

LABORATORY MANUAL FOR BIOS 308

GENETICS

by

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Almost the End

The End

INTRODUCTION

Welcome to the Genetics Lab! The lab concentrates heavily on Drosophila melanogaster, the fruit fly. This is a two credit lab, and it will take up a lot of your time. Unlike most labs in 100, 200, or 300 level classes, you yourself will have most of the responsibility and do most of the work. The TA's and the instructor are there to help you, but you will need to keep your flies alive, set up your own crosses and gather your data--all on your own schedule. This is a serious dose of middle class work ethic.

Now it's time to meet:

YOUR FRIEND, THE DROSOPHILA [Potentially amusing Freddy the Fruitfly cartoon!](#)

Drosophila melanogaster (D.m.) is a cosmopolitan species--it is found all over the world, including in your home if you have overripe peaches in the summer. Flies are among the most successful of the insects, and there are over 2500 species of *Drosophila*.

Structure of the Fly. Like all insects, *Drosophila* have three main body parts: the head, the thorax, and the abdomen. The major structures on the head are the large red (usually) compound eyes. On top of the head are two antennae (antennae for the linguistic purists)--the fly uses these to smell. The mouth is a proboscis--the fly lowers it to suck up food like a vacuum cleaner.

The thorax has 6 legs, 2 wings, and 2 halteres. The halteres are small, club-shaped organs behind the wings that function as gyroscopes to keep the fly balanced in flight. On the dorsal (top) surface of the thorax are a number of long dark bristles (hairs).

The abdomen contains the guts of the fly. On females, the abdomen is striped on every segment, but on males, the last few segments are solid black. Also on the abdomen are the genitalia. On females all you can see is a small bump, but on males, there is a distinct set of brown anal plates and claspers (see the drawings).

Life cycle. *Drosophila* go through 4 stages in their lives. [Diagram!](#)

1. Egg. Eggs are laid by the mother on the food and take about 1 day to hatch. They are small, oblong and translucent, with two "ears" sticking out.

2. Larva. Larvae are maggots which crawl through the food in a jerky motion, eating as they go. The larvae go through 3 molts: they hatch from the egg as small, first instar larvae. Then after a day they molt to become larger, second instar larvae. After another day they molt again to become even larger third instar larvae. After two days in the third instar, the

larvae climb up on the sides of the vial, glue themselves to the glass, evert their spiracles (breathing tubes), and settle down as pupae.

3. Pupa. Pupae are the cocoons in which the larvae metamorphose into adults. The larval cuticle becomes a shell, their muscles melt away, and a new adult exoskeleton

and musculature forms inside. The pupal stage lasts five days. During the last day, you can see the red eyes and the dark wings forming inside.

4. Adult. The adult emerges from the pupal case as a white, elongated thing whose wings are still folded up. After about an hour, the wings will expand and the body will take on its normal shape and coloration. The adult becomes sexually mature after 8-10 hours. After this time, the males chase the females about in an endless quest for mating. Flies can live for up to 3 months, but they are pretty decrepit after 6 weeks or so.

CULTURING DROSOPHILA

We grow flies in vials with about 2 cm of food on the bottom and a foam plug in the top. At the beginning of the semester, the food will be made by the lab preparator Pat McCarthy. However, after the initial sessions you will be required to make your own food using the Instant Food dispensers in the lab room. Empty vials are stored in racks: sometimes a few old dried up pupa cases are still in the clean vials--don't worry, they are dead. The foam plugs are found in the large metal can (garbage can) in the room: they are necessary to keep foreign flies out!

To make Instant Food: Put a vial under the spout and turn and handle all the way back then all way the forward. Sometimes it helps to knock the side of the dispenser beforehand. Then add 3 squirts of water from the dispenser bottle--the food will solidify over a period of 3 minutes or so. Finally, add a small amount of yeast--no more than 10-20 grains. Use the yeast dispenser, but only rotate the handle 1/4 turn or so, not all the way. The food should look like smooth, creamy instant mashed potatoes. If the food looks dry and flaky, it needs more water! Flies die quickly if they are dehydrated.

Food Problems. If your flies aren't growing well, one of these is probably the reason.

1. Too much yeast. If the yeast forms a solid layer over the food, the flies won't lay their eggs and they will get mired in it. Transfer the flies to a fresh vial, and don't add any yeast--they will carry enough over on their feet. To say this again--it is never really necessary to add yeast because the flies always bring enough over from their old culture. However, a little bit extra helps them grow bigger and stronger.

2. Mites. Very tiny creatures that attach themselves to the legs and backs of the adults. They like old food, so be sure to throw out vials more than a month old. To get rid of them, you can transfer the adults to fresh vials daily for 3-4 days just like for the mold. If things get really bad, we can make up mite strips that you can put in the vials to kill the mites. [Embarassing social problem](#)

3. Wet food. Flies can't swim, so make sure there are no puddles. Sometimes the food is too wet from being stored in the cold. The best solution is to put small pieces of paper towel in the vials to absorb the water and give the flies something solid to stand on

4. Dry food. This is the biggest problem with Instant Food. If the air is too dry (as it often is in the winter), you might need 3 1/2 squirts of water rather than 3. Maybe even 4 squirts. If the food looks flaky, add a little water from the squirt bottles until the food looks creamy, like mashed potatoes. This is rarely a problem with the already made food.

Incubators. Flies grow well at room temperature, although they like 25°C best. You can keep them in your drawer (or grow them at home). However, if you take them home, don't let them freeze or cook on the way. Also, keep them away from inquisitive pets, small children, roommates, etc. Development can be slowed by growing them at 19°C--there is an incubator for this purpose in the room. This is also useful for collecting virgins--see below.

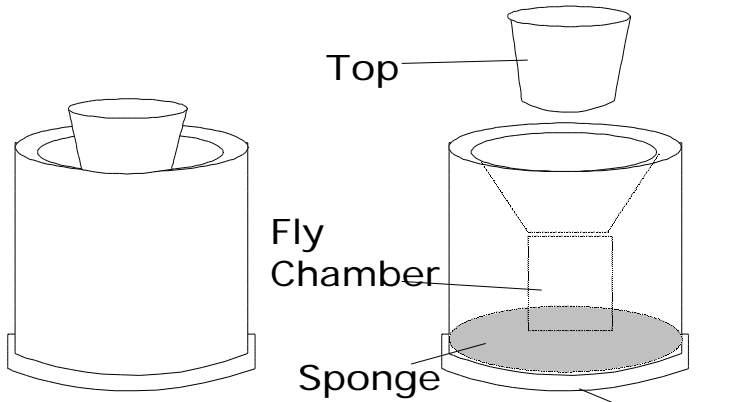
Disposing of Old Cultures. If a vial is more than 1 month old, it is probably dried up and dead. The mites love it, however, so you should get rid of it. Remove all rubber bands and tape (don't use tape ever!) and make sure the plug is in. Then, put them in the discard rack. Unplugged vials cause flies to be loose in the lab, and they can get into your vials and contaminate them. So, plug any vials you find.

WORKING WITH FLIES

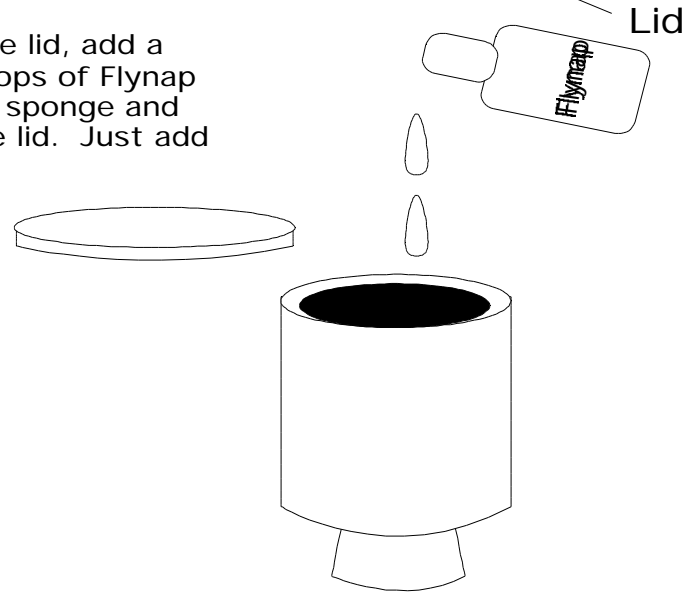
Anesthetizing Flies. To examine the flies, you need to anesthetize them--otherwise they'll fly away! [Freddy after too much FlyNap](#)

