## ***Drosophila* Lab Manual**

## Genetics Project Laboratory

## Eugenia Villa-Cuesta

## Department of Biology, Adelphi University.

Instructors:

Teaching assistants:

### Student Information:

### Introduction 3

###

###  Overview 3 Keys to Success 5

 Safety 5

 Schedule 6

### Description of Genetics Project 7

**Experiment 1: Phenotypic characterization of mitochondrial** 11

 **haplotypes and mitochondrial mutations.**

###

### Experiment 2: Analysis of inheritance 19

### Experiment 3: Molecular characterization of haplotypes 25

###  by PCR-RFLP

### Independent Project 34

### Appendices 37

### How to use a micropipettor 37

### How to calculate dilutions 38

### Chi square analysis 39

1. Prelab summaries 42
2. Article responses 42
3. Independent project proposal and literature search strategy 42
4. Independent project oral presentation 43
5. Lab reports 43
6. Guidelines for Information Literacy in Biology 44
7. Guidelines for Oral Communication in Biology 46
8. Guidelines for Lab Reports 49
9. Bio Department – Project, Paper, and Lab Report 50

 Reference Formatting

1. Rubrics 53

### Introduction

The laboratory class will meet in Science Building 124:

Mondays and Wednesdays from 9.20 to 11.50am

Instructor: Dr. Eugenia Villa-Cuesta

 Science Building, Room 111

 evilla-cuesta@adelphi.edu

### Overview of Course

This course provides students with hands-on experience in experimental principles of molecular biology and classical genetics. This course integrates chemistry, biology, and mathematics and provides a foundation for further study in the biological sciences and medicine.

**Course Description**

Develop independent investigative ability, technical skill, proficiency in scientific writing and presentation, and an in-depth understanding of research and the scientific approach through semester-long projects in genetics and molecular biology.

During the first part of the course students will learn basic techniques of molecular genetics and bio-informatics and gain hands-on experience of genetics using *Drosophila melanogaster* as a model organism. During the second part of the course, students will engage in a project that allows them to design, execute and evaluate experiments.

**General Course Objectives**

This course is a good opportunity for you to appreciate the challenges and rewards of research. At the completion of this course, we hope that you will be able to:

1. Understand the genetic approach to biological problems and the use of molecular biology techniques to address biological problems
2. Understand key features of experimental design
3. Understand the importance of proper statistical analysis of experimental results
4. Understand the organization of the scientific literature and scientific databases
5. Communicate science and give presentations on scientific subjects

**Student Learning Goals**

At the end of this course, students should be able to:

1. Interpret primary research literature
2. Design and carry out novel research projects investigating genetics of an established organismal model system.
3. Describe and explain concepts used in research in genetics and molecular biology
4. Search for, collect, and evaluate scientific research articles relating to topics of interest.
5. Statistically analyze and evaluate novel research findings
6. Describe results in written papers and in oral presentations following established conventions for the field of biology.

### Experimental Material and Projects

In both projects that you will work on, you will use the fruit fly *Drosophila melanogaster*. *Drosophila* is widely employed in laboratory studies in the field of genetic (see video *A fly in New York* posted on Moodle). In the first part of the semester you will characterize mutant flies with altered mitochondrial function. During the second part of the course you will develop your own research project. You will design, execute and evaluate your own experiments. For the class to be successful and fun for you, you will need to be organized, efficient, and aware of what you are doing at all times.

### General Organization of the Laboratory

You will work in pairs during the lab. During the first part of the semester, the instructor and TA will guide you through the techniques that you will need to learn as you carry out Experiments 1, 2, and 3. You will then work more independently to complete Experiment 4 (an experiment of your own design) during the remainder of the scheduled lab periods. Most lab periods will begin with an introductory lecture.

To encourage you to come to lab prepared, you are required to submit before each of the first 12 lab periods, via Moodle, a brief summary, in your own words, of what you will be doing in lab and a brief explanation of why you are doing it. Copying directly from the lab manual is not acceptable for this prelab summary: the proper approach is to read the entire description of the lab, and then write in your own words your understanding of what you will be doing and why. This prelab description must be at least one paragraph (three sentences) long.

The procedures you are to follow in the experiments are described in detail in this lab manual. You should keep your lab manual in a one-inch three-ring binder with pockets inside the cover. The lab manual will also serve as your lab notebook, where you must record the details and dates for the experiments that you do in lab; you will find that the more complete your lab notebook, the easier it will be for you to complete your lab reports. At the end of the semester your lab notebook will be graded, so keep it up-to-date, accurate, and complete.

Your first lab report will explain the results of Experiments 1. Your final lab report will be on your independent project. At three points during the semester, the scheduled lab periods will be devoted to student presentations. Guidelines for each exercise are described in the appendices section of the lab manual.

The final grade for the project lab course will be determined as follows:

15% Attendance

15% Participation and lab notebook

 5% Prelab summaries

 5% Article response written presentation

 5% Article response oral presentation

 5% Independent project proposal

 5% Literature search strategy

10% Independent project oral presentation

15% First lab report

20% Final lab report (Independent project)

### Keys to success in genetics lab

* Follow directions.
* Be organized: prepare for lab by reading over the lab manual and thinking about what you did previously, so that you know what you’re doing before you come to lab.
* Keep good notes of what you do in lab: every time you come to lab, write down in your lab notebook the key details of what you have done.
* Be meticulous: perform an experiment carefully and without rushing so it will be done correctly!
* Plan to complete what you need to for the week.
* Consult with the instructor or TA with any questions.

