the abdomen. The male has heavy pigmentation on the entire posterior part of the abdomen with two pigmented bands anterior, while the female has five bands of pigment along the entire abdomen. This characteristic is very clear-cut and easily distinguished in mature flies, but in newly emerged flies, the amount of pigmentation is much less and this difference is not so clear-cut. The posterior tip of the abdomen of the female is somewhat pointed, but is rounded in the male. The male genitalia are surrounded by heavy dark bristles that are absent in the female. The males also have a pair of dark, bristle-like sex combs, on the front legs. Males, as a whole, are noticeably smaller than females raised under similar conditions, but there can be some overlapping of size even in flies from the same culture.

4. Starting *Drosophila* Cultures

Make up vials with media as needed. Remove the vial plug and holding the vial on its side, use the brush to move the flies into it. IMPORTANT - keep the vial on its side after you replug it until ALL the flies are awake. If you turn it upright, the flies will fall on the sticky surface of the food and usually cannot escape, so you will lose most, if not all, your flies, if you turn the vial upright too soon. Once they are awake, however, they can take care of themselves and there is no problem.

Your cultures will grow very well at room temperature (preferably above 21 ºC and below 25ºC). Extremes of temperatures should be avoided. If the temperature goes above about 30C for several hours, the flies may become sterile and you will get no offspring. You cannot put them in a refrigerator. At temperatures below about 13 ºC, the flies become inactive and will begin dying after several days at these temperatures. Culture vials should NOT be exposed to direct sunlight and may be kept entirely in the dark. We will keep ours in a controlled environment chamber.

5. Collecting Virgin Females

As a geneticist, it is important to have controlled matings for your experiments, so it is necessary to obtain females before they have mated with males. Traditionally, we can take advantage of the fact that flies are not ready for mating until about ten hours after emerging from the pupae cases. So, if a vial is emptied of all flies, the females that emerge in the next ten hours will be virgins (i.e. have not been inseminated). They can be separated from their male siblings and used for controlled genetic crosses. As a margin of safety, it is best to use eight hours as the maximum time to leave the flies together in the vial. Usually, to collect virgin females for matings:

1. select a vial where new adults are emerging;
2. remove and discard ALL adult flies from the vial;
3. make note of the time when the flies are discarded;
4. return no more than 8 hours later and remove adults that have emerged;
5. separate those adults into males and virgin females;
6. set up vials for controlled matings.

Females will be ready for mating somewhat earlier than males, so be sure than an older male is not left in the vial. Females tend to emerge sooner than males raised in the same vial. Hence more virgin females can be obtained from a vial that has just begun producing adults than from an older culture. So it is a good idea to use vials that first start producing adults when collecting virgins. Also, you will find that most of the flies will emerge during the early part of the day. You will probably get 4 times as many flies from a vial that was emptied at 8 AM and the flies collected at about 4 PM than you would if the vial was emptied at 10 AM and the collection made at 6 PM. You may keep the virgins in isolation for several days in prepared vials with food before mating them.

Flies will die within a few hours in a dry vial. Also, do not be disturbed if you find eggs in a vial of supposed virgins. Virgin flies, like virgin chickens, will lay eggs, especially after they have been kept for several days without mating. Should you find small wiggling larvae in a vial of supposed virgins; however, you have made a mistake and they were not virgins or a male was not excluded.

***Our wild type flies are of a genetically engineered strain with an apoptosis inducing gene under a heat shock promoter on the Y chromosome. The supplier heat shocks the vial and then sends it to us. This kills all XY males and we are left with only virgin XX females and XO males.

Crosses

1. Making the Original Cross and the Reciprocal Cross

Reciprocal cross analysis is used to determine which alleles are dominant or recessive, if the genes follow simple Mendelian inheritance, and if the genes are autosomal or X-linked. Select virgin females from one of your true breeding stocks and place 3virgin females in each of two newly prepared media vials (laid on their side). In the same vials, place 3-6 males of the other true breeding stock. Mark the date and type of mating on the bottle. For example - 9/12/99, 3 Females A X 3 Males B, with the female designated on the left and the male on the right. The flies will mate soon after recovering. We should make the cross in the reciprocal (opposite)

direction (i.e. - 9/12/99, 3 Females B X 3 Males A). However, we will not since the strains are not available. **Why do you use virgin females? Why do you use true breeding stocks?**

After 7 days, anesthetize the parent flies in the vials, check the parent flies for each vial for phenotype (just to be sure), and discard them in the fly morgue. By this time, larvae should be evident in considerable numbers in the media of the vials. If you do not see larva, repeat the crosses until you have F_1 larva.

2. The F_1 Generation

About 12 days after the date of the original mating, F_1 flies should begin to emerge. Separate these F_1 flies by sex and record the observed phenotypes. **If the genes are autosomal what do you expect? If the genes are X-linked what do you expect? How can you tell which traits are dominant and which are recessive?**

3. Sib Matings

The results from the sib-mate will tell you if the genes are linked to one another or if they are independently assorting. Place at least 6 pairs of F_1 flies from the original cross in one fresh media vial and at least 6 pairs of F_1 flies from the reciprocal cross in one different fresh media vial. Because the F_1 flies are to be sib mated, the flies do not have to be virgins. Mark the vials with the date, the type of original mating, and the generation. Each of these bottles will produce about 250 flies for the next generation. Remove the F_1 flies after about 7 days. **What are your expected results from this cross if the genes are unlinked?**

4. Test Cross

The results from the test cross will also tell you if the genes are linked to one another or if they are independently assorting. Test crosses are the **preferred** way to determine map units between linked genes. A test cross is set up with a virgin female F_1 and a homozygous recessive male fly. We will NOT perform a test cross as F_1 virgin females are hard to come by. **What are your expected results from this cross if the genes are unlinked?**

5. F_2 Generation

In about 12 days after the F_1s were transferred or the test crosses were set up, the F_2 generation will begin to emerge. You need at least 100 flies from each cross, preferably over 200, to do the calculations you need for this project. Sort and count each fly based upon phenotypes. If you do not have 100 flies, request data from other groups and add this data to yours. Acknowledge them in you write up and in your notebook.

Cleanup

You are responsible for cleaning up your mess in the lab room and the area you are assigned for keeping your flies. Follow your instructor's directions on clean up.

Data analysis

1. Propose your hypothesis (unlinked genes, autosomal, Mendelian inheritance).
2. Perform a Chi square analysis using the F_2 data to accept or reject your hypothesis.
3. If your hypothesis is rejected, determine why (i.e. linkage, X-linkage, extensions to Mendelian inheritance, or nonMendelian inheritance).
4. If your genes are linked, you need to map the genes.
5. To map our genes we will calculate a Z value and use a look up table. This is LESS ACCURATE than using the test cross data, but it will suffice.