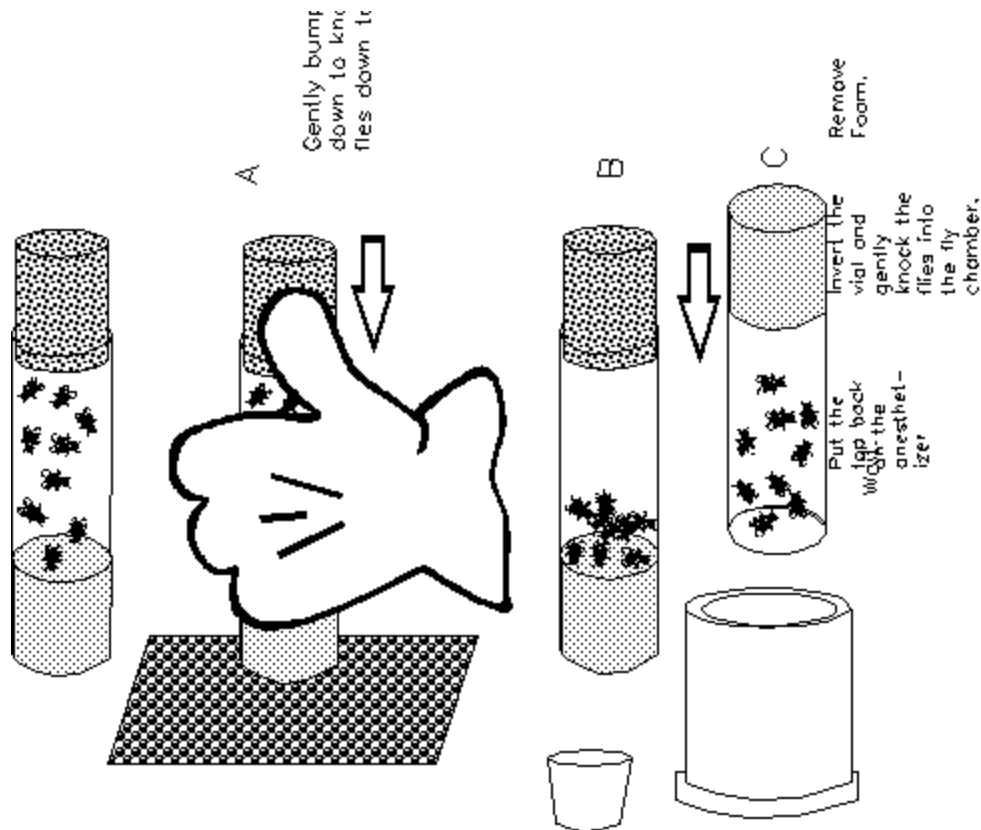
The anesthetizer is a plastic gadget with a funnel-shaped opening in one end--this goes over the vial of flies. On the other end is a foam reservoir with a cap. Put several drops of FlyNap on the foam, then close the cover.

Get the flies into the anesthetizer by banging the vial to get all the flies on the bottom, then inverting it over the anesthetizer and banging again to knock the flies down. Once the flies are in the anesthetizer, close it up with the plastic cap. After 2 or 3 minutes, the flies will stop moving. You can then shake them out onto a white card for examination. They should stay unconscious for 10 minutes or so.



Remove lid, add a few drops of Flynap on the sponge and replace lid. Just add Flies.





If the food is very wet or loose, you can transfer the flies to an empty vial first--they will walk or fly up into it as a natural reflex. Then bang them down and put them in the anesthetizer.

It is possible to kill the flies if you leave them in the anesthetizer for too long. When they die, their wings get folded straight up over their bodies instead of the normal straight back position.

While the flies are unconscious, don't put them on fresh food--they can get mired in it and drown. It is better to put the flies on the side of the vial (with the vial lying on its side) until they wake up. You can also put them temporarily in a clean vial with no food until they awaken.

Looking at Flies. Use the stereomicroscope. The power is adjustable and so is the focus. Start at a low power to get the feel of the microscope, then zoom in to get the details. For most things (sorting and counting), somewhere between 10 and 15 x (1.0 to 1.5 on the dial) works well--but experiment to find what works best for you. Move the flies around with a dissecting needle or a paintbrush or a forceps--your personal taste.

When you are done looking at them or sorting them, you can put the flies back in the vial--but remember to not put unconscious flies on fresh wet food (see above). Or, if you don't need the flies any longer, drop them into the morgue--the oil-filled glass dishes on

the benches. Here they will die peacefully in their sleep and ascend directly to Fly Heaven.

Stockkeeping. Keep all of your initial stocks going until you are completely done with the experiments. Disaster can strike at any moment, and you need to be able to start your experiment again from scratch. Keep up the stocks by transferring them to fresh food every two weeks. You can also split the stocks into 2 or 3 vials. After the vials are a month old, throw them out.

Transferring Flies to Fresh Vials. For stockkeeping, you don't need to anesthetize the flies. Instead, bang the old vial a few times to get all the flies on the bottom, then pull out the plug and cover the opening with a new vial. Invert the whole thing, then bang them so the flies fall from the old vial into the new vial. Then, pull the vials apart and quickly plug them both. Don't worry if a few flies get away--it's normal--but try to keep the numbers down. This takes a little practice, but you will get good at it.

CROSSES

The most difficult and time-consuming aspect of this lab is making sure you have virgin females to start your crosses. [The truth about fly mating](#)

Virgin females. Flies don't use birth control, so if a female has mated, she's pregnant. Also, females store sperm enough for a lifetime's worth of eggs, so you must prevent any mating other than the one you want. Flies have no morals either, so if a female is old enough to mate and there's a male present, she's going to be pregnant. Thus, it is necessary to isolate the females before they are old enough to mate. At 25°C this means before the flies are 8-10 hours old.

To collect virgin females, first remove all the adults from the vial. Kill any that don't come out easily by pushing them below the surface of the food. Make absolutely sure there are no live adults left in the vial. Then, wait 8 hours for more flies to hatch out. Anesthetize the young flies, separate out the females, and put them on fresh food.

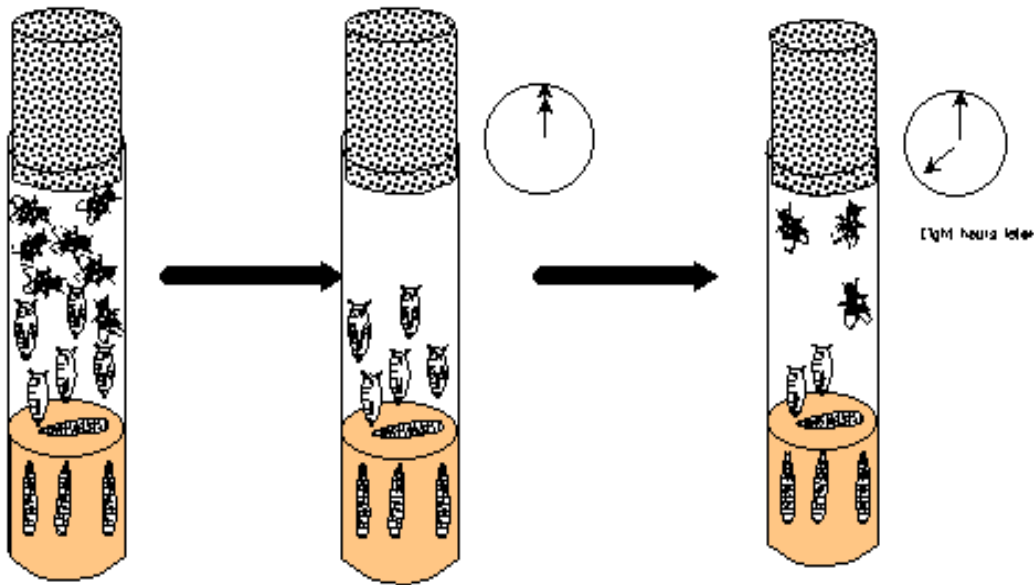
A trick for collecting flies overnight: Since you probably don't want to live in the lab, you can slow development of the flies down by putting them in the 19°C incubator overnight. At this temperature they will stay virgins for about 16 hours. Thus, you can follow a schedule like:

9 a.m. Take flies from 19°C incubator, separate the virgin females. Put the vials into 25°C.

5 p.m. Take the flies from 25°C, separate the virgin females. Put the vials into 19°C overnight.

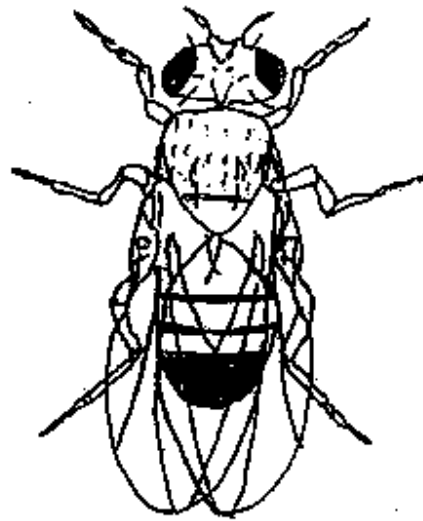
To be sure the flies really are virgin females: let them sit in a vial for 3-4 days. Then, look at the side of the vial where the top of the food meets the glass. If any of the flies was not virgin, first instar larvae will be visible here, crawling and eating. Note that even

virgin females lay eggs (just like chickens), but they don't hatch.

