

Learning Objectives

Upon completing the exercise, each student should be able:

- to understand the developmental cycle of *Drosophila melanogaster*;
- to set up genetic crosses with *Drosophila melanogaster* mutants, using the Virtual Fly Lab computer simulation;
- to use reciprocal crosses to determine if an unknown *Drosophila* mutation is dominant or recessive, and if it is autosomal or X-linked;
- to use a three-factor testcrosses to determine the linkage order and map distances between genes on *Drosophila* chromosomes;
- to use known marker genes to identify the chromosome on which unknown *Drosophila* mutant autosomal genes are located.

Background

T. H. Morgan, a geneticist who worked in the early part of the twentieth century, pioneered the use of the fruit fly, *Drosophila melanogaster*, as a model organism in genetic studies. *Drosophila* has a diploid chromosome number of eight, or four pairs of homologous chromosomes numbered 1 - 4. Chromosome 1 is the X chromosome (sex chromosome) and is responsible for sex determination in the fruit fly. Females have two X chromosomes, but males have only one X and a much smaller Y chromosome. Chromosomes 2 - 4 are autosomes.

Drosophila undergoes complete metamorphosis during its life cycle. It begins life as a fertilized egg, laid by the females on the surface of the medium. Usually, the eggs are too small to be seen without the aid of a microscope. After about three days, the eggs develop into larvae. The white, worm-like larvae undergo a series of developmental stages known as instars. Young larvae are small, about a millimeter in length. A few days after hatching, they reach a length of about five millimeters. Their anterior ends contain dark projections with which they probe the medium as they graze. At the end of the third instar, the larvae crawl onto the side of the vial and develop into pupae. The outer skin, or cuticle, hardens and becomes more translucent as the pupa matures. During this time, most of the larval body is replaced by the growth of specialized cells located on the ventral surface of the organism. These cells, located in regions called imaginal disks, develop into the structures of the adult fruit fly. About a day before the pupae are ready to hatch, the pupal case becomes almost transparent. At this time, you can see the dark structure of the adult fly.

Geneticists grow *Drosophila* on a variety of media. The easiest to use is commercially available instant medium made from instant mashed potatoes with a little yeast. *Drosophila* grows between the temperatures of 18 - 28°C. It grows best at 22 -26°C. At this temperature, the entire life cycle requires 8 - 14 days for one generation.

After *Drosophila* females have mated for the first time, they store sperm that they can use for fertilization throughout their life. Therefore, mating experiments require the use of only virgin females. Newly hatched *Drosophila* adults require about 12 hours to reach sexual maturity. Therefore, you can isolate virgin females by removing all the adults from a vial with pupae, waiting several hours (no more than 8), then removing the newly hatched adults before they reach sexual maturity. Males are distinguished by the presence of two structures not found in females: the genital arch, located on the ventral side of the last abdominal segment, and the sex combs, located on the front legs of the males.

Initially, geneticists had to rely on natural mutation to provide mutant traits to study. Later, radiation or chemically induced mutations became available, so that today hundreds of genetic loci have been identified and mapped. Each gene is given a symbolic representation based on how the mutation is expressed. By convention, if the mutant allele is recessive then the symbol begins with a lower case letter; if the mutation is dominant the the symbol begins in upper case. For either a dominant or recessive mutation, the wild type allele for the gene is designated by adding a superscript “+” to the mutant gene symbol.

For example, one of the genes that controls eye color is located on chromosome 3. A mutation at this locus produces “scarlet eyes,” a phenotype with unusually bright red eye color. The normal, or wild-type eye color, is brownish-red. The representation for the scarlet and normal alleles are:

scarlet allele: st
wild type allele at the scarlet locus: st^+

Notice that the symbol for scarlet is in lower case, because the scarlet allele is recessive to wild type.

The three possible genotypes for the scarlet gene locus are given in the table below.

<i>Genotype</i>	<i>Phenotype</i>
$st^+ st^+$	Wild type (Brownish-red) eyes
$st^+ st$	Wild type (Brownish-red) eyes
$st st$	Scarlet (Bright red) eyes

In this exercise the instructor will assign six unknown mutations to each student. Three of the unknown mutations will be located on chromosome 1 (X-linked), one on chromosome 2, one will on chromosome 3, and one on chromosome 4. Also, you will be given the names of known “marker genes” on chromosomes 2 and 3. Using the “Virtual Fly Lab” (published on the Internet by Biology Labs Online), you will be able to rapidly obtain data from simulated *Drosophila* crosses. You must:

- determine, for each mutation, whether the mutant allele is dominant or recessive, and whether it is X-linked or autosomal;
- determine, for each mutation, on which of the four *Drosophila* chromosomes the gene is located; and
- determine the map order and map distance between the three genes that are located on Chromosome 1.