   A. Figure out phenotypic numbers for the cross:

      a is the number of flies with both dominant phenotypes
      b is the number of flies with one homozygous recessive phenotype
      c is the number of flies with the other homozygous recessive phenotype
      d is the number of flies with both homozygous recessive phenotypes

   B. Figure out, do we have a coupling cross or a repulsion cross?
      A coupling cross is:
      P X P
      double dominant true breeding phenotypes x double recessive true breeding phenotypes

      A repulsion cross is:
      P X P
      one recessive true breeding phenotype X the other recessive true breeding phenotype

   C. Use this equation for a coupling cross

      $\frac{b * c}{a * d} = Z$

      Use this equation for a repulsion cross

      $\frac{a * d}{b * c}$ = Z value

6. Then use table on the following page to find the approximate map units for the cross. Look in the appropriate column and then look over to the left to get the Crossover Value. table taken from
http://lifesci.rutgers.edu/~mcguire/Toolbox-Demo/F2%20crosses/calculation_of_recombination_fro.htm

7. Multiply the Crossover Value by 100 to get the map units. Ta da. You have mapped your genes from the F_2 data.

Table for Calculating Linkage Intensities (Percentages of Crossing Over) from F_2 Data

Cross-over Value	Ratio of Products $\frac{ad}{bc}$ (Repulsion)	Ratio of Products $\frac{bc}{ad}$ (Coupling)	Cross-over Value	Ratio of Products $\frac{ad}{bc}$ (Repulsion)	Ratio of Products $\frac{bc}{ad}$ (Coupling)	Cross-over Value	Ratio of Products $\frac{ad}{bc}$ (Repulsion)	Ratio of Products $\frac{bc}{ad}$ (Coupling)
.005	.00005000	.00003361	.205	.09351	.08140	.405	.5079	.5007
.010	.00020005	.0001356	.210	.09865	.08628	.410	.5266	.5199
.015	.0004503	.0003076	.215	.1040	.09136	.415	.5460	.5398
.020	.0008008	.0005516	.220	.1095	.09663	.420	.5660	.5603
.025	.001252	.0008692	.225	.1152	.1021	.425	.5867	.5815
.030	.001804	.001262	.230	.1211	.1078	.430	.6081	.6034
.035	.002458	.001733	.235	.1272	.1137	.435	.6302	.6260
.040	.003213	.002283	.240	.1334	.1198	.440	.6531	.6494
.045	.004070	.002914	.245	.1400	.1262	.445	.6768	.6735
.050	.005031	.003629	.250	.1467	.1328	.450	.7013	.6985
.055	.006096	.004429	.255	.1536	.1396	.455	.7266	.7243
.060	.007265	.005318	.260	.1608	.1467	.460	.7529	.7510
.065	.008540	.006296	.265	.1682	.1540	.465	.7801	.7786
.070	.009921	.007366	.270	.1758	.1616	.470	.8082	.8071
.075	.01141	.008531	.275	.1837	.1695	.475	.8374	.8366
.080	.01301	.009793	.280	.1919	.1777	.480	.8676	.8671
.085	.01471	.01116	.285	.2003	.1861	.485	.8990	.8986
.090	.01653	.01262	.290	.2089	.1948	.490	.9314	.9313
.095	.01846	.01419	.295	.2179	.2038	.495	.9651	.9651
.100	.02051	.01586	.300	.2271	.2132	.500	1.0000	1.0000
.105	.02267	.01765	.305	.2367	.2228	.505	1.0362	1.0362
.110	.02495	.01954	.310	.2465	.2328	.510	1.0738	1.0736
.115	.02734	.02156	.315	.2567	.2432	.515	1.1128	1.1124
.120	.02986	.02375	.320	.2672	.2538	.520	1.1533	1.1526
.125	.03250	.02594	.325	.2780	.2649	.525	1.1953	1.1942
.130	.03527	.02832	.330	.2899	.2763	.530	1.2390	1.2373
.135	.03816	.03083	.335	.3008	.2881	.535	1.2844	1.2819
.140	.04118	.03347	.340	.3127	.3002	.540	1.3316	1.3282
.145	.04434	.03624	.345	.3250	.3128	.545	1.3806	1.3762
.150	.04763	.03915	.350	.3377	.3259	.550	1.4317	1.4260
.155	.05105	.04220	.355	.3508	.3393	.555	1.4847	1.4776
.160	.05462	.04540	.360	.3643	.3532	.560	1.5400	1.5312
.165	.05832	.04875	.365	.3783	.3675	.565	1.5975	1.5868
.170	.06218	.05240	.370	.3927	.3823	.570	1.6574	1.6446
.175	.06618	.05591	.375	.4076	.3977	.575	1.7198	1.7045
.180	.07033	.05972	.380	.4230	.4135	.580	1.7848	1.7668
.185	.07464	.06371	.385	.4389	.4298	.585	1.8526	1.8316
.190	.07911	.06787	.390	.4553	.4467	.590	1.9234	1.8989
.195	.08374	.07220	.395	.4723	.4641	.595	1.9972	1.9689
.200	.08854	.07670	.400	.4898	.4821	.600	2.0742	2.0417

Let the four observed F_2 classes be represented by

$\frac{AB}{(a)}$	$\frac{Ab}{(b)}$	$\frac{aB}{(c)}$	$\frac{ab}{(d)}$	$\frac{\text{Total}}{N}$

For repulsion, calculate $\frac{ad}{bc}$ and for coupling, $\frac{bc}{ad}$.

Determine the crossover value (expressed as a decimal fraction) by interpolation in this table.
Source: Table adapted from R. A. Fisher and B. Balmukand, The estimation of linkage from the offspring of selfed heterozygotes. *Journal of Genetics* 20 (1928–1929): 79–92.

***Drosophila* Paper Assignment**

Title: Short, but informative, include the organism studied and the experiment(s) carried out. You will get points off if your use the title "Drosophila Lab" or some such nonsense.

Abstract: Short summary of the hypotheses, experiments, results, and conclusions. Each one of the following should be answered in one to three sentences. The abstract is a mini-paper and should stand alone as a concise but complete description of the "Why?" "How?" And "What were the results?" of your experiment.

A. What is *Drosophila*? Why use *Drosophila*?
B. What is your hypothesis? How are you going to test it?
C. What SPECIFIC crosses did you do? Describe PXP to F_1 reciprocal crosses, F_1 sib mate, test cross, for both sets of phenotypes
D. What SPECIFICALLY were your results?
E. Interpret your results as they relate to your hypothesis. What phenotypes are dominant, which are recessive? Are the X-linked or autosomal? Are they linked or unlinked? How do you know?
F. If you genes are linked, indicate the map units between the genes and how you determine these (i.e. from the test cross or from the F_2 data).

ABSTRACT IS DUE AFTER THE INTRODUCTION.

Introduction: Describe the background necessary to have in order to be able to understand the experiment of the paper and describe what the experiment is attempting to discover.

A. *Drosophila* life cycle, fly genetics
B. Central Dogma of Molecular Biology, what is the ultimate product of a gene?
C. How are genes inherited in simple Mendelian inheritance? In other forms of inheritance?
D. What are X-linked or autosomal traits? How can you determine if a gene is autosomal or X-linked? What is Y-linked? How can you tell if a gene is Y-linked?
E. What are dominant or recessive traits? How can you determine if a trait is dominant or recessive?
F. What are linked or independently inherited genes? How do you determine if a gene is linked or independently inherited?
G. What are your hypotheses or purpose of the experiment?
H. What experiments are addressing which parts of your hypotheses/purpose?
I. map distance between linked genes (if appropriate)

Literature cited: There are several books on reserve in the library. You may also use any relevant journal articles in the library and/or your textbook. You may use 2 websites. Use 4 or more references in your introduction.

THIS IS AN EXAMPLE OF AN INTRODUCTION OUTLINE. YOU DO NOT HAVE TO FOLLOW IT (EXCEPT FOR THE LITERATURE CITED). USE PROPER SENTENCE STRUCTURE, GRAMMAR, AND PARAGRAPH FORMATION.

Questions to be answered in your notebook:

Parental crosses:

1. What are the genotypes and phenotypes of these strains?
2. Are these strains true breeding? If so, is this important for the experiment?
3. Why must virgin females be used?
4. Diagram the crosses that you performed.
5. Why were reciprocal crosses performed? What experimental questions(s) will these reciprocal crosses answer?
6. What results do you expect if the genes are autosomal?
7. What results do you expect if your genes are sex-linked?