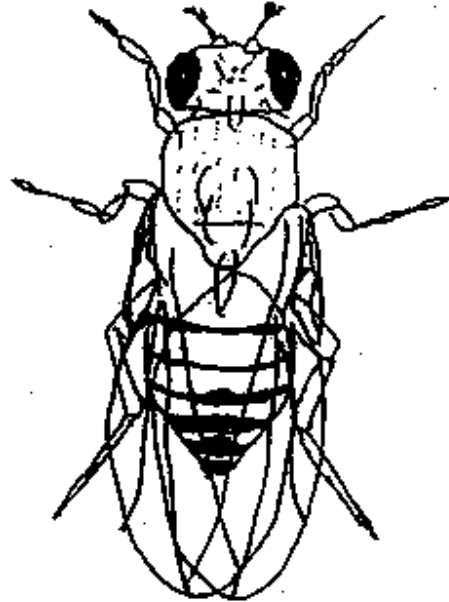


Telling Males from Females. The most reliable method is to place the flies (anesthetized!) on their backs and examine their genitalia. The males have a distinctive set of orange or brown structures, the genital arch and anal plate, while the females have just a bump at their rear end.

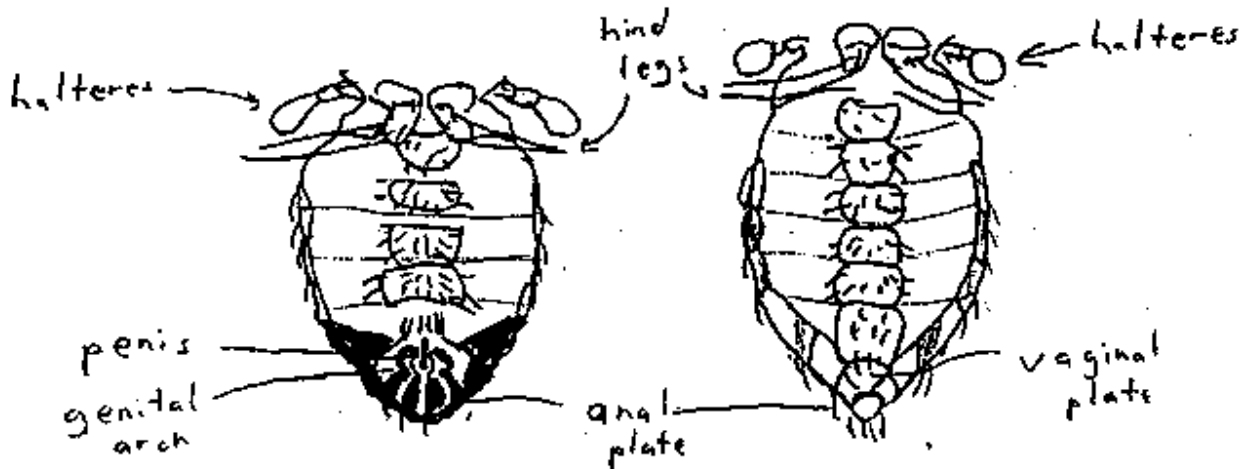
Secondary methods of distinguishing the sexes: the abdomen of the male is black on the last few segments on the top (dorsal) side, while the female is striped all the way down. Also, the male abdomen is shorter and rounder than the female. Finally, male flies have a "sex comb" on their forelegs, about 2/3 of the distance from the body to the foot. This structure is a group of dark, thick bristles--females never have them. However, you need high magnification to see them.



male, top view



female, top view



male abdomen

||

female abdomen

Setting up a Cross. Put 2-4 flies of each sex in a fresh vial. If the flies are unconscious, don't put them directly on fresh food or they'll drown! More males than females ensures fertilization. Set up several vials for any cross and keep your records for them separate. That way, if any contamination (like a stray fly of the wrong genotype or a non-virgin female) gets in, only one vial will be ruined, not the entire experiment. To get large numbers of offspring, transfer the parents to fresh vials every 3-4 days so they can lay more eggs. Don't leave the parents in the vial more than 9 days, or you will get offspring hatching out and mixing with the parents, which is usually disastrous.

Labeling. Use your Hydro-marker to label each vial with the stock or cross it contains,

and the date you set it up. Use rubber bands to hold together similar vials. You can also make small cardboard tags to attach to the rubber bands. DO NOT use tape on the vials! Also, DO NOT use felt tip markers--they don't always wash off.

Bookkeeping. The essence of genetics is keeping careful records. Keep a notebook! Spiral-bound is probably the best. Record every vial as you set it up, when you transfer or remove the parents, and when you count them. Record all counts for each vial separately, then pool the data for your lab write-up.

NOMENCLATURE

--Wild type is +

--If a mutation is recessive to wild type, it starts with a small letter e.g. w for white eyes. The dominant wild type allele is w+.

--If a mutation is dominant to wild type, it starts with a capital letter e.g. Sb for Stubble bristles. The recessive wild type allele for this gene is Sb+.

--Genes on the same chromosome are listed in a row e.g. y w f means that yellow, white, and forked are all on the same chromosome. The same chromosome also has a large number of wild type genes on it, which are not listed. However, in some cases you can put them in e.g. y w f cv+ is mutant for yellow, white, and forked, and wild type for cv (crossveinless). The exact same chromosome could also be designated y w f.

--Genes on opposite homologues are separated by a slash (/) e.g. y w f/cv means that one X chromosome has the mutant yellow, white and forked alleles, while the other X has the mutant crossveinless allele.

--Genes on separate chromosomes are separated by a semicolon (;) e.g. v;bw means that v (vermillion) is on the X chromosome and bw (brown) is on the second chromosome. It is not necessary to designate chromosomes you aren't interested in.

--Males have only 1 X chromosome, with no genes on the Y. So, a male with w on its X would be designated w/Y. The male is "hemizygous" for w. The homozygous female is w/w or just plain w. The heterozygous female is w/+ or w/w+.

LAB HOUSEKEEPING

Keep the place neat and clean. Don't let too many flies loose. Don't leave uncapped vials or other junk lying around. Keep the food area and the discard area neat.

Microscopes. You will be sharing a microscope with 3 or 4 others, so take care of it. Tell the TA or the instructor if it isn't working properly. During your scheduled lab period, you have absolute priority over the microscope. Other times, it's first come, first served.

Security. To be blunt about it, some students (a small number) will happily steal your flies and others will happily destroy your experiments. The instructor will show no mercy to anyone caught doing either of these things. To protect yourself, you can code your vials--just don't forget the code! You can also use a code name instead of your own. Be creative--instead of being Joe Smith, be Mystic Warrior or Purple Desert or Freddy Krueger. Finally, you can keep some or all of your cultures in your locked drawer. They will grow fine there, although a little slower than in the incubator.

Room Security. Keep your drawer locked when not in use. You also have a key to let you into the building and room after hours. This makes the instructor nervous. Keep the doors (room and building) locked all the time. You need to pull the building doors closed behind you--air pressure keeps them open otherwise. Remember, the red phone in the lab goes straight to the campus police. If anyone is bothering you, call the cops and let them sort it out. Also, the instructor takes the issue of harassment very seriously, so if anyone bothers you verbally, physically, or even by looking at you funny or smelling bad, tell the instructor as soon as you can and he will deal with it. This lab must be conducted in a civilized fashion, especially at night.

Keys. Keys are a major problem: the Biology Department doesn't want a lot of stray building keys floating about. There are several levels of dealing with this problem: First, you paid a deposit for the keys, so don't lose them. Second, you won't get your grade until your keys are returned--you will get an Incomplete instead. Finally, an encumbrance will be placed on your records, so you won't be able to register for classes until

you return them. See the instructor if there is a problem.

LAB REPORTS

The syllabus shows the dates when the lab reports are due. You will lose points for late reports.

The lab reports should be concise and short. Good grammar and spelling are expected. The reports should be typed or neatly handwritten, with neat diagrams. The reports should include:

1. Introduction. What did you do? What crosses were involved, genotypes, phenotypes, sexes. Use diagrams. Explain any basic principles involved, such as sex linkage, epistasis, crossing over, etc.
2. Results. What did you observe? Numbers of flies of each phenotype counted in each generation.
3. Data Analysis. What did you expect? Compare the observed numbers with the expected. Use Punnett squares, chi-square statistical tests, coefficients of coincidence,

etc.

4. Discussion. Briefly state the conclusions of the results and analysis. Explain any deviations from expectations. Be mercifully brief.

FINAL WORD

There is no doubt that this lab will be one of the longest and most tedious labs you will have at NIU. The Biology Department thinks that it is important for you to have a lab experience of this nature so you can understand that science is more than memorizing and regurgitating lectures, and more than having brilliant ideas. To minimize the effort, be neat and well organized--just keep grinding away at it. Also, you have the opportunity to get to know one another, as well as the TA's and the instructor. These contacts might be able to help you throughout your career in biology, so make the most of them. If all else fails, this lab will provide good horror stories to scare younger students or your grandchildren. Good Luck!