### Safety in the Laboratory

Because there is the possibility for accidents and danger in every laboratory, you must pay careful attention to the instructor’s and TA’s directions with regard to laboratory safety. Protective equipment such as safety goggles and gloves will be available and should be used when so directed by the instructor. The instructor will point out and explain the use of safety equipment such as an eyewash, a safety shower, and a fire blanket. The laboratory can be kept a safe place by using common sense and following directions.

#### Laboratory Rules

**1. No eating, drinking, or smoking in the laboratory.**

**2. Wear safety glasses or goggles** for any procedure in which your eyes could be endangered, such as handling a hot solution or hazardous chemicals or viewing UV light.

3. **Wear protective gloves** for any exercise involving hazardous chemicals or hot liquids.

4. Report any injury to the instructor or TA immediately.

**5. Closed-top non-mesh shoes and long pants** are recommended attire in the lab. No sandals or other types of open shoes are permitted. Long hair should be tied back.

**6. Keep the laboratory clean.** Discard your trash in the wastebaskets before you leave. Broken glassware or glass items such as slides go in the broken glass containers only. Do NOT put other kinds of trash into the broken glass container. **Don't mix glass and non-glass items in the trash.** For pipet tips, tubes, etc. there will be special waste containers.

**7. Discard waste liquids in the proper container, as designated by the instructor.** Some hazardous wastes must be kept separate during disposal.

**8. Rinse out dirty glassware and then place it in the areas designated by the instructors.**

9. Return and put away all equipment and supplies when finished.

**10. Listen carefully** to the instructor’s directions regarding any hazards in the lab.

11. The instructor or a TA must be present for you to work in the lab; never work alone.

12. Wash your hands before leaving the laboratory.

**Biology 224 Tentative Lab Schedule, Spring 2015**

| **Class** | **Expt 1:**  | **Expt 2:**  | **Expt 3:**  | **Expt 4- Project**  | **Due dates** |
| --- | --- | --- | --- | --- | --- |
| 1/26 | Introduction.Overview of *D. melanogaster.*Developmental time. |  |  |  |  |
| 1/28 | Climbing assay.Flying assay. | Introduction.Set up F1 cross. |  |  |  |
| 2/2 | Succinate Dehydrogenase. |  |  |  |  |
| 2/4 | Graph results.Experiment 1 overview.Lab report discussion. | Clear F1 parents |  | Introductionliterature search & project development |  |
| 2/9 |  | Set up F2 cross. |  | article discussion | **Written article** **response** |
| 2/11 |  |  | DNA extraction Set up PCR |  |  |
| 2/16 | **1st Lab report due** | Clear F2 parents |  Gel electrophoresis and RFLP |  | **1st Lab report due** |
| 2/18 |  |  | RFLP gel electrophoresis.Discussing results | Article discussion |  |
| 2/23 |  **Article response** **Oral Presentation** | **Article Oral** **Presentation**  |
| 2/25 |  |  F2 climbing assayIdentify chromosome location of *sdhBEY12081* |  | Work on Proposal Presentation |  |
| 3/2 |  **Proposal**  **presentation** | **Proposal** **presentation** |
| 3/4To4/27 |  |  |  | Work on project |  |
| 4/29 |  |  |  | Work on project | **Lab notebook** |
| 5/4 |  |  |  | Work on project |  |
| 5/6 | **Oral presentation** | **Oral presentation** |
| 5/8 |  **Final lab report due** | **Final lab report**  |

**Description of the Genetics Project**

Preface

In the Genetics project you will be using *Drosophila melanogaster* as a genetic model to study mitochondrial disease. The first three experiments are based on published work and on-going work being done at Adelphi University. Those experiments are already prepared for you and you will be guided by the instructors while doing them. In Experiment 4 you will have the freedom to do a research project of your own. Of course, the instructors and TAs will be there to help you and advise you during your independent work. It might be difficult for you right now to come up with a topic to study, but I assure you that by the middle of the semester you will have a better idea about what you would like to study.

 I strongly recommend you to talk to your instructors and TAs if you have any problems, question or curiosity. We are here to help you and to motivate you about research. We enjoy doing it. Think about us as a collaborative team working for your education. As long as you are engaged with the class, we will be there to help.

An encouraging note

You might be thinking: “*50 confusing pages about a complex project in genetics. Am I ever going to read the whole lab manual and understand it?”* Don’t worry; we will explain the project to you as many times as needed. However, you should read the project descriptions several times during the semester. You will understand more each time and it will be (hopefully) rewarding to you to feel that you understand and participate in a complex research project such as this one.

Acknowledgments

Thanks to Dr. Lawrence Hobbie and Dr. Aram Stump for their help in the design of the Biology 224 genetics project laboratory, the writing of lab manual and for sharing with us their experiences teaching this class.

Welcome to genetics project lab 224!!

### Introduction

How genes affect health span, disease and life span is of critical importance in medicine, society and biology. In this laboratory you will use the genetic model system *Drosophila melanogaster* (frequently recognized as fruit flies) to study mutations that are known to cause disease in humans, and conditions shown to be important in the regulation of health homeostasis. In this project in particular we will focus on two genomes, the mitochondrial genome and the nuclear genome.