Procedures

A. Accessing the Virtual Fly Lab program

Using Internet Explorer or a similar Web browser, open the following location:

<http://biologylab.awlonline.com/>

This will give you the introductory screen for the Virtual Fly Lab. **You should set up your own account to use the Virtual Fly Lab.**

When you begin the Virtual Fly Lab program, you see a welcome screen with a button that says “Start Lab.” When you click on the button, a new window opens with the Virtual Fly Lab program. You should maximize this window. In the screen shots below, I have pointed out some of the key features of the program with red text labels.

The program is a simulation, not a tutorial. This means that you need to figure out what it is that you want to cross before you start clicking on buttons. You will have to determine what the correct crosses are to answer the questions about your unknown mutations. The program simply allows you to “set up” and “collect the data” from the crosses without actually having to come into the lab and mating live flies.

Read the information about starting crosses. Note that each class of trait (eye color, wing shape, etc.) can be altered in the cross by checking the round circles (“radio buttons”) in front of the trait. You will have to be careful to make sure that the sex of the flies is what you want. In the initial cross, each cross must be done by crossing the mutant trait of one gender with the wild type alternative of the opposite gender. Also, read carefully the note about the symbols used by the program. The program does not use the standard convention regarding lower/upper case and dominance. Instead, students must do crosses to determine dominance of a trait.

B. General Features of the Program

The Main Screen

The screenshot shows the FlyLab main interface. At the top, there's a navigation bar with links like 'INTRODUCTION', 'NOTEBOOK', 'ASSIGNMENTS', 'GENETIC ABBREVS', 'GLOSSARY', and 'HELP'. Below this is the 'FlyLab' logo and a decorative banner with fly illustrations and mathematical formulas like $(O_1 - E_1)$ and $\sum_{i=1}^n (O_i - E_i)$.

The main area is divided into several sections:

- Mating Area:** At the top, there are two fly icons labeled 'Female' and 'Male'. Between them is a 'Mate' button. To the left of the female fly is a dropdown menu labeled 'Each Mating Creates' with '10000 Offspring' selected. A red arrow points to this dropdown with the text: 'Set this box to 10000 offspring.'
- Design Area:** Below the mating area are two 'Design' buttons, one for the female and one for the male. Below these are labels 'Female: + N=3832' and 'Male: + N=3724'.
- Results Area:** In the center, there are two fly images representing offspring. Above them is 'Cross #4' and 'Offspring'. Below the offspring images are two '2 fly images' labels with up and down arrows, indicating scrollability. A 'Select' button is located below the right offspring image.
- Control Buttons:** At the bottom left, there are buttons for 'Analyze Results', 'New Mate', and 'Select'.

Annotations on the right side:

- 'Mating Area' Press "Mate" after male and female for the cross have been designed or selected.
- Use these buttons to "Design" flies from the Design screen.
- 'Results' Area The gender, phenotype, and number of each class of offspring are given. Arrows indicate you can scroll up or down to see more images. You can "Select" an offspring to use in the next cross.

Annotations on the left side:

- This button takes you to the "Analysis" screen.
- This button clears the program for a new cross.

At the bottom left, it says 'Java Applet Window'.

The Design Screen

The screenshot shows the FlyLab design screen. It features a vertical list of mutation categories on the left:

- Bristle
- Body Color
- Antennae
- Eye Color
- Eye Shape
- Wing Size
- Wing Shape
- Wing Vein
- Wing Angle

At the top right, there's a row of six fly body segments showing different bristle phenotypes. Below them are radio buttons for: Wild Type (selected), Forked, Shaven, Singed, Spineless, and Stubble.

In the center, there's a fly icon labeled 'Female: +'.