F_1 Generation (answer test cross questions even if you didn't do a test cross):

8. What are the genotypes and phenotypes (with sex ratios) of the F_1 generations?
9. Are any of these traits X-linked? How do you know? If the genes are autosomal what do you expect? If the genes are X-linked what do you expect?
10. Which traits are dominant? Which are recessive? How do you know? Does the inheritance follow simple Mendelian rules? How do you know?
11. What are your hypotheses?
12. What are the expected ratios based upon your hypotheses?
13. What are your chi-square values? What are your degrees of freedom? Do you accept or reject your hypotheses?
14. Diagram the crosses you made.
15. Why did you perform each cross?
16. For each sib-mate, what are your expected results if your genes are unlinked?
17. For each sib-mate, what are your expected results if your genes are linked?
18. For each test cross, what are your expected results if your genes are unlinked?
19. For each test cross, what are your expected results if your genes are linked?

F_2 Generation:

20. What are the genotypes and phenotypes with sex ratios of the F_2 generation?
21. What are your hypotheses?
22. What are the expected ratios based upon your hypotheses?
23. What are your chi-square values? What are your degrees of freedom? Do you accept or reject your hypotheses?
24. Are any of your genes linked? If so, how do you know?

Test Cross:

25. What is a test cross?
26. What is the ratio expected for a test cross if there is no linkage?
27. Why didn't we do a test cross?
28. What equation do we use to map test cross data?
29. What does this equation tell us?

Mapping:

30. If any of your genes are linked, how far apart are they? How did you determine this?
31. If you mapped using the F_2 data, why does this work?
32. In a coupling cross, draw the chromosomes from the parents and following them through to the F_2 (if there's linkage, assume no crossing over).
33. What phenotype combinations should never be present if there is no crossing over in the coupling cross, if there's linkage? If that/those phenotype(s) is/are present, what does this indicate?
34. In a repulsion cross, draw the chromosomes from the parents and following them through to the F_2 (if there's linkage, assume no crossing over).
35. What phenotype combinations should never be present if there is no crossing over in the repulsion cross, if there's linkage? If that/those phenotype(s) is/are present, what does this indicate?
36. What does the Z value tell you?
37. The higher the Z value, the closer or further apart are the two linked genes?
38. One of the overarching themes of this lab and accompanying class is the Central Dogma of Molecular Biology. How does this lab fit into the Central Dogma?

Lab 4: Analysis of Variable Phenotypes in *Drosophila* Mutants

Objectives:

1. To investigate alleles that demonstrate extensions of Mendelian inheritance, using *Drosophila* mutants as our model.
2. To practice scoring traits, including those that are subtle and those that do not conform to the expected norm of binary phenotypes.
3. To gain more experience manipulating and sexing flies.

As we saw last week, many mutants of *Drosophila* are easily identifiable. We have also witnessed this phenomenon in corn two weeks ago. However, many mutant alleles are not so easily scorable. In some cases, it can be difficult to identify if the organism in question has a mutant phenotype, or to what degree the mutation is causing a phenotype. In this lab exercise, we will study some mutant *Drosophila* with difficult to score phenotypes, while reinforcing the concepts of five extensions of Mendelian inheritance: **variable expressivity**, **incomplete penetrance, multiple alleles, dosage compensation**, **gene dosage**, and **temperature sensitivity**.

Expressivity is the degree to which a trait is expressed. Some mutant alleles show **variable expressivity**, in which the individuals have the same genotype but differ in their phenotype. An example in humans is the dominant myotonic dystrophy (http://www.mda.org.au/specific/mdamyt.html). Each individual who has the dominant M phenotype develops the disease, characterized by muscle wasting and other symptoms, such as hair loss, GI problems, mental retardation, but each individual develops the disease at a different age and some become more seriously affected than others (they may only have very slight muscle wasting, for example, or may show all of the above symptoms and more). That variation in the DEGREE (severity) of the disorder is due to variable expressivity. We will look at two fly mutants, eyeless and lobe, whose phenotypes demonstrate variable expressivity.

Incomplete penetrance is a phenomenon is which the phenotype does not always match the genotype. A dominant allele or alleles are present, but the phenotype may still be wild type. This causes the phenotype to skip a generation even though the dominant allele is present. Lobe also exhibits incomplete penetrance.

Some genes are found in three or more alleles; these genes thus exist in **multiple alleles** that differ from each other in genotype and phenotype. These alleles can exhibit different relationships of dominance and recessiveness. In this lab today we will look at four X-linked alleles of a *Drosophila* eye color gene (*white*): wild type, eosin, apricot, and white. Wild type is dominant over all of these alleles. White is totally recessive. Eosin is dominant over white and recessive to wild type. Apricot is dominant to white and recessive to wild type.

Most of the time, X-linked alleles demonstrate **dosage compensation**. Dosage compensation refers to the phenomenon that in males, the protein expression levels from the hemizygous genes on the X chromosome are the same as those from the XX female. In *Drosophila*, the expression of the genes on the one X chromosome of the male is “turbo charged” so that the protein level of most of the X-linked genes in the male is the same as the XX female. We will look at apricot

eye color and wild type eye color that demonstrate dosage compensation-the hemizygous XY male and the XX female have the same phenotype, even though the male only has one copy of the X-linked gene.

In a few cases, the **gene dosage effect** is seen in which the number of copies of a certain X-linked allele affects the phenotype of the allele-generally the more copies, the more intense the phenotype (like incomplete dominance but on the X chromosome). This occurs because each allele will influence the amount of protein made, so zero alleles will have no protein made, one allele will have 50% protein made, two alleles will have 100% of protein made, etc. In an X-linked trait, male flies will always have one copy of the gene (hemizygous genes only) and female flies will always have two. Thus, with genes that demonstrate the gene dosage effect, a male with the dominant allele (one copy, since it's X-linked) has a different phenotype than the female with two copies of the dominant allele. An example of this is the eosin fly eye color, in which the female fly's eye color (two eosin alleles) is darker than the male's eye color (one eosin allele) or the heterozygous female's (one eosin allele). We will look at eosin eye color mutant flies whose phenotype demonstrates gene dosage., the apricot allele also demonstrates gene dosage, as the heterozygous female (apricot/white heterozygote) has a lighter eye color than the homozygous female or the hemizygous male. We will also look at the gene dosage effect in the apricot allele.

Conditional alleles only show the mutant (variant) phenotype under certain conditions. For example, in humans there is a loss of function mutation in the phenylalanine hydroxylase gene that, in the homozygous form, leads to the inability to breakdown phenylalanine from dietary protein; this disorder is called PKU or phenylketonuria. A diet high in phenylalanine leads to a toxic buildup of this amino acid and metabolic breakdown byproducts and subsequent mental retardation and other detrimental phenotypes. However, a diet low in phenylalanine does not lead to the buildup and the phenotypes are not seen. Thus, this disorder is conditional; under one set of conditions (high phenylalanine diet) one phenotype is seen (mental retardation), and under another set of conditions (low phenylalanine diet), a different phenotype is seen (wild type). **Temperature sensitive** alleles are a type of **conditional** allele. With temperature sensitive alleles, the phenotype varies based upon the temperature at which the mutants are grown or incubated.

PHENOTYPES:

Wild type flies:
Wild type flies have bright red eyes with approximately 800 facets per eye. The wild type eye is a spherical shape, with a smooth surface and smooth edges. The wild type eye color allele is dominant (dominant wild type *white*+ gene) over all other eye color alleles we will be observing in this lab.

At both temperatures we will be testing, wild type flies fly and hop around the vials.

Mutants:

Eyeless is a recessive allele in which the eye size and number of facets is reduced, as compared to wild type. The eye may be only slightly reduced in size or may be less than one half of its normal size. This is an example of **variable expressivity**, because all of the true breeding stock has the same genotype but varies in phenotype. These flies have red eyes, but the eyes vary in size. Eyeless is an autosomal gene.