Mitochondria are small cellular organelles that consume ~90% of oxygen we breathe and produce ~90% of the ATP we need for normal daily functions (Scheffler, 2007) . Over 100 genetic diseases are known to affect mitochondrial activity, making this one of the most common classes of human pathologies (Scheffler, 2007).Originally, mitochondria were proteobacteria with their own prokaryotic genome. Over 2 billion years ago, this proteobacteria and an eukaryotic cell established a symbiotic relationship that led to eukaryotic organisms having genetic information in both the nuclear and mitochondrial genome (Rand et al., 2004).

**Coordination of two genomes**

During these 2 billion years some of the genes in the mitochondrial genome have been transferred to the nuclear genome. This means that some of the mitochondrial proteins are encoded in the nuclear genome, transcribed in the nucleus and translated in the cytoplasm. Therefore, mitochondria require the coordination of nuclear and mitochondrial genomes to produce all the proteins that mitochondria need to function properly (Rand et al., 2004). In terms of the genetic basis of disease, this coordinated system has two important implications:

1.) The genes regulating mitochondrial function are very abundant and, therefore, a large mutational target.

2.) The proper interaction between the two genomes is essential for normal physiological performance.

The genetic basis and functional significance of these nuclear-mitochondrial interaction effects (epistatic interactions: interactions between genes to control a phenotype) are very poorly understood and are the basis of much current research in biology. In the genetic project you will work with some examples of mitochondria and nuclear epistasis. You will characterize the pathology of disrupted coordination between mitochondrial and nuclear genomes.

**Mitochondrial function**

Mitochondria convert metabolic substrates into adenosine triphosphate (ATP), the energy currency of cells, via the process of oxidative phosphorylation (OXPHOS). During OXPHOS, NADH and FADH2, which are derived from the mitochondrial tricarboxylic acid (TCA) cycle and fatty acid oxidation, pass electrons through an electron transport chain (ETC). The ETC is formed by five complexes: complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome bc1), complex IV (cytochrome *c* oxidase) and complex V (ATP synthase) (Scheffler, 2007) (Figure 1).

Because part of the original mitochondrial DNA has been transferred to the nucleus, genes that code for proteins of the ETC might be located in the nuclear or in the mitochondrial genome. The only exception to this is complex II or succinate dehydrogenase. All the proteins that form the succinate dehydrogenase enzyme are nuclear encoded. The rest of the complexes have some subunits that are encoded in the nuclear genome and others encoded in the mitochondrial genome (Rand et al., 2004) (Figure 1).

**Coordination of mitochondria and cytosol**

Although mitochondria act as independent specialized organelles separated by lipid membranes, mitochondrial functions are tightly regulated by environmental signals in order to accommodate cellular requirements. These interactions need continuous communication between mitochondria and the cytosol (Woodson and Chory, 2008). Succinate dehydrogenase (complex II of the ETC) lies at the intersection of pathways that connect cell metabolism with mitochondrial respiration (Scheffler, 2007) suggesting an important role in cytosolic and nuclear communication.



***Figure 1****. Enzymatic complexes of the electron complex chain. The figure indicates which proteins are encoded in the Mitochondrial and nuclear genome. Obtained from Rand et al. 2004.*

**Mitochondria haplotypes and mutant strains.**

In this laboratory we will study a strain that is mutant for a subunit of succinate dehydrogenase (Complex II; the gene in *Drosophila* is called *sdhB* (Walker et al., 2006)*)* as well as a strain of *Drosophila melanogaster* carrying mitochondria from another species, *Drosophila simulans* (Montooth et al., 2010)*.* A haplotype is a group of genes within an organism that are inherited together. Since all the genes in the mitochondrial DNA are inherited together from the mother, the different mtDNAs that we will use in this lab manual are also refereed to as mitochondrial haplotypes.

 With the first strain of flies we will study a Drosophila model of a disease called succinate dehydrogenase deficiency. With the second strain of flies we will study how disruptions of co-evolved nuclear and mitochondrial genome impact heath (Montooth et al., 2010). Because *D. melanogaster* and *D. simulans* have been in reproductive isolation since 2.5 million years ago, we anticipate that flies with mitochondria and nuclear genomes from the same species (*D. melanogaster)* will perform better than those carrying a nuclear genome from *D. melanogaster* and a mitochondrial DNA from *D. simulans*. Table 1 summarizes the genotypes of the three strains of flies that we will use.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Name | Mitochondrial genotype | Species | Nuclear genotype | Species  |
| Wild type | Wild type *D. melanogaster* | Wild type *D. melanogaster* |
| *sdhBEY12081* | Wild type *D. melanogaster* | Mutant *sdhB* *D. melanogaster* |
| *simW501;OreR* | Wild type *D. simulans*  |  Wild type *D. melanogaster*  |

*Table 1. Description of the nuclear and mitochondrial genotype of the mitochondrial haplotypes and mutant strains.*

As a complement to this lab manual, you should read the paper poster on Moodle about fly husbandry:

**Getting started: An overview on raising and handling *Drosophila*** byStocker & Gallant.

**References**

Le Bourg, E., and Lints, F.A. (1992). Hypergravity and aging in Drosophila melanogaster. 4. Climbing activity. Gerontology *38*, 59–64.

Montooth, K.L., Meiklejohn, C.D., Abt, D.N., and Rand, D.M. (2010). Mitochondrial-nuclear epistasis affects fitness within species but does not contribute to fixed incompatibilities between species of Drosophila. Evolution *64*, 3364–3379.