EXERCISE NUMBER ONE

SEX-LINKED AND AUTOSOMAL INHERITANCE, AND GENE INTERACTION

(WHITE EYES)

INTRODUCTION: [The relationship between white eyes and eternal love](#)

The normal brick-red eye color of *Drosophila* is due to the presence of two separate pigments in the eyes, a brown pigment and a red pigment. The two pigments are made by separate biochemical pathways, and mutations can prevent the formation of either pigment without affecting the other pigment at all. Thus, if a mutation prevents the synthesis of the red pigment, only the brown pigment is made; this mutant fly has brown eyes. Similarly, if the brown pigment pathway is blocked, only the red pigment is made and the fly has bright red eyes. If both pigment pathways are blocked, neither pigment is made and the fly has white eyes.

In this experiment you will deal with several different mutants. All of these mutants are recessive; wild-type (+) is dominant.

-- Brown (**bw**) prevents synthesis of the red pigment, giving flies with brown eyes.
--Vermillion (**v**) blocks the brown pathway, so **v** flies have bright red eyes.

-- Scarlet (**st**) also gives bright red eyes; the **st** gene is on a different chromosome from **v**.

-- White (**w**) mutants block both pigment pathways, giving white-eyed flies; **w** is on the X chromosome.

The pigment pathways are blocked independently, and only when the mutants are homozygous. Blocking both pathways gives white eyes.

EXPERIMENT:

You will be given four stocks of flies, a wild type (**OR**) and three white eye stocks:

1. **w** stock. The white eyes in this stock are due to a single mutation in the white gene, located on the X chromosome.

2. **v**; **bw** is homozygous for **v** (vermillion) on the X and and **bw** (brown) on chromosome 2 (an autosome). Since 2 separate mutations block the two pigment pathways, the eyes are white. However, the F₂ will show white, bright red, brown, and wild type eyes because most F₂'s will not be homozygous for both mutations.

3. **bw**; **st** is homozygous for **bw** (brown) and **st** (scarlet). Both of these mutations are on autosomes; **bw** is on chromosome 2 and **st** is on chromosome 3. The

F2 of this stock will show the same 4 colors as **v; bw**.

4. OR is the wild type stock. OR stands for Oregon-R, a strain of flies captured in Roseburg, Oregon in 1925. It has been maintained in the lab since then. The eye color is brick red.

The three stocks have code numbers; your job is to figure out which stock has the **w** mutation, which stock is **v;bw**, and which stock is **bw;st**. The key to this exercise is that genes on the X chromosome segregate differently from genes on the other chromosomes: genes on the X are sex-linked.

DETAILS:

1. Set up 3 vials containing 2-4 virgin white eye females and 2-4 wild-type males for each of the 3 white stocks.

2. Remove parents after 7 days.

3. Count at least 30 F1 offspring from each unknown, classifying by sex and eye color. Make a separate count for each vial. Be sure you can tell the eye colors apart! Examine both sexes side by side--if you look at your Punnett squares you will note that the females are always wild type, but the males are white, bright red, or wild type, depending on the cross. This happens because the male only has 1 X chromosome, so it expresses any mutants on that chromosome. You should be able to tell which stock number contains which stock after examining the F1.

4. Set up at least 5 vials containing 2-4 pairs of F1 flies for each of the 3 white crosses. Discard the parents after 7 days to avoid genetic contamination.

5. Count at least 200 F2 offspring for each of the 3 white crosses. Score the flies for eye color and sex. Your lab notebook should have separate counts for each vial. This will allow you to tell if one of the vials was contaminated. Pool the data for your lab report. Also, for the F2, combine the numbers of males and females for the analysis.

IF YOU ARE COLORBLIND--just score dark eyes vs. white eyes. The dark eyes will include wild type, vermilion, scarlet and brown. The F2 ratios will differ enough for you to identify the lines. However, you must count 400 of each F2 to be certain of which vial is which.

LAB REPORT:

General comments:

--Reports are to be typed, except for difficult tables and diagrams.

--Points will be taken off for poor spelling, grammar and punctuation, for incorrect usage of genetic terminology, and for sloppiness.

--Use genetic symbols properly: "/" separates homologues, ";" separates different

chromosomes, "Y" is the Y chromosome, mutants are written in small (not capital) letters, wild type is +

--The report should include the following sections:

1. Introduction--Brief intro. What is the experiment about.

2. Methods:

- Summary of the crosses you did: for each generation, the number of vials set up, the number that produced offspring, and the number examined and/or counted.
- Crosses drawn properly.
- Punnett squares for F1 and F2, with statement of expected phenotype ratios. A total of 6 Punnett squares: F1 and F2 for each of the 3 crosses.

3. Results and Analysis:

- Overall counts of flies of each phenotype in each cross, both F1 and F2. For the F2's, pool males and females--don't count them separately.
- Chi-square tests for F2's. Carry the calculations to 2 decimal places only. Show your work. Do the observed ratios fit the expected?
- Identify which genotype goes with which stock number. Don't be wrong!

4. Discussion:

- Explain how genes interact to get observed phenotypes, X vs. autosomal segregation, phenotypes of the individual genes.
- Whether your data support the null hypothesis for each cross; what you could do to further test the cross if your data don't fit the null hypothesis.
- Any problems you encountered.
- Be mercifully brief!

Chi-square formula:

- The observed (obs) number of F2 offspring in each class comes directly from your data. The expected number of offspring for each class comes from multiplying the expected fraction from the Punnett square by the total number of offspring. You need to determine the observed and expected numbers for each phenotypic class (different eye color) present in the F2 for each unknown.
- As an example, say you have counted 547 F2 offspring, of which 91 have brown eyes. The observed number in the brown class is 91. From the Punnett square you determine that 3/16 of them should have brown eyes. The expected number

of offspring in the brown class is $547 \times 3/16 = 102.6$.

- The chi-square formula is:
$$\frac{(obs - exp)^2}{exp}$$

You must calculate the chi-square value for each class of offspring, then add them to get the chi-square value for the experiment. The critical values for the chi-square distribution are given in the table below.

A very important point concerning the chi-square test: it is designed so that 1 time out of 20 (on the average) perfectly correct, properly performed results will be REJECTED by the test. That is the meaning of using a probability value of 0.05: there is a 0.05 (5%) chance of rejecting the null hypothesis even when it is true. So, don't be alarmed if your data doesn't fit the expected chi-square value.

Table. Critical Values of the Chi-Square Distribution.

df	P = 0.10	P = 0.05	P = 0.01
1	2.706	3.841	6.635
2	4.605	5.99	9.210
3	6.251	7.815	11.345
4	7.779	9.488	13.277
5	9.236	11.070	15.086
6	10.645	12.592	16.812
7	12.017	14.067	18.475
8	13.362	15.507	20.090
9	14.684	16.919	21.666

Further values can be found in your textbook.

EXERCISE NUMBER TWO

CROSSING-OVER

Social Difficulties

INTRODUCTION

Crossing-over (recombination) occurs during the first meiotic prophase. On the average, there is one recombination event per chromosome arm in *Drosophila*. In the case at hand, there is an average of one crossing-over per X chromosome. However, some flies have 0 recombinations, some have 2, and occasionally some have 3. The frequency with which 2 genetic markers recombine (that is, have a cross-over between them) is proportional to the distance between them. Thus, recombination can be used to map genes on a chromosome.

If two genes are one map unit (centimorgan) apart, then 1% of the offspring of a suitable cross will be recombinant. If two genes are unlinked (on separate chromosomes or far apart), they will recombine 50% of the time: the other 50% of the flies will be non-recombinants.

Crossing over only occurs in female *Drosophila*, not in males. Therefore, you will examine the offspring of females heterozygous for different marker genes, in order to figure out the order of the genes and the distances between them. You will need to count a lot of flies.

You will be looking at genes on the X chromosome, because the X chromosome is hemizygous in males, so it is easy to score the results of crossing-over by looking at the males (only) in the F₂.

EXPERIMENTAL DETAILS

This is a two part experiment: 1. Triple mutant x single mutant (normal crossing-over), and 2. triple mutant x inversion (crossing over with an inverted chromosome region).

Part 1. Triple mutant stock x single mutant stock.

You will be given two stocks initially: one with 3 mutant genes and one with 1 mutant gene. Different stocks will be given to different students. **PLEASE DON'T TRADE FLIES OR DATA--YOU WON'T GET ANY CREDIT IF YOUR DATA DOESN'T MATCH THE FLIES YOU WERE GIVEN!!!**

1. Make sure you can score the mutant phenotypes.

List of Possible Mutants:

y yellow yellow body color

w	white	white (sometimes pink) eyes
f	forked	twisted bristles
ct	cut	incised rear wing margin
m	miniature	wings shorter than usual
sn	singed	twisted bristles
cv	crossveinless	wings have no cross-veins
v	vermillion	bright red eyes

Look at your flies to be sure you can tell these mutants apart from wild type (OR is wild type). Ask the TA for help if you need it.