Lobe is a dominant autosomal allele that exhibits **variable expressivity**, in which the eye size and number of facets is reduced; the eye may also have a nick or nicks in the edge in this mutant. Lobe also demonstrates **incomplete penetrance**; in the true breeding strain, the eye may also be wild type in shape and size. So, the eye may be wild type in size, only slightly reduced in size, or may be much smaller than wild type. These flies have also red eyes, but the eyes vary in size.

Antennapedia is a dominant gain of function mutation in which legs grow where antennae should be. This mutation causes developmental pathways to be misexpressed (in the wrong body location) and exhibits **variable expressivity** and **incomplete penetrance**.

The **white** eye allele is X-linked and recessive to all eye color alleles we will be looking at in this lab. We will only be looking at the white eye allele in respect to the eye colors, not the eye shape. This mutation is the total loss of function allele of the *white* gene.

The **eosin** allele causes the eye to appear a pinkish-yellow, darker in color than wild type. Eosin is dominant to white and recessive to wild type, so it is an example of an allele of a gene with **multiple alleles** (the *white* gene). Eosin is an X-linked eye color mutation in which the **gene dosage effect** is best seen. In true breeding females, the eyes are darker in color than the true breeding males' eyes. The eosin/white heterozygous female has the same eye color as the male with eosin eyes.

The **apricot** allele causes the eyes to appear a peachy color, lighter than wild type. Apricot is dominant to white and recessive to wild type, so it also is a **multiple allele** of the *white* gene. Apricot is an X-linked eye color mutation in which both **dosage compensation** and **gene dosage** are also seen. For the example of **dosage compensation**, hemizygous males (one copy on one X chromosome) and homozygous females (two copies on two X chromosomes) have the same phenotype. For the example of **gene dosage**, The apricot/white heterozygous female (one copy of the apricot allele) has a phenotype that is half as intense as the homozygous apricot female or the hemizygous male.

Shibire is an autosomal **conditional (temperature sensitive)** allele of the dynamin gene. Dynamin function is required for synaptic vesicle fusion. At the nonrestrictive temperature (low temperature, full protein function), the flies are wild type in terms of behavior. Incubation at the

restrictive temperature (high temperature, loss of protein function), the flies are no longer able to release neurotransmitters, become paralyzed, and can have seizures. The flies will return to the wild type phenotype if transferred back to the nonrestrictive temperature.

Methods:

1. Each lab group will obtain one vial of flies, available on the side counter. Double check with your instructor and then use Flynap® to anesthetize the flies. CLEARLY LABEL the files and place under a dissection microscope for observation. Go around to each lab group station (each will be a different mutant or cross) and observe, as indicated in this lab handout.

2. For the eye mutants, observe and record each phenotype as indicated in this manual. Make sure you differentiate between the sex of flies in eosin and apricot flies. White, apricot, and eosin affect the eye color; and eyeless and lobe affect the eye shape. Eyeless and lobe are autosomal. White, apricot, and eosin are X-linked.

VARIABLE EXPRESSIVITY AND INCOMPLETE PENETRANCE

Eyeless

A. For true breeding eyeless, observe the **variable expressivity** of the trait. You are looking at the size and shape of the eyes. **Count** and record how many flies have almost a wild type eye, how many have a very small eye, and how many are in between, observing at least 25 total flies. Look at both eyes, not just one, as the phenotype will vary between individuals but also can vary between eyes on the same individual!

Lobe

B. For true breeding lobe, observe the **variable expressivity** and **penetrance** of the trait. You are looking at the size and shape of the eyes. **Count** and record how many flies have a wild type eye, an eye almost wild type (for example, may only have a small nick in the edge), how many have a very small eye, and how many are in between, observing at least 25 total flies. Look at both eyes, not just one, as the phenotype will vary between individuals but also can vary between eyes on the same individual!

Antennapedia

C. For true breeding antennapedia, observe the **variable expressivity** and **penetrance** of the trait. You are looking at the size and shape of the antenna/leg (where the antenna should be). **Count** and record how many flies have a wild type antenna, a small structure where the antenna should be, how many have a full leg in the antenna region, and how many are in between, observing at least 25 total flies. Look at both antenna, not just one, as the phenotype will vary between individuals but also can vary between antennae on the same individual!

MULTIPLE ALLELES

C. Observe and record the eye color of the true breeding strains of wild type (red), eosin, apricot, and white, **looking at both sexes**. These are all alleles of the X-linked *white* gene.

GENE DOSAGE EFFECT

Eosin

D. For the true breeding eosin mutant strain, observe the **gene dosage effect** in the flies. Note that the females and males differ in their eye color phenotypes. Record how much darker the females' eyes are than the males'. You are looking at the eye color not shape in eosin flies.

E. Observe the **gene dosage effect** in flies from the mixed stocks. These are $_1F$ and F_2 generation flies from the following cross: eosin eyes crossed to white eye (crossed to white so that we can see eosin as eosin is dominant to white, but recessive to wild type). This cross was not performed with virgin females, and males and females of each phenotype were in the same vial, so the offspring are not all of the same genotype. Carefully sex, observe, **count**, and record the differing phenotypes of at least 25 different flies, noting when the females and males differ in their phenotypes. Compare these to the true breeding stocks, if necessary. In the mixed eosin stocks, you will be able to find a heterozygous female. What does the heterozygous female look like? How do you know this female is heterozygous? Show this heterozygous female to your instructor.

Apricot

F. For the true breeding apricot mutant strain, observe **dosage compensation**. Note that the eyes of the females and males are basically the same color, even though females have two copies of the X-linked allele and the males have only one copy. You are looking at the eye color not shape in apricot flies.

G. Each lab group will observe the **gene dosage effect** in flies from the mixed stocks. These are F_1 and F_2 generation flies from the following cross: apricot eyes crossed to white eye (crossed to white so that we can see apricot as apricot is dominant to white, but recessive to wild type). This cross was not performed with virgin females, and males and females of each phenotype were in the same vial, so the offspring are not all of the same genotype. Carefully sex, observe, **count**, and record the differing phenotypes of at least 25 different flies, noting when the females and males differ in their phenotypes. Compare these to the true breeding stocks, if necessary. In the mixed apricot stocks, you will be able to find a heterozygous female. What does the heterozygous female look like? How do you know this female is heterozygous? Show this heterozygous female to your instructor.

CONDITIONAL (TEMPERATURE SENSITIVE) ALLELE

3. Now observe shibire mutant flies and wild type flies. Do not anesthetize the flies!
   A. Record the activity pattern of both flies stocks at room temperature.
   B. Now we will warm up the wild type flies and the shibire mutants. Carefully time and record how long it takes for the shibire mutants to fall to the bottom of the vial and become paralyzed. Compare the shibire mutant activity to wild type.
   C. Then we will return the shibire and wild type flies to room temperature. Carefully time and record how long it takes for the shibire mutants to resume wild type activity.