Rand, D.M., Haney, R. a, and Fry, A.J. (2004). Cytonuclear coevolution: the genomics of cooperation. Trends Ecol. Evol. *19*, 645–653.

Scheffler, I.E. (2007). Mitochondria (Scheffler, Mitochondria) (Wiley-Liss).

Walker, D.W., and Benzer, S. (2004). Mitochondrial “swirls” induced by oxygen stress and in the Drosophila mutant hyperswirl. Proc. Natl. Acad. Sci. U. S. A. *101*, 10290–10295.

Walker, D.W., Hájek, P., Muffat, J., Knoepfle, D., Cornelison, S., Attardi, G., and Benzer, S. (2006). Hypersensitivity to oxygen and shortened lifespan in a Drosophila mitochondrial complex II mutant. Proc. Natl. Acad. Sci. U. S. A. *103*, 16382–16387.

Woodson, J.D., and Chory, J. (2008). Coordination of gene expression between organellar and nuclear genomes. Nat. Rev. Genet. *9*, 383–395.

**Experiment 1**

**Experiment 1: Phenotypic characterization of mitochondrial haplotypes and mitochondrial mutations.**

Prelab preparation: Read through the instructions for this lab and complete Tables 2,5,8 and 10.

Defects of mitochondrial function cause damage to cells in tissues that have high energy requirements. Because fruit flies need a lot of energy to fly and climb, muscle tissue is highly affected by mitochondrial pathology. In this experiment you will measure the climbing ability, flying capacity, developmental time and *in vitro* activity of succinate dehydrogenase of mitochondria-altered strains. We will use the following strains:

Wild type (WT)

*sdhBEY12081 (sdhB)*

*simW501;OreR (W501)*



***Figure 2****. A picture representing the Drosophila life cycle.*

Developmental time

Cell division and cell differentiation are energy costly developmental processes. During development, a fertilized egg of *Drosophila melanogaster* undergoes 4 stages: egg, larvae (1st, 2nd and 3rd instar), pupae and adult (Figure 2). The length of the developmental stages depends on the temperature at which they are raised. The warmer the temperature, the quicker the development will be. At 25 degrees Celsius an egg will develop into an adult in around 11 to 14 days.

Here you will observe and compare vials of wild type, *sdhB* and *W501* at different developmental times: 3rd instar larvae, pupae and adults.

You will work in pairs. In each table you have two vials per fly strain: one vial per each pair.

In each vial there will be white larvae wandering the walls of the vial. These are 3rd instar larvae which are preparing to pupate. Pupae have a light or dark brown color and are immobile and attached to the walls of the vial. From some of the pupae, adult flies already emerged and are freely flying. You will anesthetize and count the adult flies using FlyNap or CO2 pads.

Based on what you know about each strain, state a hypothesis about the development of each strain in the following table:

|  |  |
| --- | --- |
| Strain Name | Hypothesis:  |
| Wild type |  |
| sdhBEY12081 |  |
| simW501;OreR |  |

Table 2. Hypothesis on the developmental time of different *Drosophila* strains

Record in Table 3 how many larvae, pupae and adults flies are in each vial.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Name | larvae | pupae | adults | comments |
| Wild type |  |  |  |
| sdhBEY12081 |  |  |  |
| simW501;OreR |  |  |  |

Table 3 Numbers of flies at different stages (individual results)

To be able to statistically analyze the results you need more than 1 measurement for each strain. You will share your data with the rest of the class. Record the class data in Table 4

Class data

|  |  |  |  |
| --- | --- | --- | --- |
| Name | larvae | pupae | adults |
|  1 2 3 4 5 6 1 2 3 4 5 6 1 2 3 4 5 6 |
| Wild type |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| sdhBEY12081 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| simW501;OreR |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

Table 4 Numbers of flies at different stages (class totals)

Now we will analyze the class data. After the analysis, what do you conclude?

Climbing assay

# Fruit flies have negative geotropism (away from the earth), which means they have a tendency to climb to the top of the vial. To study motor function we can use this negative geotropism and count the number of flies that can climb to the top of the tube in a specific amount of time. The ability to climb a vial changes with age; older flies take longer to climb to a certain height or may climb only lower heights than younger flies (Le Bourg and Lints, 1992). Impaired climbing ability is also a symptom of mitochondrial dysfunction.

Here you will test the climbing ability of the strains wt, *sdhB* and *w501*. Based on what you know about each strain, state a hypothesis about the climbing ability of each strain in the following table:

|  |  |
| --- | --- |
| Strain Name | Hypothesis:  |
| Wild type |  |
| sdhBEY12081 |  |
| simW501;OreR |  |

Table 5: Hypotheses on climbing ability of different *Drosophila* strains

**Procedure:**

As before, you will work in pairs. In each table you have two vials per fly strain: one vial per each pair. Also, you have 2 empty vials per strain.

1-Draw two lines that will separate the vial into 3 sections of 3 cm each. Label the sections A,B,C. Section A will be further away from the bottom, B in the middle and C closest to the bottom (see figure 3)

2-Transfer the flies (approximately ten flies) to an empty plastic vial. Use a cotton stopper to cover the vial.

3-Gently tap the vial until all the flies are in the bottom.