2. Mate virgin females from one stock to males of the other stock. Do this both ways: triple mutant females by single mutant males and triple males by single females. Set up at least 5 vials and remember to clear the parents after 7 days.

3. When you get the F1 flies, transfer some of them to fresh vials (2-4 of each sex) to get the next generation. There is no need for virgin females or for anything else fancy at this point. Set up lots of vials--you need to count at least 300 F2 males (females aren't used)--10-15 vials is probably a good number.

4. When you get the F2 offspring (of course by this time you remembered to clear the parents after 7 days without needing to be reminded), score each male for the presence of the mutant genes. Count at least 300 males. Don't count the females: because females have 2 X chromosomes, mutant genes that are heterozygous will not be expressed. Score each vial separately--in case by some chance one vial was contaminated.

Part 2. Triple mutant x inversion.

You will be given a stock whose X chromosome contains a small inversion. The stock is marked with scute (sc), which eliminates some of the bristles--it may be difficult to see, but you don't need to score it. The inversion should almost completely eliminate recombination nearby, but not affect distant markers too much. Inversions prevent crossing over by disrupting the pairing of homologues and by giving non-viable offspring if crossing over does occur.

1. Perform this experiment exactly as in part one, substituting sc for the 1-mutation stock. Cross sc flies to flies of the 3-mutation stock.

2. Set up 10 vials of F1 to get the F2.

3. Count at least 300 male F2.

4. Analyze as in Part 1--except that you only need to consider 3 genes, so the analysis is simplified. You don't need to score or analyze the sc mutation at all--just ignore it.

DATA ANALYSIS

1. Organize your classes of offspring into reciprocal pairs.

2. Calculate the genetic distance between each pair of genes. Be sure to count all the recombinants for each pair: there are 8 classes of recombinant for each pair of genes in part 1, but only 4 classes with the inversion.

3. Make a tentative map based on these distances (a separate map for part 1 and part 2).

4. Check your gene order by looking at the genes in groups of 3. The double cross-over classes should be the smallest classes.

5. Calculate the coefficient of coincidence and interference for all 3 regions. That is, if the genes are a--b--c--d, you need to calculate for a--b + b--c, for a--b + c--d, and for b--c + c--d. Only one coefficient of coincidence and interference is necessary for part 2.

6. Compare the maps from part 1 and part 2 by determining the ratio of crossovers for each region. For example, what is the map distance for the a--b region in the inversion cross as compared to the distance in the normal cross? From this estimate the extent of the inverted region.

LAB REPORT

.

1. Introduction. What is crossing-over, interference? How can crossing-over be used to map gene loci on a chromosome? What is an inversion and how does it affect crossing-over and map distances?

2. Methods. Brief procedure and why only F2 males are counted. Punnett square for F1 but not F2. Description of the traits you scored.

3. Results.

--diagram of the the crosses, giving genotypes.

--table of all F2 males counted, organized by reciprocal classes. Include all possible male phenotypes, even those types you didn't see any of.

--table of map distances between all 6 pairs of genes.

--a nice map, showing all the distances, with the genes in the proper order.

--observed number of double crossovers, expected numbers, coefficients of coincidence and interferences, for ALL THREE pairs of intervals. Give the formulas you used for the calculations.

--No chi-square or "expected" values are needed.

--a second map for the inversion.

--How much was crossing-over reduced in each interval by the inversion? Estimate the extent of the inversion.

4. Discussion. How you constructed your map. How the interference values fit the map. How did what you found compare to what you expected, including sources of error.

EXERCISE NUMBER THREE

BACTERIAL CONJUGATION

The source of our gut instinct

INTRODUCTION

This exercise will take up the lab period for 3 weeks. In the first week you will count the number of bacteria in a culture of *E. coli* by serial dilution and plating. This experiment will familiarize you with the basic microbiological procedures you will need for the actual conjugation experiment to be done in the second period. The third period will be devoted to counting the plates resulting from the conjugation experiment.

In the main experiment you will map 3 genes in *E. coli* by determining the time that each gene enters the recipient during conjugation. The genes are his, pro, and trp; they are biosynthetic genes for histidine, proline, and tryptophan. The Hfr (donor) is his⁺ pro⁺ trp⁺ and the F⁻ (recipient) is his⁻ pro⁻ trp⁻.

It is also necessary to select against the Hfr itself (so only the F⁻ exconjugants grow). To do this, streptomycin is used to kill the Hfr's. Thus, the F⁻ is str^R (resistant to streptomycin) and the Hfr is str^S (streptomycin sensitive).

In the original work, conjugation was stopped by putting the bacteria in a Waring blender. This isn't so practical for us, so we are using an alternative system, where naladixic acid stops the conjugation by killing the Hfr. The Hfr is thus nal^S and the F⁻ is nal^R (otherwise, the naladixic acid would kill the recipient).

The actual cross can be written:

Hfr: his⁺ pro⁺ trp⁺ str^S nal^S x F⁻: his⁻ pro⁻ trp⁻ str^R nal^R

To count the number of bacteria containing the various markers, the bacteria are plated onto selective media. The plates contain minimal medium plus two of the three required nutrients. For example, to count the number of his⁺ bacteria (ignoring the other two markers), plates containing proline and tryptophan are used. Since no histidine is present, his⁺ bacteria will grow but his⁻ bacteria won't. Since tryptophan and proline are in the medium, both trp⁺ and trp⁻ bacteria, and pro⁺ and pro⁻ bacteria will grow. These plates will only determine the number of his⁺ bacteria.

There are 3 types of selective media used. The plates can be distinguished by colored stripes on the side.

1. proline/ tryptophan plates for counting his⁺
2. proline/ histidine plates for counting trp⁺

3. histidine/ tryptophan plates for counting pro+

In addition, a fourth type of plate containing minimal medium only is used as a control. Nothing should grow on these plates.

All plates also contain streptomycin and naladixic acid to kill the Hfr's and stop conjugation, as discussed above.

Conjugation takes place over a period of time (100 minutes under good conditions), with different markers entering the F- at different times. Thus, this experiment involves mixing the Hfr with the F-, then stopping conjugation at various time points and plating the bacteria on selective media.

TITERING A CULTURE

The first week's experiment is designed to familiarize you with basic microbiological lab procedures. You will be given an E. coli culture, perform serial dilutions on it, then plate the bacteria and finally count them. This procedure is called "titering" a culture. See Figure 1 for a visual depiction of the procedure.

1. Serial dilutions.

a. Take 9 culture tubes containing 9 ml of water. Arrange them in a row. Using a pipet, sterilely transfer 1 ml of bacteria from the original culture into the first tube. Vortex at low speed or slap the bottom of the tube to mix. This tube now contains bacteria diluted 10x (10^{-1}).

b. Using a fresh pipet, sterilely transfer 1 ml from the 10^{-1} tube into the next tube. Mix. This tube is 100x less concentrated than the original culture (10^{-2}).

c. Repeat this process until all the tubes have been used. use a fresh pipet every time! The final tube is 10^{-9} as concentrated as the original culture.

2. Plating.

a. Pipet 1/10 ml of the 10^{-9} tube onto a agar plate.

b. Flame the glass hockey stick to sterilize it.

c. Spread the bacteria out in an even layer with the hockey stick.

d. Repeat with the 10^{-8} , 10^{-7} , and 10^{-6} tubes. DON'T plate the higher concentrations.

e. Allow the plates to dry before putting them in the incubator.

3. Counting.

a. After the plates have incubated overnight you can count them. The TA may have put them in the refrigerator for storage .

b. Choose plates with 30-300 colonies on them to count.

c. Using a marker, touch the back of the plate on top of every colony as you count it.

4. Figuring the bacterial concentration in the original tube.

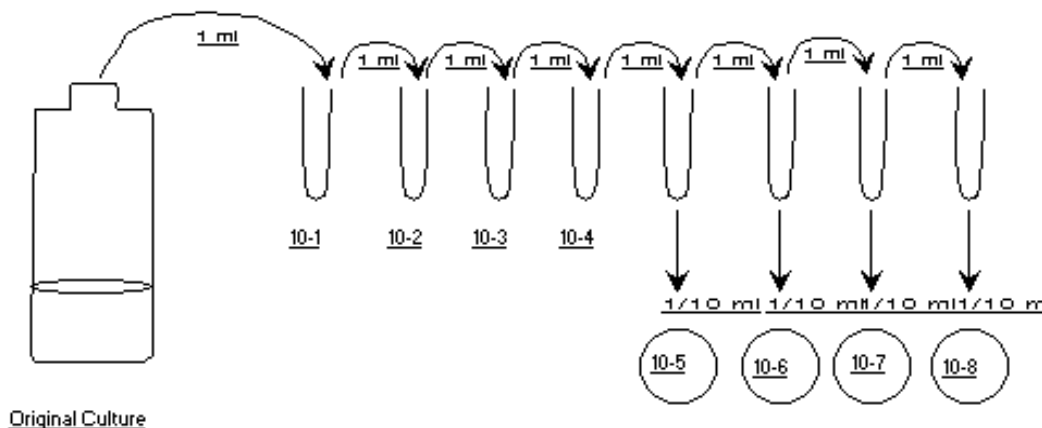
a. The concentration of bacteria is measured as the number of bacteria per ml.

b. The number of colonies on a plate is equal to the number of bacteria that were in 1/10 ml of the tube you got them from. Thus, the number of bacteria per ml in that tube is 10 times the count.

c. To get the concentration in the original tube, multiply the concentration for the plate you counted by the dilution factor for that tube. For example, if you counted a plate from the 10^{-6} tube, multiply the concentration by 10^6 .

d. Example: You count 84 colonies on the 10^{-7} plate. Since 1/10 of a ml was put on the plate, the concentration of bacteria in the 10^{-7} tube is 840 per ml. Multiplying by the dilution factor, you find the original culture had 840×10^7 bacteria per ml. This could be better written as 8.4×10^9 .