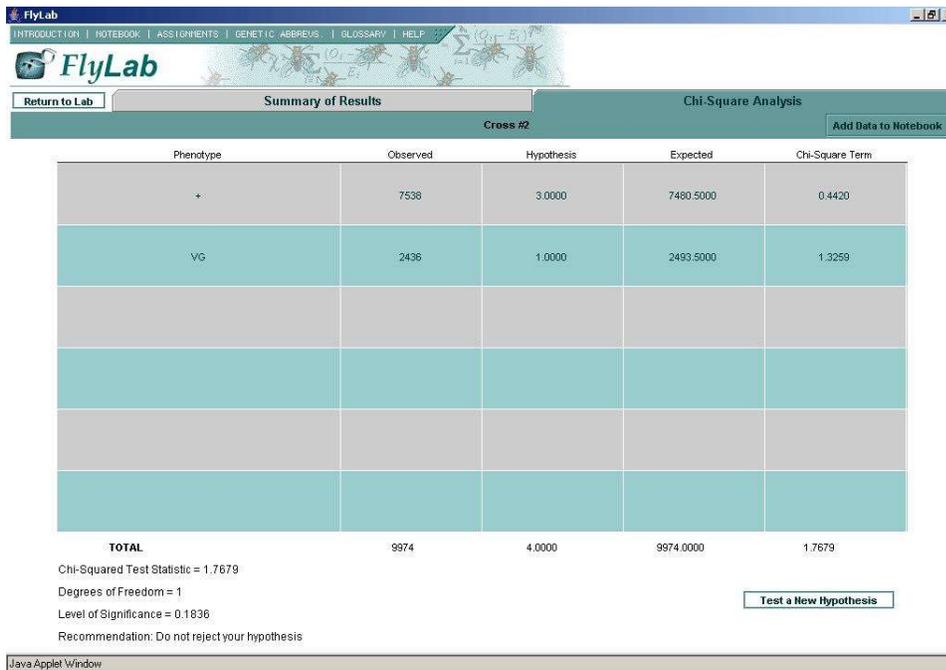
At the bottom left, there is a 'Reset All' button. At the bottom center, there is a 'Select' button.

Annotations on the right side:

- Mutations are grouped into nine different categories.
- Within each category, either "wild type" or one of the mutant phenotypes can be selected.
- This button will reset all of the categories to "wild type."
- Press this button when you are finished designing your fly.

At the bottom left, it says 'Java Applet Window'.

The Analysis Screen: Chi Square Results



Analysis "Chi square" results screen displays the results of the chi square calculation, including a recommendation of whether or not to reject the hypothesis.

C. Determining if a mutant is dominant or recessive, and if it is X-linked or autosomal

To determine if a mutant is dominant or recessive, and if it is X-linked or autosomal, you perform a pair of reciprocal crosses. In a pair of reciprocal crosses, the gender of the parents is reversed. If the gene is autosomal, then you get identical results in both crosses, but if the gene is X-linked, then the results of the two crosses is different. The expected results for reciprocal crosses is summarized in the table below.

Question: Is an unknown *Drosophila* mutation dominant or recessive, and is it X-linked or autosomal?

Hypothesis: The offspring of a pair of reciprocal *Drosophila* crosses, mutant ♀ x wild type ♂ and mutant ♂ x wild type ♀, is determined by whether the mutation is dominant or recessive, and whether it is X-linked or autosomal, as shown in the following table.

<i>If the mutation is:</i>	<i>Offspring of the cross :</i>	
	<i>Mutant ♀ x Wild type ♂</i>	<i>Wild type ♀ x Mutant ♂</i>
Recessive and autosomal	Wild ♂ & Wild ♀	Wild ♂ & Wild ♀
Dominant and autosomal	Mutant ♂ & Mutant ♀	Mutant ♂ & Mutant ♀
Recessive and X-linked	Mutant ♂ & Wild ♀	Wild ♂ & Wild ♀
Dominant and X-linked	Mutant ♂ & Mutant ♀	Wild ♂ & Mutant ♀

Experiment: Reciprocal crosses, Mutant ♀ x Wild type ♂ and Wild type ♀ x Mutant ♂

Independent variable: Phenotypes and genders of the parents

Dependent variables: Phenotypes and genders of the offspring

Procedure for Reciprocal Crosses:

1. Log onto the Virtual Fly Lab.
2. Set 10000 offspring. Click “New Mate,” if necessary.
3. Click the “Design” button under the female fly. This should take you to the Design screen.
4. Find the category and the button for the mutation you are testing, then press “Select.” This should take you back to the main screen. Note: You have to test each mutation separately in reciprocal crosses with the wild type.
5. From the Main screen, click the “Design” button under the male fly. This should take you to the Design screen.
6. Click “Reset all” to make sure you have wild type, then click “Select” to get back to the Main screen.
7. Click “Mate.”
8. Click “Analyze results.”
9. Make sure the “Use sex” button is checked, and record the results on a “Reciprocal Cross” data sheet.
10. Click “Return to Lab.”
11. Click “New Mate.”
12. Repeat steps 3 – 10, except this time “Design” a wild type female and a mutant male.
13. Compare the results for your unknown mutation with the expected results to determine if the unknown is dominant or recessive, and if it is X-linked or autosomal.

<i>If the mutation is:</i>	<i>Offspring of the cross :</i>	
	<i>Mutant ♀ x Wild type ♂</i>	<i>Wild type ♀ x Mutant ♂</i>
Recessive and autosomal	Wild ♂ & Wild ♀	Wild ♂ & Wild ♀
Dominant and autosomal	Mutant ♂ & Mutant ♀	Mutant ♂ & Mutant ♀
Recessive and X-linked	Mutant ♂ & Wild ♀	Wild ♂ & Wild ♀
Dominant and X-linked	Mutant ♂ & Mutant ♀	Wild ♂ & Mutant ♀

14. Record your conclusion in the summary chart in the worksheet.

D. Determining the order and map distances of Chromosome 1 genes

Three of your unknown mutations are X-linked, which means that they are located on Chromosome 1. In order to determine the order and map distances between these genes, you will perform a three factor testcross.

In a three factor testcross, you cross a female that is heterozygous for all three genes with a male that is homozygous recessive for all three genes. From the numbers of recombinant progeny, you can determine the order of the genes on the chromosome and the distance between the genes.

In testcrosses with *Drosophila*, the heterozygous parent **must** be female, because in *Drosophila* crossing over occurs only in the females, not in the males. Also, be aware that the male must be recessive for all three genes. If the mutation is dominant, then the male must be homozygous recessive wild type for that trait when the testcross is made.

To set up a testcross, you must first do a preliminary cross between a female with all three unknown mutations and a wild type male. Then, you **select** a female offspring from this cross (she is heterozygous for all three genes), **design** a male that is recessive for all three traits, and then **mate** them to get the results for the three factor testcross.

Procedure for Three Factor Testcross: Using M, F, W genes as an example

1. Log onto the Virtual Fly Lab.
2. Set 10000 offspring. Click "New Mate," if necessary.
3. Click the "Design" button under the female fly. This takes you to the Design screen.
4. On the Design screen, click "Bristle," then click "Forked." Click "Eye color," then click "White." Click "Wing size," then click "Miniature." Click "Select" to go back to the Main screen.
5. On the Main screen, click the "Design" button under the male fly. This takes you back to the Design screen.
6. On the Design screen, click "Reset all," then click "Select" to go back to the Main screen.
7. On the main screen, click "Mate."
8. At the bottom of the Main screen in the "Results" area, click "Select" under the **female** offspring.
9. At the top of the Main screen in the "Mating" area, click "Design" under the **male** fly. This takes you back to the Design screen.
10. On the Design screen, click "Bristle," then click "Forked." Click "Eye color," then click "White." Click "Wing size," then click "Miniature." Click "Select" to go back to the Main screen. **Note:** Forked, white, and miniature are all recessive to the wild type. If any of the mutations are dominant, then you must set up the male as wild type (recessive) for that trait, because the male must be recessive for all three traits.
11. On the Main screen, click "Mate."
12. Click "Analyze results," then click "Ignore sex."

13. Record the number of offspring in each of the eight phenotypes. The following table shows data from the example with miniature, forked, and white. Notice that the program only shows the mutant phenotype in each class; it implies that everything else in the phenotype is wild type.

Phenotype	Number
+	3223
F	632
W	1051
F;W	226
M	229
F;M	1019
W;M	617
F;W;M	3091
Total	10088

14. Arrange the phenotypes into pairs, with each different phenotype represented in each pair. Identify the nonrecombinant (largest) and double crossover (smallest) classes.