4-Measure the number of flies in section A,B and C after 20 seconds

5-Combine the results from your table and for the rest of the class

A

B

C

Cotton stopper

***Figure 3****. Schematic representation of the vial for the climbing assay. A,B and C sections are around 3cm each.*

|  |
| --- |
| **Your results** |
| # flies that climbed/total flies | 20 seconds | A |  |  |  |
| B |  |  |  |
| C |  |  |  |

 Wild type *sdhBEY12081* *simW501;OreR*

Table 6: Climbing assay results for one group

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Class results**  | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| # flies that climbed/total flies | 20 seconds | A |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

 Wild type *sdhBEY12081* *simW501;OreR*

Table 7: Climbing assay results for the class

Now we will analyze the class data.

After the analysis, what do you conclude?

Flight assay

Because fruit flies require a lot of energy to fly, decreased flight capability is associated with mitochondrial dysfunction (Walker and Benzer, 2004). In this experiment you will test the flight capability of the strains wild type, *sdhB* and *W501.*

Based on what you know about each strain, state a hypothesis about the flight capability of each strain in the following chart

|  |  |
| --- | --- |
| Strain Name | Hypothesis:  |
| Wild type |  |
| sdhBEY12081 |  |
| simW501;OreR |  |

Table 8: Hypothesis on flying ability of different *Drosophila* strains

Procedure:

1. Each table has two 500-ml graduated cylinders and one vial for each strain.
2. Coat the inside of the cylinders with paraffin oil. To do that, use a long pair of forceps and a piece of cotton.
3. With the help of a funnel, drop the flies of a vial (not the vial) into the graduated cylinder. Keep the vial and the funnel as vertical as possible so that the flies won’t accidentally hit the walls of the cylinder.
4. The distribution of the levels at which the flies hit the wall and become stuck in the oil reflects their flying ability. Normal flies quickly initiate horizontal flight, striking the wall close to the entry level (top of the cylinder), whereas poor fliers land at lower levels or at the bottom of the cylinder.
5. Record where each fly landed as # flies that landed/total number of flies. You can take advantage of the cylinder graduation to take the measurements. Record the measurements in the table below.

|  |  |  |  |
| --- | --- | --- | --- |
| >500ml |  |  |  |
| 250-500ml |  |  |  |
| 0-250 ml |  |  |  |

Wild type *sdhBEY12081* *simW501;OreR*

Table 9: Number of flies at different sections of the cylinder

Based in your data, analyze and graph the results. Write your conclusion from the experiment here:

In vitro activity of Succinate dehydrogenase (Complex II )



***Figure 4****. Schematic representation of the reaction catalyzed by Complex II of the electron transport chain.*

In this experiment you will be measuring the in vitro activity of the enzyme succinate dehydrogenase. Because this enzyme is a member of the electron transport chain (Complex II), it is located in the mitochondrion. Hence you will first isolate mitochondria from the rest of the cellular components of the *Drosophila* strains *wt, sdhB and w501*. After mitochondria are isolated, you will perform the enzymatic assay.

 Succinate dehydrogenase catalyses the oxidation of succinate to fumarate in the Krebs (tricarboxylic acid) cycle. It transfers the electrons from succinate to the electron carrier FAD, which is reduced to FADH2. FADH2 is then oxidized back to FAD and the resulting electrons are transferred to ubiquinone (Figure 4).
In the lab you will be measuring the transferring of these electrons as indicators of the enzymatic reaction. To do that, you will add to the reaction a blue compound, **DCPIP** (dichlorophenol-indophenol) to the reaction mixture. DCPIP intercepts the electrons before they get to the electron transport chain. When DCPIP picks up an electron, the blue color disappears and it becomes colorless, therefore, the catalytic activity of the enzyme is monitored by the reduction (and therefore change of color) of DCPIP using a spectrophotometer (Figure 5). You used a similar procedure in Introductory Biology class (Bio 111) when you measured respiration using lima beans.

***Figure 5****. Schematic representation of the path of electron in the succinate dehydrogenase in vitro enzymatic reaction.*

Based on what you know about each strain, state a hypothesis about the SDH activity of each strain in the following chart

|  |  |
| --- | --- |
| Strain Name | Hypothesis:  |
| Wild type |  |
| sdhBEY12081 |  |
| simW501;OreR |  |

Table 10: Hypothesis on the *in vitro* activity of complex II of different *Drosophila* strains

**Procedure:**

Before you start the procedure you should read appendix A) how to use a micropipettor

Extraction of mitochondria

1. In a 1.5ml Eppendorf tube add 1000μl of Mitochondria Isolation Buffer (MIB) and approximately 20 whole female flies. Use a different tube for each strain (*wt, sdhB and w501*).
2. Using a blue pestle as a grinder, grind by hand the flies until the solution turns red due to the eye pigment going into solution.
3. Centrifuge 5 minutes at 300g. Make sure the centrifuge is at 4C
4. Transfer the supernatant into a new tube and discard the pellet
5. Centrifuge supernatant (from previous step) 10 minutes at 6000g. Make sure the centrifuge is at 4C
6. Discard supernatant. Resuspend the pellet in 100 µl of Mitochondrial Respiration Buffer (MRB)
7. Keep your mitochondria suspension on ice at all times. Label each tube with the name of the strain and your initials. You can freeze the mitochondrial suspension samples or proceed with the *in vitro* assay

**In vitro activity of succinate dehydrogenase**

In a separate tube, mix the reagents in the order listed.

|  |  |
| --- | --- |
| **Reagents** | **Volume Added** |
| **Sdh activity Buffer** | 520µl |
| **50 mM Succinate** | 160 µl |
| **2 mM DCPIP** | 40 µl |
| **2 mM Decylubiquinone** | 40 µl |

# *Table 8. Components of the in vitro succinate dehydrogenase assay mix*

# Running the Assay

We will use a 96-well plate for the whole class. Each group will fill three wells (1 per strain). Add the following to each well.