Questions to be answered in your notebook:

1. Why did the eyeless and lobe true breeding stocks show such variability of phenotype?
2. At the molecular level, what do you think is causing the phenomenon of variable expressivity? Answer in terms of protein function at a cellular or organ level.
3. At the molecular level, what do you think is causing the phenomenon of incomplete penetrance? Answer in terms of protein function at a cellular or organ level.
4. What is the gene dosage effect? How does it differ between males and females? At the molecular level, what is causing the phenomenon of gene dosage?
5. Design a series of crosses in which you can tell a fly with a lobe allele but wild type phenotype from a true wild type fly.
6. What does hemizygous mean?
7. For the multiple alleles, white is a total loss of function allele and the wild type (red) codes for a fully functional protein. What do you think is happening at the molecular, cellular, and organ (eye) level in the apricot and eosin mutants? Describe what you think is occurring as it relates to protein activity/function.
8. Eosin, apricot, white, and wild type are all alleles of the same gene. You know that wild type is dominant over all other alleles and white is recessive to all other alleles. Design a cross or series of crosses that would allow you to determine whether eosin is dominant over apricot (or vice versa).
9. In the true breeding stocks, why did the female eosin mutants have darker eyes than the eosin males? Answer in terms of protein levels as they relate to activity/function.
10. Why do the true breeding flies with apricot eyes have the same color eye regardless of sex, when apricot is X-linked? HINT: Think about dosage compensation in flies. Answer in terms of protein levels as they relate to activity/function.
11. In the mixed eosin stocks, you will be able to find a heterozygous female. How can you tell the female is heterozygous? Why does she look that way? What are her protein levels? How do they compare to the homozygous female and the hemizygous male? How do her protein levels relate to activity/function/phenotype?
12. In the mixed apricot stocks, you will be able to find a heterozygous female. How can you tell the female is heterozygous? Why does she look that way? What are her protein levels? How do they compare to the homozygous female and the hemizygous male? How do her protein levels relate to activity/function/phenotype?
13. Why were eosin and apricot crossed to white rather than wild type?
14. Why don't all X-linked genes demonstrate the gene dosage effect? Answer in terms of protein levels and protein activity/function in *Drosophila*.
15. Antennapedia is a gain of function mutation. What is a gain of function mutant? How is the dominant gain of function antennapedia mutation working in the cell?

16. Describe how the antennapedia phenotype could demonstrate incomplete penetrance and/or variable expressivity.
17. What developmental pathway is antennapedia regulating?
18. How does the wild type Antp protein work in *Drosophila*?
19. Which phenotype(s) was/were the hardest to score? Why?
20. In this experiment, we looked at some mixed stocks and some true breeding stocks. Why do geneticists use true breeding stocks rather than mixed for their experiments?
21. What does restrictive temperature mean? What does nonrestrictive temperature mean?
22. What function does the protein dynamin specifically perform in the cell?
23. What is happening to the dynamin protein in the shibire mutants at the molecular level at the restrictive and nonrestrictive temperatures? Think in terms of protein structure, function, and activity.
24. Find and describe one other human conditional allele (not PKU).
25. One of the overarching themes of this lab and accompanying class is the Central Dogma of Molecular Biology. How does this lab fit into the Central Dogma?

Special thanks to Dr. Deutch for the initial idea for this lab.

Lab Five: Chromosomal Structure in Wild Type and Abnormal Cells

Part 1: Disorder Detectives
Modified from Carolina Biological Supply Company Disorder Detectives©

Objectives:
You should be able to:
1. understand what a karyotype is and what it is used for.
2. analyze a karyotype for human genome disorders.
3. develop a hypothesis about human genetic disorders based upon karyotypes you analyze.

You and a partner will take on the role of a cytogeneticist working in a hospital. A case study will be given to you for review, along with a set of patient chromosomes. You and your partner will arrange the chromosomes into a completed karyotype on a prepared board. You will analyze the karyotype and diagnose your patient. Your patient may have one of the many types of recognized chromosomal abnormalities, though normal karyotypes are also represented. Be careful and use your observation skills—things are not always as simple as they seem.

Background
In order to think like a cytogeneticist, here are a few things you will need to know.

What is a chromosome? The DNA of all living organisms is organized into discrete packets called chromosomes. Most human cells contain 46 chromosomes, grouped into two sets of 23—a maternal set contributed by the mother's egg and a paternal set contributed by the father's sperm. The maternal and paternal chromosomes of a pair are called homologous chromosomes, or homologs. Within each set of chromosomes there is one sex chromosome and 22 other chromosomes, called autosomes. There are two types of sex chromosomes, classified as "X" and "Y." Typically, a male has both an X and a Y chromosome, while a female has two X chromosomes.

What is the structure of a chromosome? Most of the time, the chromosomes are present as long, tangled chromatin strands composed of DNA tightly wrapped around histone proteins and further condensed and stacked. At this stage, individual chromosomes cannot be distinguished from one another.

During cell division, the DNA is replicated and even further condensed. The two copies of each chromosome, called sister chromatids, are temporarily held together at a specific location on the chromosome called the centromere. At this point, individual chromosomes can be identified.

How are chromosomes classified? Chromosomes vary in size and shape. Centromere location is another feature used to distinguish one chromosome from another. Metacentric chromosomes have arms of roughly equal lengths. The arms of submetacentric chromosomes are more unequal. It is easier to distinguish the shorter arm, called the p arm, from the longer arm, called the q arm. Acrocentric chromosomes have a centromere that is even closer to one end of the chromosome,

making their p arms even shorter in relation to their q arms. At each end of a chromosome is a protective region called a telomere.

When stained with Giemsa stain, different chromosomes have different banding patterns. These patterns of dark and light bands uniquely identify each chromosome. The bands do not indicate genes—for some chromosomal regions, hundreds of genes may be present in one band, while in other regions, there may be relatively few genes per band.

What is a karyotype? A karyotype is an organized profile of an individual's chromosomes. Generally the chromosomes have been stained, identified, and organized in a specific order. This allows a scientist called a cytogeneticist to examine the chromosomes and quickly identify alterations that may result in a genetic disorder. Chromosomes are typically prepared for karyotyping with the sister chromatids so closely aligned that they appear as a single structure (in other words, they look like an "I" rather than an "X").

What types of samples are often obtained for karyotyping?
Cells may be obtained from various sources for karyotype analysis, including

- blood.
- skin or other tissues.
- chorionic villi (part of the placenta). Chorionic villus sampling (CVS) involves removing some of the chorionic villi so the cells can be analyzed. This test, which can be conducted at 10-13 weeks' gestation, carries a 1-2% risk of miscarriage.
- amniotic fluid. Amniotic fluid surrounds the fetus and contains fetal cells that have been shed. The process of withdrawing this fluid using a hollow needle is called amniocentesis. It is conducted at 14-20 weeks' gestation and carries a 1% or less risk of miscarriage.

Examples of Findings Commonly Identified by Karyotyping

Common Karyotype Findings	**Associated Clinical Symptom**
9/22 translocation	Chronic myelogenous leukemia
5p deletion	Cri du chat
22q11.2 deletion	22q11.2 deletion syndrome
Trisomy 21	Down syndrome
Trisome 18	Edwards syndrome
XXY	Klienfelter syndrome
46 chromosomes (XX)	Wild type female
46 chromosomes (XY)	Wild type male
3p25q21 inversion heterozygote	No clinical symptoms present
9p11q12 inversion heterozygote	No clinical symptoms present
Trisomy 13	Patau syndrome
14/21 translocation heterozygote	Down syndrome, Robertsonian translocation
Monosomy X	Tyrner syndrome
Trisomy X	XXX syndrome
XXY	47, XYY male

What benefits are provided by karyotyping? Prenatally, the results of a karyotype can provide answers or a diagnosis. Parents may utilize this information to identify a care team to be present at the birth or to make an informed reproductive decision. In postnatal cases, the results can be used to diagnose complicated syndromes.