Serial Dilution and Plating Scheme



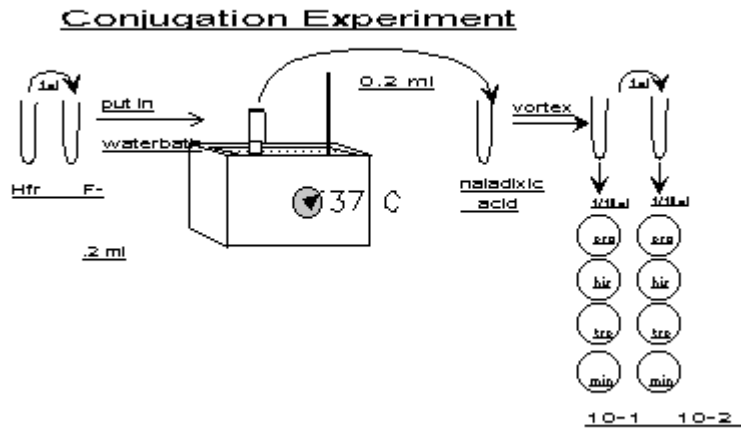
CONJUGATION

This experiment will be started in the second lab period. This experiment will take 2 hours or more, so be prepared in advance and get started right away. The basic procedure is to mix the two strains of bacteria and allow them to grow at 37^o. At different time points, a small sample is removed and put into a naladixic acid buffer to stop conjugation. Then samples of this are spread onto the proper Petri plates at various dilutions. The bacteria are allowed to grow for 48 hr, and then the resulting colonies are counted. The number of colonies for each marker is plotted versus time, and from this, the order of genes can be determined. See Figure 2. Each student will be assigned one or two time points to take care of. Each time point will be tested by several students in each lab period, and all the class's data will be pooled and passed out to everyone.

Here is the basic protocol to be followed at your assigned time points:

1. Mix 1 ml of Hfr with 9 ml of F-. Immediately put them in the 37^o water bath and start the timer. This should be done at the very start of class. (This step is done in groups).
2. At the indicated time intervals (0, 20, 40, 60, 80, and 100 minutes) pipet 0.2 ml of bacteria into the tube containing 1.8 ml of naladixic acid buffer. Vortex for 30 sec. Immediately put the rest of the bacteria back in the water bath.
3.
 - a. Pipet 0.1 ml of the naladixic acid bacteria on the plates for that time point. This is the "10⁻¹" dilution, meaning that the tube of bacteria in naladixic acid is 1/10 as concentrated as the conjugation tube (remember you did a 1/10 dilution by putting 0.2 ml of bacteria into 1.8 ml of naladixic acid). Since you only plate 1/10 of a ml, the number of colonies that you count on the plate will be equal to 1 /100 of the number of bacteria per ml in the conjugating culture. There are four types of plate--do one of each type.
 - b. Serial dilution: put 1 ml of the naladixic acid bacteria into 9 ml of dilution buffer (tubes containing 9 ml of dilution buffer are available for you). Then plate 0.1 ml of this onto the four types of plate as in step 3. This is the "10⁻²" dilution--the number of colonies is 1/1000 of the bacteria per ml.
4. Streak out the controls (Hfr alone and F- alone) at some point during the experiment--after things settle down a bit. Do one plate of each type with each of the 2 strains. The exact time isn't that critical for this part. Nothing should grow on these plates. (This step is also done in groups).
5. Let the plate incubate upside down at 37^o for 2 days. The TA will then move the plates into the refrigerator.
6. Count the number of large colonies on each plate, using a marker on the

plastic to be sure you get them all. Ignore air bubbles--if you use the colony counters you will easily see that air bubbles are clear while colonies are opaque white. It is essential that you get a count for each plate type at each time point: don't use "TNTC" (too numerous to count) for both dilutions. Hand in your data to the TA, including your name, the counts for each type of plate, and the time points you did.



Data Analysis:

You will be given data for all the time points from the entire class. Your job is to examine this data, determine the mean and the standard deviation of the counts for each time point, plot the data on a graph, and determine the order of the genes on the chromosome.

1. For each marker, convert the colony counts to cells/ml. Only use the count on one plate, either 10^{-1} or 10^{-2} for each person's data--use the plate with the highest count. This is generally the 10^{-1} plate, but sometimes there will be so many colonies that this plate will be uncountable. In this case, use the 10^{-2} plate.

2. For each marker, plot all the available data for each time point. Look at the data points and decide whether any of them are obviously a mistake. BE VERY CAUTIOUS about throwing out any data points !!! Use all the data unless some particular point is very wrong, for example, if one point has 1/10 or 10 times as many colonies as any other. Generally you should use all the data, but you must exercise your judgement here to find data that is clearly attributable to experimental error.

3. Determine the mean and the standard deviation for each marker at each time point. The mean is simply the average number: the sum of all counts for that marker and time point divided by the number of data points. The standard deviation is:

$$\sqrt{\frac{(X_i - \mu)^2}{n - 1}}$$
 where X_i is each individual count (for that time point and marker), μ is the mean, and n is the number of counts. The \sum means to take the sum for all data points.

4. Plot the data. The x-axis is time in minutes, and the y-axis is cells/ml. The mean for each time point should be plotted, and error bars should extend above and below the mean point for a distance of one standard deviation. For instance, if the mean at 60 min is 52 cells/ml and the standard deviation is 13, there should be a point at 52 cells/ml with a vertical bar extending from 65 (= 52 + 13) to 39 (= 52 - 13).

5. Draw a line through the means. You can smooth the line if you like. The line will not be straight--it is basically logarithmic. Extrapolate the lines back to 0 to determine the initial time of entry for each marker. You will end up with one graph with 3 lines on it. ((Advanced analysis--plot a regression line for the data after linearizing it by using the logarithm of time))

6. Draw a rough map of the E. coli chromosome, showing the positions of the markers and the Hfr.

EXERCISE NUMBER FOUR

PLASMID MAPPING

INTRODUCTION

In this lab you will map a bacterial plasmid. This plasmid is a small circle of DNA that grows in *E. coli*; it is sometimes used in genetic engineering work. You will locate several sites in the plasmid DNA where restriction enzymes cut. The restriction enzymes cut the DNA only at particular base sequences, and the enzymes you will be using will cut 1, 2, or 3 times in the DNA circle. Because these enzymes cut the DNA in defined locations, they make good markers for the DNA.

Very important message below. Be careful--we are using some dangerous things here.

WARNINGS!!!!

DANGEROUS THINGS

WARNINGS!!!!

1. Electricity in the electrophoresis gels. Always keep the gel boxes covered and your fingers out when the power is on.
2. Ethidium bromide, a potential carcinogen, used as a DNA stain in the gels. Always wear gloves when handling the gels.
3. Ultraviolet light from the light box used to examine the gels after they have run. UV can burn your retinas. Always keep the plastic shield between the light box and your eyes (or use goggles).

Other cautions:

1. The pipettors are expensive and fragile. Don't drop them or treat them roughly.
2. The DNA and enzymes are also expensive--be frugal and careful with them.
3. You will be working in small groups--make sure everyone does some of the work. Also, exchange names and phone numbers so everyone gets a copy of the

data.

4. The procedure has many steps--be organized and prepared. That is, read it over before you do it.

The basic procedure is to digest the DNA with each of these enzymes separately and also with each possible combination of 2 enzymes (single and double digests). The enzymes break the circular DNA molecule into one or more linear fragments of DNA, whose size depends on how far apart the restriction sites are. The DNA must be mixed with the enzyme plus a buffer solution, then incubated for several hours at the proper temperature (37°C).

The digested DNA is then mixed with a dense dye solution ("Blue Juice"), which helps in loading the DNA on the gel and then helps track the movement of the DNA down the gel. Electricity is then applied, and the negatively charged DNA moves towards the positive pole. Due to friction in the gel, smaller DNA fragments run faster than larger fragments. The distance each band has migrated can be converted into size (in kilobases) by comparison to standards of known size that are run on the same gel. The size standards are called Hind-lambda: they come from DNA of bacteriophage lambda that has been digested with Hind III.