Add Mix volume to each well: 190µL

Mito volume : 10µL

* Set the spectrophotometer to 30°C
* Run a kinetic assay at **Absorbance** 600nm.
* Shake the plate for 3 seconds before reading.
* Use the rate from the first 60-150 seconds.

Graph and analyze your results. Paste here the graph.

What do you conclude regarding the activity of succinate dehydrogenase in the *wt, sdhB and w501* strains?

**Experiment 2**

**Experiment 2: Analysis of inheritance**

In this experiment you will determine the pattern of inheritance of the allele *sdhBEY12081* .One thing you need to remember before you proceed with this experiment is that the *sdhBEY12081* is an allele of a gene encoded in the nuclear genome of *Drosophila melanogaster* and has a Mendelian inheritance pattern. On the other hand, the haplotype s*imW501* has the same nuclear DNA as the wild type but differs in its mitochondrial DNA. Because mitochondria are extranuclear organelles they are inherited only through the cytoplasm of the egg. Therefore, mitochondrial genomes are only transferred to the offspring by the mother (called maternal inheritance). Maternal inheritance produces non-mendelian results in reciprocal crosses (check your textbook)

Pre-lab: Maternal Inheritance. Answer the question:

Which cross or crosses below will give progeny that have a developmental delay?

Cross A: ♂ *sm21W501* X ♀ *sm21W501*

Cross B: ♂ *wt* X ♀ *sm21W501*

Cross C: ♂ *sm21W501* X ♀ *wt*

Mendelian Inheritance

**Prelab preparation.** Read through the instructions for this lab, answer questions 1 and 2 and complete the blank Punnett square (Table 12).

In the first part of this experiment, you will determine where the *sdhBEY12081* allele is located in the genome of *D. melanogaster.* To determine the location you will cross *sdhBEY12081*with a fly that has genetic dominant markers and balancer chromosomes on both the second and third chromosomes. A marker is essentially a DNA polymorphism that allows the inheritance of a piece of a chromosome to be followed through crosses. A balancer chromosome is a modified chromosome so that suppresses homologous recombination during meiosis. Also, a balancer chromosomes have a dominant marker in it. We will call that strain the double balancer/ marker, and its genotype is:

 If MKRS

 ;

*Figure 5. Double balancer/marker. Each line represents a homologous chromosome.*

*Only the 2nd and 3rd chromosome are represented.*

 CyO TM6B

 2nd Chromosome 3rd Chromosome

The phenotype of the double balancer/marker is:

**If:** Dominant marker. Flies have irregular and smaller eyes.

**MKRS:** Dominant marker. Flies have short bristles.

**CyO:** Balancer Chromosomes with dominant marker Cy. Flies have curled wings.

**TM6B:** Balancer chromosomes with the dominant marker Tubby (Tb). Tubby larvae are smaller and chubbier.

All of the makers are homozygous lethal. This means that a fly homozygous for the markers will die and therefore you won’t be able to observe it.

The schematic representation of the cross you will perform is presented in this diagram:

Parental ♀ *If/CyO ; MKRS/TM6B* X ♂*sdhBEY12081*/ *sdhBEY12081*

F1♂ ?/CyO ; ?/MKRS X ♀ ?/CyO ; ?/MKRS

 F2 Punnett Square

Knowing that *sdhBEY12081* is a recessive allele:

1-what is the expected phenotypic ratio of the F1 for wild-type climbers: mutant climbers?

2- and for the F2? Read below before you answer the questions.

To answer the questions 1 and 2, think about a Punnett square of the F2 cross. Suppose, for example, that the *sdhBEY12081*allele is on the second chromosome. The Punnett square would be as follows.

 F1♂ *sdhB*/CyO ; +/MKRS X ♀ *sdhB*/CyO ; +/MKRS

GAMETES

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  ♀♂  | *sdhB, +* | *sdhB*,MKRS | CyO, + | CyO, MKRS |
| *sdhB, +* | *sdhB/sdhB;* *+/+* | *sdhB/sdhB;**MKRS/+* | *sdhB /CyO;**+/+* | *sdhB /CyO;**MKRS/+* |
| *sdhB*,MKRS | *sdhB/sdhB;* *MKRS/+* | *Lethal* | *sdhB/CyO;* *MKRS/+* | *Lethal* |
| CyO, + | *sdhB /CyO;**+/+* | *sdhB /CyO;**MKRS/+* | *Lethal* | *Lethal* |
| CyO, MKRS | *sdhB /CyO;**MKRS/+* | *Lethal* | *Lethal* | *Lethal* |

*Table 11. Punnett square assuming that the sdhBEY12081allele is on the second chromosome:*

Now do the Punnett square assuming that the *sdhBEY12081*allele is on the third chromosome:

 F1♂ *+/CyO ; sdhB/MKRS X ♀ +/CyO ; sdhB/MKRS*

 GAMETES

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  ♀♂  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

*Table 12; Punnett square assuming that the sdhBEY12081allele is on the third chromosome:*

**Procedure**

Since *Drosophila* females flies store sperm for days, virgin flies are needed to perform crosses. Virgin flies have recently eclosed from the pupae and they are recognizable by morphological characteristic. They often have folded wings, they are lighter in color, and puffier than non-virgin flies.