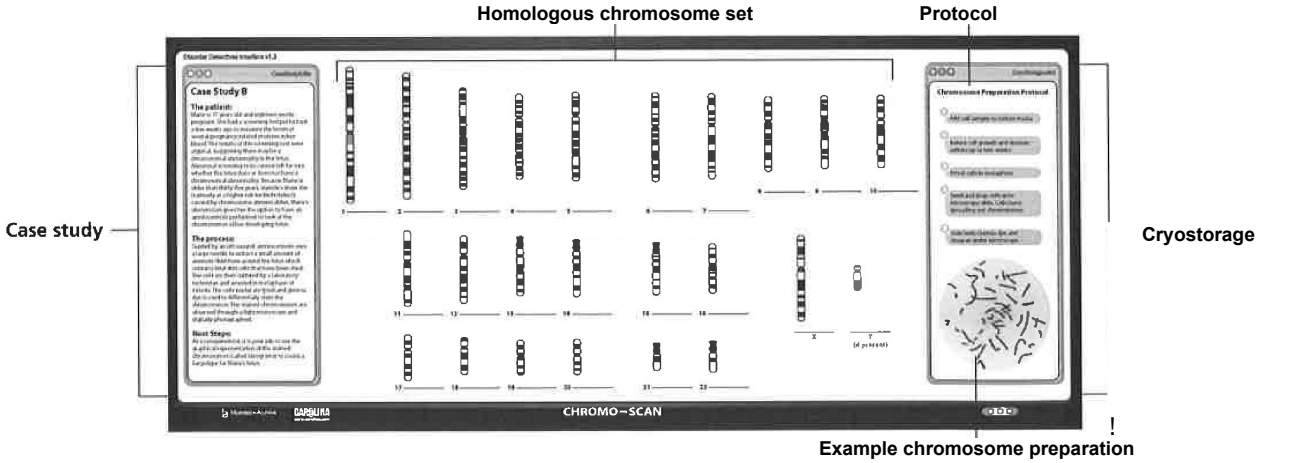
Often a pregnant woman is offered a blood test to help identify certain chromosome disorders. Is this a karyotype? Pregnant women are offered a screening test for a number of disorders, including neural tube defects and certain chromosomal disorders. Although the word "screening" is often used loosely as a synonym for "testing," the two are not identical and the maternal blood test is not used to create a karyotype for the fetus.

The screening tests measure specific proteins found in the blood of pregnant women to identify who should be offered more extensive (and often more invasive and expensive) testing, such as amniocentesis to obtain fetal cells for karyotyping. The screening test is not 100% sensitive and often women who are carrying a normal fetus have an abnormal screening test and must deal with the stress of deciding whether or not to undergo amniocentesis. At the same time, in a small number of cases, truly abnormal pregnancies go undetected by the maternal blood screen.

Procedure for the Karyotyping Activity

1. You will need one copy of the "Cytogenetics Report for G-Banded Karyotype" for each case study you perform. It will need to be copied into your notebook. Please perform at least 3 case studies.
2. You will also receive a Chromoscan board containing a case study and set of patient chromosomes (reusable decals). Each case study has a Case ID (letters A-0) and a unique color. The color of the patient chromosomes matches the color that is printed around the case study section of the Chromoscan board. Confirm that the colors of the chromosomes and the board match.
3. Select a chromosome decal from the cryostorage area of the board and sketch it on your Cytogenetics Report, noting the centromere, telomere, and p and q arms. Note the centromere position and identify the chromosome as metacentric, submetacentric, or acrocentric.
4. Read the case study found on the left side of the board.
5. On the Cytogenetics Report, record patient information, including name, case ID, reason for referral, patient age, and source of cells.
6. To make the process of karyotype assembly less complex, one of each of the homologous chromosomes is already illustrated on the board. Identify the other homolog and place it on the board in the proper position.
7. Once the karyotype is completed, analyze it for chromosomal anomalies, paying particular attention to chromosome number and structure.
8. Record chromosome number, gender, and chromosomal findings on the Cytogenetics Report.
9. Determine the suggested diagnosis by looking at the table "Examples of Findings Commonly Identified by Karyotyping."
10. Complete the Cytogenetics Report on your patient to include patient diagnosis.
11. Briefly explain on the Cytogenetics Report how a karyotype is prepared. A summary of the technique can be found on the right-hand side of the board where the chromosomes are stored.
12. Discuss the questions found at the bottom of the Cytogenetics Report and write out your answers.

13. At the end of the activity, return the chromosome decals to the cryostorage region of the Chromoscan board in random order, to prepare the board for the next group's use. Check carefully around your desks and lab tables to make sure all the chromosomes have been collected and returned to the board.



Cytogenetics Report for G-Banded Karyotype (record in notebook)

1. Select a Chromosome from the cryostorage area (abnormal if there is one). Sketch the chromosome, abeling the p arm, the q arm, centromere, and telomere. Is this chromosome metacentric, submetacentric, or acrocentric?

2. Fill out the table below

Patient Name	Case Study ID	Age
Why is the patient being referred for karyotyping?	Source of Cells for Karyotyping	
Total # of Chromosomes Observed	Gender	
Chromosomal Findings	Patient Diagnosis	
What are the implications for the patient's caregiver with this genomic diagnosis? i.e. life expectancy, complications, available treatments, etc		

3. briefly explain how a karyotype is prepared.
4. Why do you think relatively few fetuses with chromosomal trisomies survive to birth?
5. Why are microdeletions and insertions difficult to diagnose with karyotyping?
6. Sometimes a genetic change is too small to visualize through G-banding alone. For example a disease such as 22q11.2 Deletion Syndrome which is caused by a deletion on chromosome 22 might not be detected by G-banding. Other methods have been created to take advantage of new technology and equipment and recognize these smaller abnormalities. One method is **Fluorescent In Situ Hybridization** (FISH). What is FISH?
7. What are other ways to identify micro-abnormalities?
8. What are pros and cons of CVS and amniocentesis?

Part 2: Chromosomal Structural Aberrations in a Cancer Cell Line
adapted from CellServ@FAES/NlH (www.cellservkits.com)

Objectives:
You will learn how to:
1. fix, stain, and visualize human chromosomes mounted on microscope slides.
2. recognize two chromosomal features: sister chromatids and centromeres.
3. recognize size differences among human chromosomes.
4. recognize the three basic human chromosome morphologies based on centromere position: metacentric, submetacentric, and acrocentric.

Each somatic cell in the human body contains 23 pairs of chromosomes. During the interphase stage of the cell cycle each of these chromosomes is duplicated and consists of two chromatids joined by a common centromere (spindle attachment region). During mitosis the chromatids separate and become independent chromosomes that move to opposite ends of the cell. The subsequent division of the cytoplasm results in the formation of two new daughter cells each containing the same diploid number of chromosomes as the parent cell. Cells grown in tissue culture are used for most human chromosome studies. For this exercise, a human tumor cell line, HeLa, (which is highly aneuploid), is grown in culture and subsequently treated so as to allow for the microscopic examination of the chromosomes. Colchicine, a plant alkaloid, has the unique property of arresting cells in metaphase of the mitotic cycle by interfering with formation of the mitotic spindle that is needed for the movement of chromosome during the metaphase to anaphase progression. This blockage increases the frequency of metaphase cells. Metaphase chromosomes are most readily observed with the light microscope and various chromosomal features such as sister chromatids and centromeres are evident. In summary, the procedure for chromosome visualization (and karyotyping) entails arresting a fraction of a log phase population in metaphase, treating the cells with a hypotonic saline solution to swell the cells and increase their fragility, fixation with acetic acid-methanol, splattering onto slides, and staining. This is followed by a search for ideal chromosome spreads for the study of chromosome number and structure.

The 46 chromosomes and mitochondria located in each somatic cell of the human body contain the entire human genetic complement. Located within the nucleus, these 23 pairs of homologous chromosomes are comprised of 22 pairs of autosomes (non-sex chromosomes) and 1 pair of sex chromosomes (XX or XY). The genetic material, or DNA (deoxyribonucleic acid), exists within the chromosomes and contains the entire genetic blueprint for development of an individual. It exists in a highly coiled and condensed state, due in part to the action of a class of DNA binding proteins called histones. All normal human cells contain identical numbers and types of chromosomes. Aberrations in the chromosomal number and/or structure will most likely result in some type of genetic defect. The analysis of human chromosomes has allowed researchers to identify specific genetic diseases and abnormalities that are attributed to this disruption in the normal complement and structure of the chromosomes. Each chromosome pair contains unique physical attributes which distinguishes them from all others. The three main criteria used to distinguish and identify individual chromosomes are:

length of the chromosome
position of the centromere (the primary constriction)
staining (banding pattern of a chromosome when exposed to stains)

Using these criteria, cytogeneticists (individuals who analyze and research chromosome structure and function) have set up a classification system for chromosomes which labels each chromosome with a number, or for the sex chromosomes, as X or Y. This system of standardization allows for accurate communication among scientists.