Phenotype	Number	
+	3223	} NR
F;W;M	3091	
F;W	226	} DC
M	229	
W	1051	
F;M	1019	
W;M	617	
F	632	
Total	10088	

15. Compare the nonrecombinant class with the double crossover class to determine the one gene that is switched in the double crossover class. This is the gene that is in the middle. In this example, it is miniature (M), so the order is forked-miniature-white (F-M-W). You could also state the gene order as white-miniature-forked (W-M-F).
16. Each of the remaining classes is a single crossover class between a gene on the end and the gene in the middle: either white and miniature (W-M singles) or miniature and forked (M-F singles). Compare each singles class with the nonrecombinant class to see which gene is switched to determine which one is the W-M singles class, and which is the M-F singles class.

Phenotype	Number	
+	3223	} NR
F;W;M	3091	
F;W	226	} DC
M	229	
W	1051	} W-M Singles
F;M	1019	
W;M	617	} M-F Singles
F	632	
Total	10088	

17. Calculate the distance between each pair of genes.

$$W - M \text{ distance} = \frac{1051 + 1019 + 226 + 229}{10088} \times 100 = 25.0 \text{ map units}$$

$$M - F \text{ distance} = \frac{617 + 632 + 226 + 229}{10088} \times 100 = 16.9 \text{ map units}$$

18. Calculate the expected double crossover frequency, the obtained double crossover frequency, the coefficient of coincidence, and the interference.

$$\text{Expected double crossover frequency} = (0.25)(0.169) = 0.04225$$

$$\text{Obtained double crossover frequency} = \frac{226 + 229}{10088} = 0.04510$$

$$\text{Coefficient of coincidence} = \frac{\text{Obtained double crossover frequency}}{\text{Expected double crossover frequency}} = \frac{0.04510}{0.04225} = 1.07$$

$$\text{Interference} = 1 - \text{Coefficient of Coincidence} = 1 - 1.07 = -0.07$$

E. Determining the Chromosomal Location of Autosomal Genes

The X-linked genes are located on the X-chromosome, or chromosome 1. Each of the autosomal mutations in your unknown is located on either chromosome 2, 3, or 4. To determine which, you need to know the location of known “marker genes” on at least two of the chromosomes. If the unknown is **linked** to the marker gene, then it **does not assort independently** from the marker, and therefore it must be located on the same chromosome as the marker.

Here are the markers for chromosomes 2 and 3.

Chromosome 2: Purple eye color (PR) and Apterous wing shape (AP)

Chromosome 3: Incomplete wing veins (RI) and Spineless bristles (SS)

Two markers for each chromosome are necessary because you must select a characteristic that is different from the unknown gene you are testing. For example, if you are testing the unknown wing shape vestigial (VG), you cannot use incomplete wing veins (RI) for a chromosome 2 testcross; instead, you must use spineless bristles (SS).

For each of your unknown autosomal mutations, you will need to develop hypotheses and design your own experiments to determine if the autosomal mutations are on chromosome 2, 3, or 4. In testing your hypotheses, you should use the chi square test to determine if the unknown mutation assort independently from chromosome 2 or chromosome 3 markers, based on the expected frequencies in your cross.

**Linkage Mapping in Drosophila
Laboratory Report Sheet**

Name _____

A. Reciprocal Cross Data

For each of your unknown mutations, perform a set of reciprocal crosses and record the data in one of the tables below. Based on the data, determine whether each mutation is dominant or recessive, and whether it is X-linked or autosomal.

1. Mutation: _____

<i>Offspring</i>	<i>Reciprocal Crosses:</i>	
	<i>Mutant</i> ____ ♀ <i>x Wild type</i> ♂	<i>Wild type</i> ♀ <i>x Mutant</i> ____ ♂
Female		
Male		

2. Mutation: _____

<i>Offspring</i>	<i>Reciprocal Crosses:</i>	
	<i>Mutant</i> ____ ♀ <i>x Wild type</i> ♂	<i>Wild type</i> ♀ <i>x Mutant</i> ____ ♂
Female		
Male		

3. Mutation: _____

<i>Offspring</i>	<i>Reciprocal Crosses:</i>	
	<i>Mutant</i> ____ ♀ <i>x Wild type</i> ♂	<i>Wild type</i> ♀ <i>x Mutant</i> ____ ♂
Female		
Male		

4. Mutation: _____

<i>Offspring</i>	<i>Reciprocal Crosses:</i>	
	<i>Mutant</i> ____ ♀ <i>x Wild type</i> ♂	<i>Wild type</i> ♀ <i>x Mutant</i> ____ ♂
Female		
Male		

C. Determining the order and map distances of Chromosome 1 genes

1. In the table below, record the results of the three factor cross experiment with your three unknown Chromosome 1 (X-linked) mutations.