1-Set up the Parental crosses. Set up a cross between 5 virgin females flies (provided by the TA) of the *double balancer/marker* strain *If/CyO; MKRS/TM6B* and five males *of* the strain *sdhBEY12081.* Label the vial with details of the cross, the date, and your names.

The cross will be as follows:

♀ *If/CyO ; MKRS/TM6B* X ♂ *sdhBEY12081*/ *sdhBEY12081*

2- (~ one week later) clear adult flies from vials. Remove all live adults from the parental cross and dispose of them in a fly morgue.

3- (~ one week later). Set up the F1 crosses. Set up a cross between approximately five virgin females and five males from the F1 with the **phenotype normal sized eyes, curled wings and short bristles**. Because we don’t know in which chromosome the allele *sdhBEY12081* is located yet, we will write a “?” indicating a potential location. The genotypes of the cross will be as follows:

♂ ?/CyO ; ?/MKRS X ♀ ?/CyO ; ?/MKRS

Again, label the vial with details of the cross, the date, and your names.

4- (~ one week later) clear adults flies form vials. Remove all live adults from the F1 cross and dispose of them in a fly morgue

 5) Phenotype F2 progeny: You will sort the phenotypes and screen for climbing deficiency.

Now sort and count how many flies have the following phenotypes:

|  |  |  |
| --- | --- | --- |
| Phenotype | # flies | Climbing ability |
| A- Wild type: straight wings and normal bristles |  | Normal or Abnormal |
| B- short bristles  |  | Normal or Abnormal |
| C- curled wings |  | Normal or Abnormal |
| D- curled wings and short bristles |  | Normal or Abnormal |

*Table 13: number of flies with different phenotypes*

6) Keep the flies separated by phenotypes in different vials. You can label them A, B, C and D according to the previous table. Next you will assay their climbing ability. You will do that next week when the flies are old enough for you to see a clear difference in their climbing performance. In the meantime, determine the location of the *sdhBEY12081*(following page).

7) Assay the climbing ability as explained before. Consider “Normal” if 50% or more of the flies climb to section A of the vial. (Figure 3). Conversely, consider “Abnormal” if <50% of flies climb to section A. Record this in the previous table as # flies climbed to A/total number of flies

Determine on which chromosome, 2nd or 3rd, the allele *sdhBEY12081* is located.

Let’s remember what we know about the *sdhBEY12081* allele:

 1) it is a recessive allele

 2) homozygous *sdhBEY12081* flies have abnormal climbing ability

Now answer this question:

Did some of the abnormal climbers from the F2 offspring display curled wings or short bristles?

Review the Punnett squares (Table 11 and 12) made assuming the location *sdhBEY12081*  was either on the 2nd or 3rd chromosome. In Table 11, one of the genotypes is possible only with the assumption that the *sdhBEY12081* mutation is on chromosome 2. Which genotype is this? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ What is the corresponding phenotype?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

In Table 12, one of the genotypes is possible only with the assumption that the *sdhBEY12081* mutation is on chromosome 3. Which genotype is this?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ What is the corresponding phenotype? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Thus, the phenotype (either curled wings or short bristles) of the flies that also have abnormal climbing ability will tell you the chromosomal location of the *sdhBEY12081* mutation.

On which chromosome is the allele *sdhBEY12081*  located within the genome of *D. melanogaster?*

Now you will practice chi-squared analysis to test the hypothesis that *sdhBEY12081* is indeed a recessive allele. Focusing on the in the data obtained in Table 13, calculate the expected frequencies from Table 11 if you determined that the allele *sdhBEY12081* is located on chromosome 2, calculate the expected frequencies form Table 11. If you concluded that is located on chromosome 3, use Table 12. Please read the chi-square test appendix and fill the following table:

|  |  |  |  |
| --- | --- | --- | --- |
| Class | Observed (O) | Expected (E) | (O-E)2/E |
| Normal Climbers |  |  |  |
| Abnormal Climbers |  |  |  |
| Total |  |  |  |

*Table 14: calculations for the chi-square test*

For the observed data, add the total number of flies that are normal or abnormal climbers. For example, if phenotype A (wild type) and phenotype C (curly wings) have normal climbing ability, the Observed Normal climbers is # flies of A + # flies of C and the Observed Abnormal climbers is # flies of B + # flies of D.

Calculate the expected values for the different classes of progeny (write it in Table 14). Don’t forget to use the actual total number of progeny that you observed to calculate the expected values.

How many degrees of freedom are there in this cross? Use this number and the total chi-squared value obtained from Table 14 above, in the “Chi-Squared Probabilities”(appendix), the *p* value for your results and your hypothesis.

What is the *p* value obtained by this test? \_\_\_\_\_\_\_\_\_\_\_

Does this support or fail to support the hypothesis?

If your *p* value doesn’t support the hypothesis, it may mean several things:

a. Your hypothesis is incorrect (the obvious choice): for example, you thought the mutation was recessive, but it was actually dominant.

b. One of your “hidden” assumptions is incorrect. For example, it could be that your scoring was inaccurate (whereas you assumed that you could tell the difference between the different phenotypes); it could be that one of the classes of progeny has reduced viability (whereas you were assuming that the genotype did not affect viability); or it could be that your crosses were not correctly performed, e.g. you thought you used virgin females but one or more of them had already mated at the time that you set up the cross.