Many genetic diseases have been associated with a specific change or abnormality within the chromosomes. These abnormalities can include: an increase or decrease in the amount of chromosome material or the translocation of one piece of a chromosome to another chromosome. Several kinds of cancer are associated with chromosomal abnormalities. Some examples of genetic diseases and their respective chromosomal aberrations are:

1. Down Syndrome - characterized by an extra chromosome #21 (trisomy 21).
2. Cru du Chat - characterized by a deletion of the short arm of chromosome #5.
3. Turner's Syndrome - characterized by the absence of one X chromosome; these females only have 45 chromosomes.

On the other hand, there are many genetic diseases that result from a defect within a particular gene. The abnormal genotype may result in an abnormal phenotype. Such defects may be more subtle and more difficult to analyze. Recent advances in recombinant DNA technology and genetics, however, have allowed researchers to identify specific locations of genes on chromosomes. This information is useful for researchers from around the world who are constructing a genetic map of the human genome. Although the human genome sequence has been “officially completed”, work is ongoing to continue to refine and correct mistakes in the initial analysis.

In order to analyze an individual's chromosomes, or prepare a karyotype, the chromosomes must be in a state in which they can be easily observed. This is accomplished by treating the cells with a chemical called colchicine. The action of colchicine causes the arrest of mitosis in the metaphase stage of the mitotic cycle. It is during this stage that the chromosomes are in their most condensed state and the most visible with the light microscope. Once the cells have been "arrested" in metaphase, the cells are placed in a hypotonic solution. Since the osmotic pressure is greater inside the cell as compared to the outside, water will enter the cell until a state of equilibrium between the cell and its environment has been reached. Movement of water into the cells causes the cells to swell in size. The hypotonic solution is then replaced with a fixative that preserves the existing cell architecture. The cells are now ready to be "splatted" onto microscope slides, stained, and observed. When preparing a karyotype, the investigator will take a photograph of a chromosome spread that shows clear and distinct chromosomes. The photograph is enlarged and the individual chromosomes are cut out and arranged based on the physical criteria stated earlier (size, centromere location, and banding patterns). This representation of an individual's chromosomes is called an idiogram and is pictured in Figure 1.

One practical application of karyotype analysis is in the early detection of genetic defects through amniocentesis. In this process some of the amniotic fluid surrounding the fetus is

removed by a physician. This fluid contains fetal cells that will propagate under very specific laboratory conditions. Once the cells have increased in number a karyotype can be performed on these fetal cells. The results of the karyotype analysis may alert the physician to potential problems or abnormalities of the fetus. In karyotyping not involving fetuses the cell type most often used for analysis are lymphocytes. As with fetal cells these cells are grown in culture, treated with hypotonic solution, and fixed prior to performing a karyotype.

In this exercise, the human tumor cell line, HeLa, is used for karyotyping. The HeLa cell line originated in the early 1950's from the cancerous cervical cells of a woman named Henrietta Lacks. Prior to her death, some of the cancerous (tumor) cells were removed from her body and grown in culture. The cells were allowed to divide repeatedly in this artificial culture environment and resulted in formation of what is known as a cell line. These HeLa cells (the abbreviation coming from *He*nrietta *La*cks) live on today and are used for research on cell and tumor growth. They are also useful for showing chromosomal structure in the biology lab. Because the cells are of tumor origin they have continued to divide and multiply over the last 35 years and will continue to do so for an indefinite period of time. Furthermore, since these cells are of tumor origin, they will not contain the normal diploid number of chromosomes characteristic of human beings (46). Instead, these cells are considered aneuploid and generally possess a chromosome number greater than the diploid number, with three, four, five, or more copies of a particular chromosome being present in some cells. When you make a chromosome count of your spreads you may find chromosome numbers of 50 to 60 per cell, or more (see Figure 2 for an example of one cell's chromosomal components)! You might also see cells with less than the normal diploid number, as sometimes chromosomes will fuse together to form one very large chromosome.

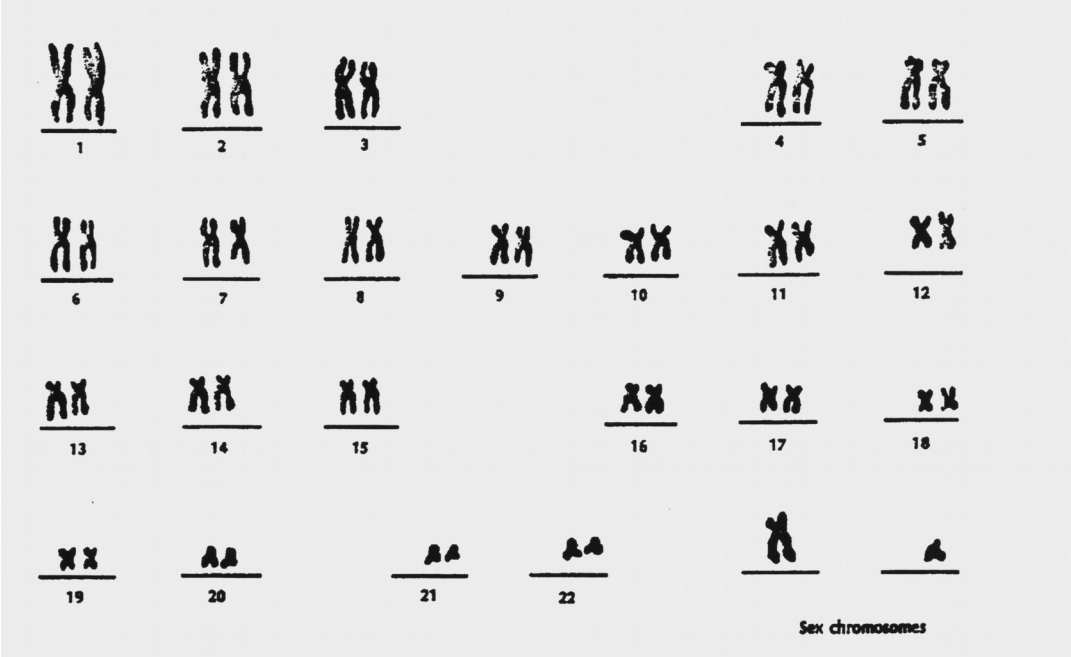


Figure 1. An idiogram of a human metaphase arrested cell.

HeLa cells, a human tumor cell, shows the typical aneuploid condition common to transformed cells. Normal human diploid cells will contain 46 chromosomes but it is evident that this cell

shown in Figure 2 below contains more than that number. Note the typical chromosome structure with the centromere evident in each chromosome. The sister chromatids are also evident. The position of the centromere is used to classify chromosomes as either: metacentric, submetacentric, acrocentric. A chromosome of a fourth category, telocentric chromosome, has the centromere terminally situated. However, there are no NORMAL human telocentric chromosomes. Close examination will show the presence of all four types in Figure 2.