Although we may change the restriction enzymes to be used, currently we are using:

Pst I: cuts at DNA sequence CTGCAG

Pvu II: cuts at CAGCTG

Hind III: cuts at AAGCTT

These enzymes will cut the DNA only when incubated at the proper temperature and pH, with appropriate levels of Na⁺ and Mg²⁺ ions, as supplied in the buffer solution.

List of the digestions to be done:

1. Pst I
2. Pst I + Hind III
3. Hind III
4. Hind III + Pvu II
5. Pvu II
6. Pst I + Pvu II

Schedule:

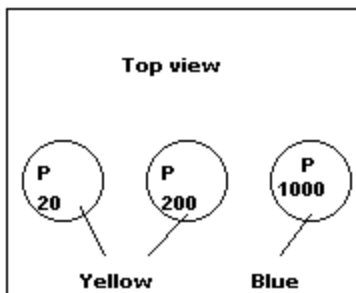
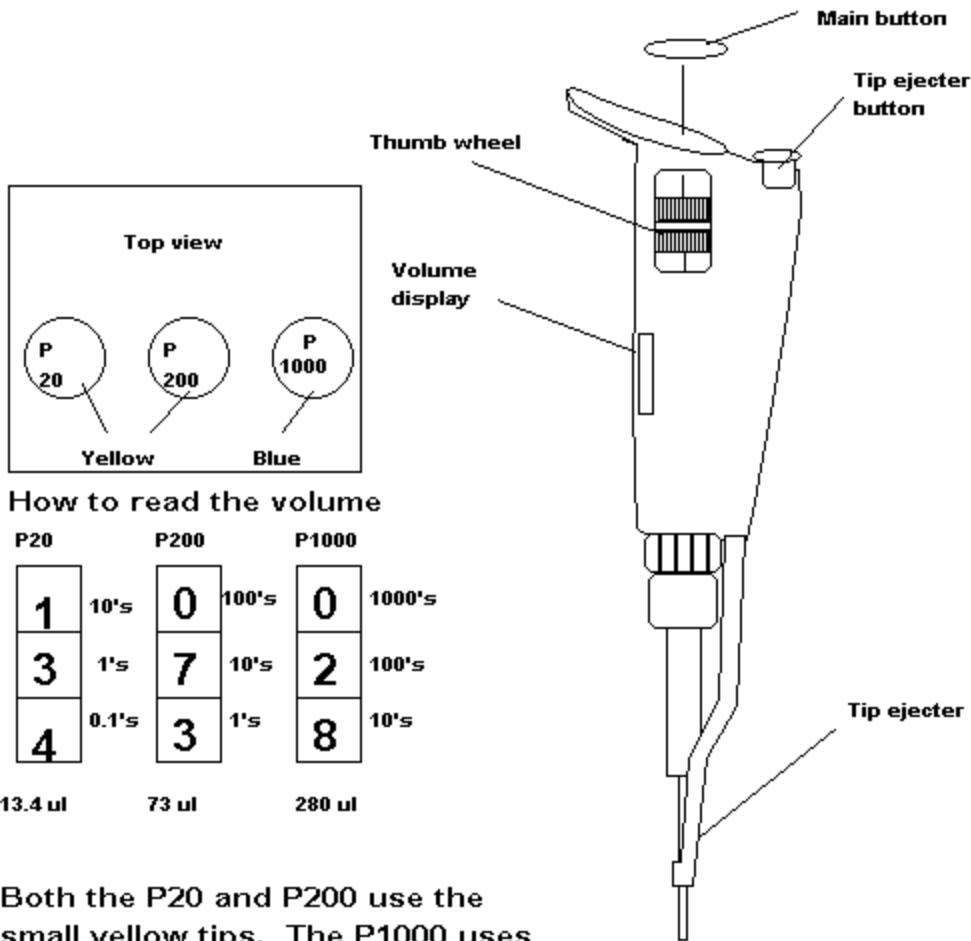
Week 1. Learning to use the pipettors, then digestion of the DNA. The TA will demonstrate the pipetter, but you should look at the diagrams on the following pages first. Each person will set up 2 or 3 digestions, assigned by the TA. The digestions are run at 37°C for several hours, so you will leave them for the TA after you have them set up in the water bath. The TA will store them in the freezer until next week.

Week 2. Pouring and running electrophoresis gels. Each group will pour its own gel, load the samples, and start the run. Electrophoresis takes 2-3 hours, so the TA will stop the electrophoresis and photograph the gel for examination next week.

Week 3. Analysis. You will measure the distance each band has migrated on the gel (using the photograph). The TA will make sure everyone has a set of relatively clean data. You will also get a lecture on converting migration distances to band sizes, and on creating a map from the data.

Pipeters

Pipeters come in three sizes: P20, P200, and P1000.



How to read the volume

P20	P200	P1000
1	0	0
3	7	2
4	3	8
10's	100's	1000's
1's	10's	100's
0.1's	1's	10's
13.4 ul	73 ul	280 ul

Both the P20 and P200 use the small yellow tips. The P1000 uses the larger blue tips.



Detailed Instructions:

Digestion.

1. Use the felt marker to label eppendorf tubes with the digestions you have been assigned.

**** Actual amounts to be pipetted in steps 2-4 may vary: get instructions from the TA.

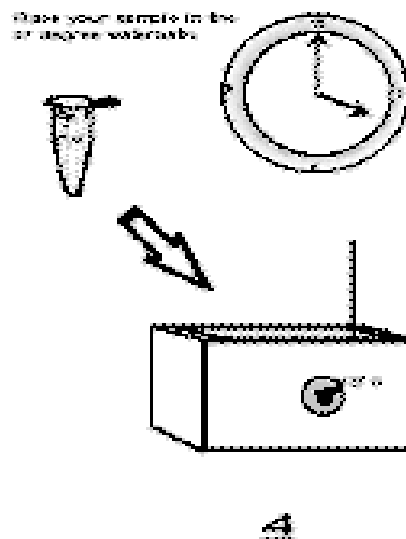
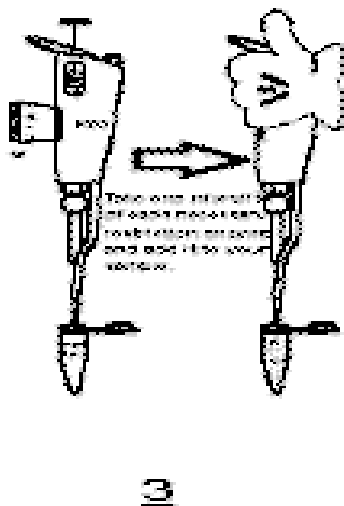
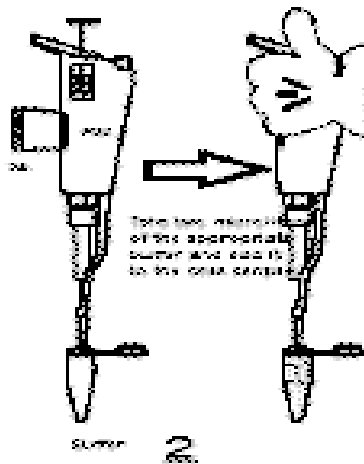
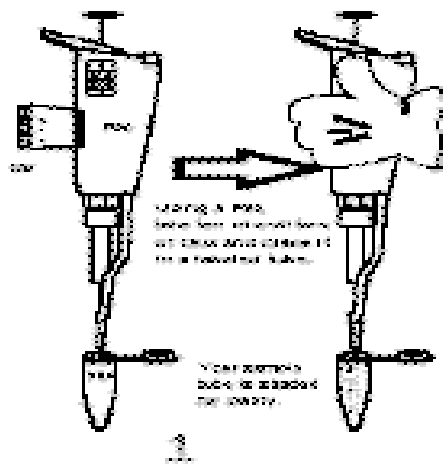
2. Put 10 ul of DNA into each tube. The TA will supervise your initial pipettings, so wait for him/her before beginning.

3. Put 2 ul of buffer into each tube

4. Put 11.5 ul of deionized water in each tube.

5. Put 1 ul of the appropriate enzyme(s) into each tube. **USE A FRESH TIP EACH TIME!!!** Never put a dirty tip into the enzyme tubes. Mix the contents of each tube by gently pipetting up and down a few times.

6. If the liquid is not at the bottom of the tube, close the caps and spin it down in the microcentrifuge for a few seconds. Put the tubes in the 37°C water bath. Be sure you can find your samples again (i.e. label them!).