<i>Phenotype</i>	<i>Number</i>
Total	

2. In the space below, determine the linkage order, map distances, coefficient of coincidence, and interference.

E. Formal Lab Report for the Maize Lab

For your grade on the maize lab, you will write a formal lab report that summarizes the principles, methods, analysis, results, and conclusion that you reached. The report will be graded according to the rubric shown on the next page. The report should contain the following five sections: Introduction, Methods, Results, Analysis, and Conclusion. Basic instructions on the lab report format, as well as specific instructions for each section, are given below.

Basic Format

- **Each individual student must write his or her own individual laboratory report.**
- Any direct quotation from another work should be properly cited and referenced.
- The laboratory report should be typed (preferred) or neatly printed.
- All typographic conventions (Greek letters, special symbols, italics, mathematical formulas, superscripts and subscripts, etc.) must be correctly used.
- There should be no spelling or grammatical errors.
- Diagrams should be neatly drawn.
- Each section of the laboratory report should be clearly indicated with a boldfaced label.

Introduction

The “Introduction” should consist of :

- a brief one or two paragraph background on the use of *Drosophila* in genetics;
- an introduction to the mapping of unknown mutations by reciprocal crosses, testcrosses, and linkage mapping;
- clear statements of each of the questions to be answered in this lab exercise;
- clear statements of each hypothesis to be tested, with the hypotheses based on the principles of reciprocal crosses and linkage mapping.

Methods

The “Methods” section should:

- contain a detailed description of the crosses that were performed;
- contain mating diagrams showing the crosses to aid in the description of the crosses.

Results

The “Results” section should:

- have a separate tables for the data from each cross;
- have each table clearly and accurately labeled.

Analysis

The “Analysis” section should:

- include a complete analysis appropriate for each cross;
- for the three factor testcross, the analysis should include a determination of the gene order, map distances, coefficient of coincidence, and interference;
- for the linkage analysis to determine the location of autosomal genes, the analysis should include use of the χ^2 test.

Conclusion

The “Conclusion” section should:

- begin with an introductory paragraph that briefly restates the questions that were answered in the lab exercise;
- summarize the essential data, including the chromosomal location of each unknown mutation, and the map order and distances of the three chromosome 1 mutations;
- indicate how the results illustrate the genetics principles covered in the exercise;
- have an explanation of any sources of error, if there were any errors or discrepancies in the counts.

Lab Report Rubric for Lab IV. Patterns of Inheritance in *Drosophila* (40 pts)

	Highest pts	Lower pts	pts	Professor I Evaluation	Professor II Evaluation
Introduction (section 1)	The questions to be answered during the lab are stated	One or more questions are missing	7		
	The hypothesis is stated based on the linkage theory	Hypothesis is incorrect			
Methods (section 2)	A description of how the experiment was performed	Description is unclear	7		
	Mating diagrams are drawn to aid the description	Diagrams are drawn incorrectly			
Results (section 3)	Results and data are well organized in Tables	Wrong data entry	7		
	Tables are complete	Tables are incomplete			
Analysis (section 4)	Lists of genotypes and phenotypes are correct and complete	The lists are incorrect and incomplete	7		
	The calculation of recombinants is correct	Calculation is incorrect			
	The calculation for genetic distance is shown and correct	Calculation is not shown and/or incorrect			
Conclusions (section 5)	Summarizes the essential data and derives conclusions based on the data	Conclusions are unclear	7		
	Conclusions are supported by the data	Data does not support the conclusions			
	Hypothesis is rejected or accepted based on the data	Wrong hypothesis is accepted			
	If there was an error, explain the possible source of the error	No explanations			
Format	Few spelling and grammar errors	Multiple spelling and grammar errors	5		
	Neat, with few smudges	Not neat, multiple smudges			
	Chromosomes and gametes are drawn correctly	Drawings incorrect			
	Each section is clearly highlighted	Sections unclear			