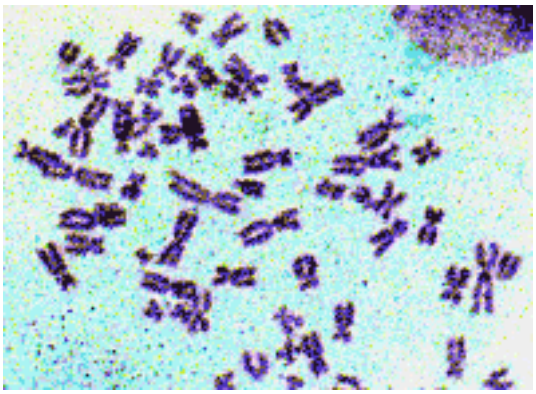
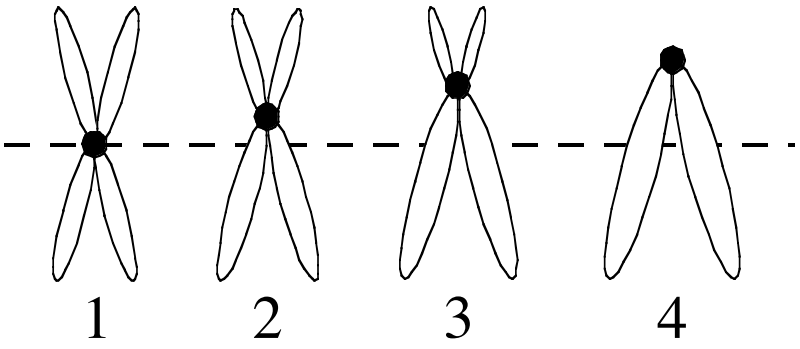


Figure 2.
Representative HeLa cell chromosome spread, note the aneuploidic nature and the telocentric chromosomes.

http://mccarterbiology.edublogs.org/labs/human-chromosome-spreads/



1. Metacentric Chromosome: centromere is centrally situated.
2. Submetacentric: centromere is moderately off center.
3. Acrocentric: centromere is grossly off-center.
4. Telocentric: centromere is terminally situated.

Figure 3. Centromere location.

Materials:

microscope slides	Pasteur pipettes	tube of cells
Stain #1	Stain #2	Permount (mounting medium)
glass coverslips	compound microscope	forceps

Karyotype slides from wild type humans

Methods, One Slide Made per Lab Group:

1. Due to the variable nature of slide preparation, you are STRONGLY encouraged to make several slides (3-5) simultaneously rather than make one slide and then notice it didn't work and have to and have to start over from scratch!
2. Wash your slide with soap and water and then dry thoroughly.
3. Place the slide vertically at a 45° angle.

4. With a pipette, gently resuspend the cells in the tube provided. Remove a small sample of cell suspension with a pipette and hold the pipette **at least 2 feet** above the slide. Allow one drop of cell suspension to "splat" onto the slide about 3/4 inch from the upper end and tumble down the slide. Carefully apply 6 - 8 more drops from various heights, one drop at a time, onto the same region of the slide. It is important to release the cell suspension ONE DROP AT A TIME. Do not expel all of your cell suspension in one squirt, or you will obtain poor results. Gently blow across the slide for 2 - 3 seconds. The drying will help "spread" the chromosomes. Mark on which side of the slide you have dropped the cells.
5. Allow the cells to AIR DRY COMPLETELY.
6. Dip (using forceps) the slide into the tube containing STAIN #1 for 1 SECOND ONLY. Remove the slide and dip into STAIN #1 again for 1 SECOND ONLY. Remove the slide and dip into STAIN #1 again for 1 SECOND ONLY.
7. Drain off stain and dip the slide into tube containing STAIN #2 for 1 SECOND ONLY. Remove the slide and dip into STAIN #2 again for 1 SECOND ONLY. Remove the slide and dip into STAIN #2 again for 1 SECOND ONLY. Caution should be taken to avoid carryover of stains (wipe the bottom of slide with a paper towel before transferring).
8. Remove slide from stain and thoroughly rinse with distilled water.
9. Add a coverslip and begin to view your slide.
10. Under low power, scan your spread for cells that appear to have ruptured and released their chromosomes. Shift to 400X to examine your spread more carefully. An ideal chromosome spread will contain chromosomes which appear distinct, do not overlap with adjacent chromosomes, and whose sister chromatids are separate and distinct (see Figure 1). This exercise requires careful observation so take your time when viewing.
11. Once you have found what appears to be a clear and distinct set of chromosomes, place a small drop of immersion oil on the coverslip over that area and switch to 1000X. You will need to find TEN total ruptured cells for analysis in the questions section.
12. After you have completed answering questions 1-4 in your notebook, look at one of the demonstration slides of wild type human chromosomes (male or female, your choice), and then answer questions 5 and 6. You will need to find THREE representative well spread nuclei to answer questions 5 and 6.

Questions to answer and exercises to do in your notebook:

1. Count the number of chromosomes present in **TEN** different cells on the slide and try to identify and locate the characteristic chromosomes based on the location of the centromere. Remember that this cell line is aneuploid and each cell should contain a different number of chromosomes, each different than the diploid number (46). Fill out the following table in your notebook for each of the TEN cells:

	Total number of Chromosomes per cell	**Number of Metacentric Chromosomes**	**Number of Submetacentric Chromosomes**	**Number of Acrocentric Chromosomes**	**Number of Telocentric Chromosomes**
Cell one					
Cell two					
etc					

2. When you have completed the table in question one, calculate the average number of chromosomes per cell. Then calculate the average of each of the chromosome types. Fill out the following table in your notebook:

Average number of Chromosomes per Ten Cells	Average Number of Metacentric Chromosomes	Average Number of Submetacentric Chromosomes	Average Number of Acrocentric Chromosomes	Average Number of Telocentric Chromosomes

3. Draw **THREE** representative cells from the HeLa sample with well spread chromosomes.
4. Document and label the number of chromosome types in one representative cell DRAWN IN QUESTION NUMBER THREE.
5. From one wild type human slide, look at **THREE** representative cells and count the chromosomes from the three cells and fill out the following table in your notebook:

	Total number of Chromosomes per cell	Number of Metacentric Chromosomes	Number of submetacentric Chromosomes	Number of Acrocentric Chromosomes	Number of Telocentric Chromosomes
Cell one					
Cell two					
Cell three					

6. Draw ONE representative cell from the wild type slide.
7. In the wild type cells, did you see 46 chromosomes, or a different number? If you saw a different number that 46, why do you think you saw a different number (remember these are wild type cells)?
8. Specify the location and function of the centromere.
9. Why is it necessary to expose the cells to a hypotonic solution when preparing cells for karyotyping?
10. Define the role of histones in the chromosomes.
11. Explain the difference between an aneuploid cell, a haploid cell, and a diploid cell.
12. During which stage of mitosis are the chromosomes in their most condensed state and thus best suited for karyotyping? Why is this so?
13. Name the three types of chromosomes found in NORMAL human cells based on the location of the centromere.
14. State and describe an example of a genetic disease (not mentioned in this exercise) that is caused by an abnormal chromosomal condition.
15. How do you think the HeLa cells acquired telocentric chromosomes?
16. How do you think the HeLa cells became aneuploid?

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Genetics Lab Practical Study Guide
Open lab notebook

Mathematical Review
Dimensional Analysis-calculating molarity, moles, grams
Metric system-conversion from one unit to another

Lab Skills
Microscopy-finding something focusing
Pipettes-setting a pipette to a given volume and pipetting said volume
Gel analysis- what size is the band on this gel? DNA and protein gels

Concepts
Central Dogma-Transcription, translation using codon table, including DNA mutation
Central Dogma-coding strand and template strand
Punnett squares-including two gene interaction and epistasis
Chi square
Linkage
Operons and operon regulation
PCR
Karyotype analysis
Transformation efficiency
Column chromatography
Restriction Enzyme Mapping
Mendelian Genetics
Extensions to Mendelian Genetics