Pouring a gel. (The TA may do this before class)

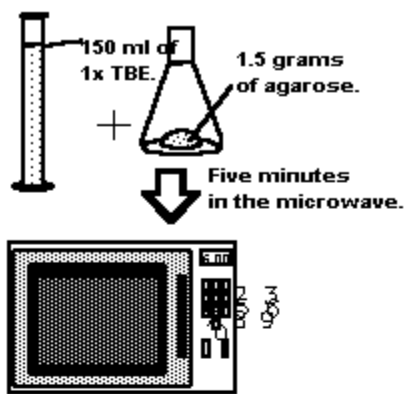
1. Weigh out 1.2 g of agarose. Mix with 120 ml of TBE buffer in a 500 ml flask.
2. Microwave the mixture 2 minutes, or until the agarose is completely dissolved. This requires boiling.
3. Cool the flask using running water until you can hold it in your hand.
4. **PUT GLOVES ON!!** Add 6 ul of Ethidium bromide (DNA stain) to the gel mix

and swirl to mix it in.

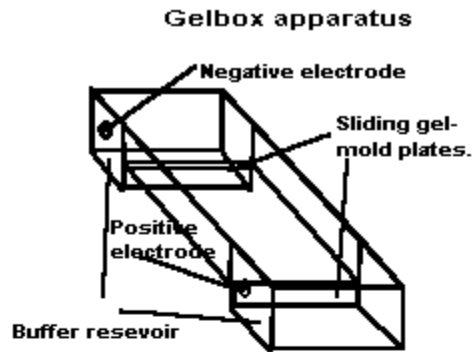
5. Seal the edges of the gel box with a little bit of agarose, then pour in the rest.

6. Flame the top of the gel with a Bunsen burner to remove bubbles, then put in the comb at the end.

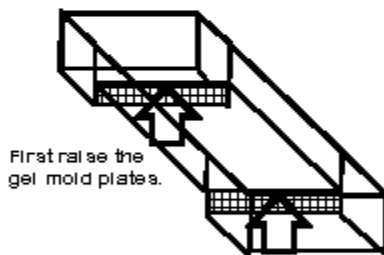
7. When the gel has solidified, cover it with TBE buffer and gently remove the comb.



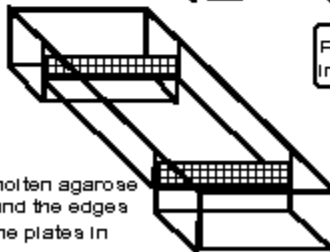
1



2

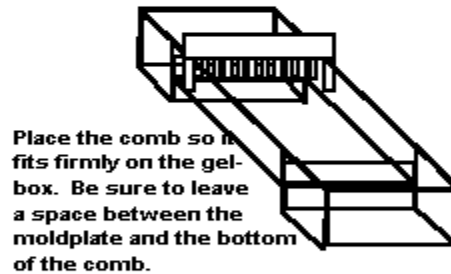


First raise the gel mold plates.

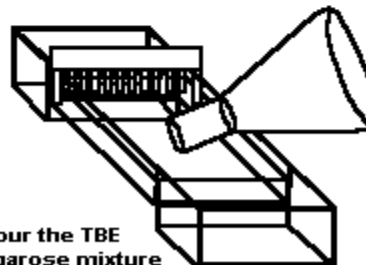


Pour molten agarose in around the edges to fix the plates in place.

3



Place the comb so it fits firmly on the gel-box. Be sure to leave a space between the moldplate and the bottom of the comb.

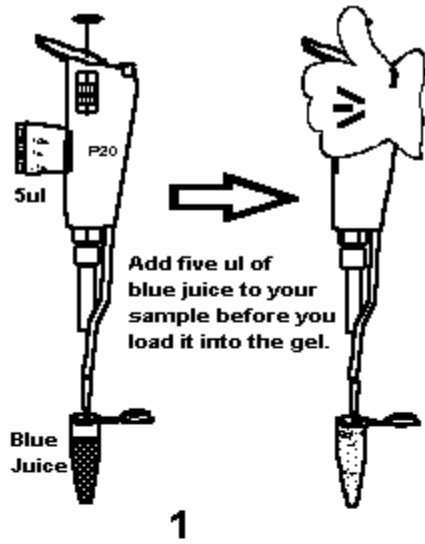


Pour the TBE agarose mixture into the gelbox.

4

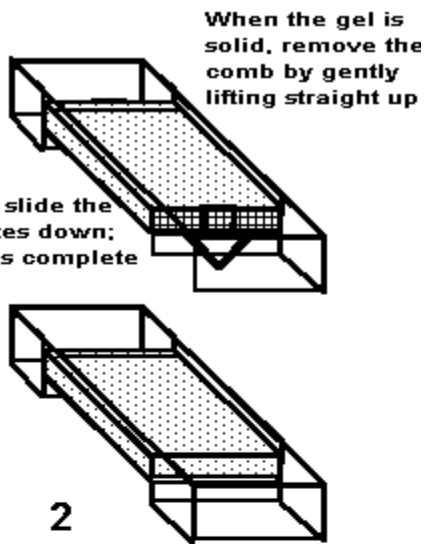
Loading the gel.

1. Put 5 ul of Blue Juice in each tube. Mix by gentle pipetting.
2. Using the pipetter, carefully transfer the DNA into the proper wells. Make sure you know which sample is in which well!
3. Be sure the Hind-lambda size standard is also loaded in one lane of the gel.
4. Run the gel at 100 V. from negative to positive. Black is negative and red is positive. Run until the blue dye is at the bottom of the gel. CAREFUL OF ELECTRICITY!

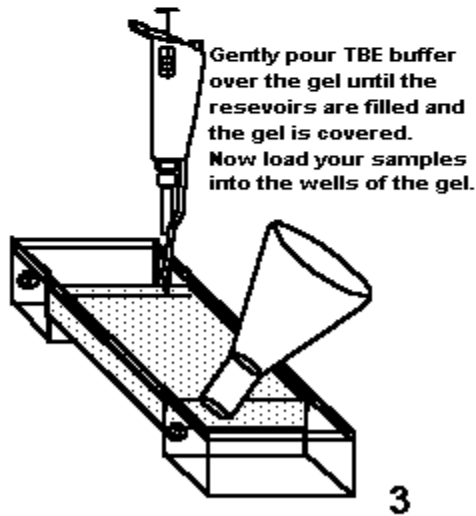


Add five ul of blue juice to your sample before you load it into the gel.

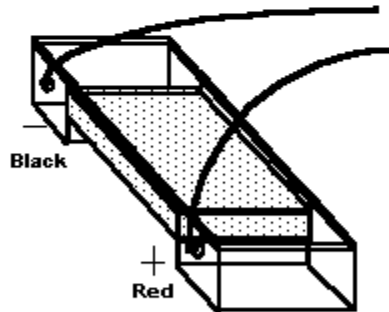
After that slide the mold plates down; your gel is complete



When the gel is solid, remove the comb by gently lifting straight up



Gently pour TBE buffer over the gel until the reservoirs are filled and the gel is covered. Now load your samples into the wells of the gel.



Hook the gel rig up to the power supply. Be sure the negative electrode is on the side that has the wells or you will run your DNA backwards. Turn on the power and set the voltage to 100.

Examining the gel.

1. Put the gel on the UV light box. USE THE SHIELD--UV CAN HURT YOUR EYES!!!

2. Measure the distance each band has traveled from the well. Be sure to measure the size standards as well. Measure every band in every lane of the gel. There may be some spurious bands--the instructor will point these out--don't use these.

3. Draw a diagram of the gel, noting bands that have migrated to the same position.








4. Convert the migration distances to sizes:

a. With the semi-log graph paper, put distance migrated (cm) on the X axis and size (kb) on the Y axis. Make sure you know how to use semi-log paper, especially the Y axis.

b. Plot the distances of the Hind-lambda standards against their sizes, as given in the diagram.

c. Draw a line between the Hind-lambda points, and use this to convert the distances of the plasmid bands to sizes.

Hind- lambda standards

	23.1 kb (often smeared)
	9.5 kb
	6.6 kb
	4.3 kb (usually faint)
	2.3 kb (These two bands are
	2.0 kb an easy-to-spot pair)
	0.56 kb (This band is quite far down the gel and usually faint)

CONSTRUCTING A MAP

Your TA will cover plasmid mapping during a lecture. Converting the fragment sizes to a circular genetic map is an exercise in logic and trial-and-error. Here are some hints to

help you out:

1. The sum of the fragment lengths should be the same for all digestions (i.e. they will add up to the total length of the circle).
2. The number of fragments is equal to the number of restriction sites.
3. To make the map, start with the simplest data, i.e. the enzymes that give the fewest fragments, and work to the more complex.
4. Realize that the data you collect is not going to be perfect--you have to make allowances for this fact when you are trying to fit your data into a consistent map.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Jack Bennett, who wrote the original (and now much modified) version of this manual, and also the late Dr. Sidney Mittler, who devised the original versions of several of these experiments. Thanks also to the many Genetics students whose comments, mistakes and complaints have led to the improvement of this manual.

Many thanks to Glenn Smith, who did the original versions of the computer graphics. "Freddy the Fruitfly" character and storyline were developed by Rick Johns.

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