**Experiment 3**

**Experiment 3: Molecular characterization of haplotypes by PCR-RFLP**

In this experiment you will characterize and idenfity the mitochondrial haplotypes of the strains *wt, sdhB and w501*. To do that you will use the following molecular genetics techniques: **Polymerase Chain Reaction (PCR)** and **Restriction Fragment Length Polymorphism (RFLP).** By PCR you will amplify genomic DNA. By RFLP these DNA samples will be digested (cut into pieces) by restriction enzymes and the resulting restricted fragments will be separated by length using gel electrophoresis.

But first of all, we need to extract the genomic DNA from the three strains. We will use a protocol that will isolate all genomic DNA, mitochondrial and nuclear. The extraction of DNA from biological material requires several steps. First you will lyse the fly cells to release the DNA by grinding and incubation in the lysis buffer, and then separate the DNA from the other macromolecules by binding it to a special DNA-binding chemical on a “spin column”, washing, and then eventually releasing the DNA.

Genomic DNA extraction from a whole fly

1. In a 1.5ml Eppendorf tube add 180μl Lysis/Binding Buffer and 20 μl of proteinase K and approximately 5 whole female flies. Use a funnel to transfer the flies to the tube. Female flies have a large number of oocytes that contain lots of MtDNA and typically yield greater amounts of MtDNA than males.
2. Using a blue pestle as a grinder, grind by hand the flies until the solution has gone red with the eye pigment in solution. This will be approximately enough time for the majority of the flies to have been homogenized. There should be no large chunks of tissue at this stage but there may be some exoskeleton present.
3. Centrifuge briefly to ensure all homogenized tissue is in solution.
4. Place the Eppendorf tube in a water bath/tube heater for 45 minutes at 55ºC. After 25 minutes, mix the solution well, and then continue incubating until 45 minutes is up.
5. After 45 minutes incubation centrifuge the sample at ~8,000 RPM for 1 minute. Transfer the supernatant to a fresh 1.5 ml microcentrifuge tube being careful not to get any of the pellet. The supernatant now contains genomic DNA.
6. Add 120µL 100% ethanol and mix by vortexing for 5 seconds.
7. Add the lysate to a spin column placed in a collection tube.  Centrifuge at top speed for 1 minute.  Discard the collection tube and place the column in a new collection tube.
8. Add 500µL Wash Buffer 1 to the column.  Centrifuge the column at top speed for 1 minute.
9. Discard the liquid in the collection tube and place the column back in the collection tube.
10. Add 500µL Wash Buffer 2 to the column.  Centrifuge the column at top speed for 3 minutes.
11. Discard the collection tube and place the column in a sterile 1.5mL tube.
12. Add 50µL Elution Buffer directly onto the membrane within the column.  Let the column stand for 1 minute.  Centrifuge the column at top speed for 1 minute.
13. Discard the column but keep the tube it was in.  The purified DNA is suspended in the buffer in the 1.5mL tube.
14. Add 100µL sterile water to the DNA to reach a working concentration.  Mix by flicking the bottom of the tube.  Label your tube with the strain and initial.  Place your tube in your group’s freezer box and store it in the freezer.

Planning Polymerase Chain Reactions

Prelab summaries: Read through the instructions for this lab and complete Tables 15 and 16.

The PCR was developed in 1984 by Kary Mullis. It uses a DNA polymerase enzyme and specific DNA primers to amplify (make lots of copies of) a small region of the genome. You can find more details on PCR in your textbook.

Each polymerase chain reaction (PCR) requires a number of components:

* template DNA
* DNA polymerase (in our case, a thermostable version called *Taq* polymerase)
* DNA precursors (deoxynucleoside triphosphates, or NTPs)
* primers (oligonucleotides=short single-stranded pieces of DNA): two per reaction, binding close together on the chromosome and on opposite strands
* buffer (to ensure optimal conditions of pH and ionic strength for the polymerase, includes magnesium)

The region to amplify by PCR is determined by the primers. The primers are designed to bind, through complementary base pairing, a region of the DNA that we are interested in. In our particular case, because we want to distinguish between different mitochondrial haplotypes, this region encodes the Cytochrome c oxidase subunit I (Montooth et al., 2010).

PCR primer sequences for Cytochrome c Oxidase subunit I locus:

3593F(FORWARD PRIMER): GAACAGTTCCCGCTTTAGGAG

4528R (REVERSE PRIMER): GCAGTTAATCGGACAGCTAATGTTCCC

Planning Polymerase Chain Reactions

First, you need to plan what will go into each PCR. You should complete this section **before** coming to lab. Read this explanation, and then fill out the last column of Table 16 below.

As already mentioned above, each polymerase chain reaction (PCR) requires a number of components. These components are supplied as concentrated stock solutions, and are therefore added in small amounts to the reaction to give the correct final concentration:

* template DNA: while it is possible to use too much or too little template DNA, in general this amount is flexible within a range of 10 to 100 ng, or even wider. We will simply use 2 µL of our DNA preparation.
* *Taq* DNA polymerase: concentration of this enzyme is measured in activity units; we will use 0.5 activity unit (at 5 units/µL, this is 0.125 µL of the *Taq* polymerase).
* DNA precursors (dNTPs): generally a final concentration of 200 µM is adequate; our stock solution is 10 mM.
* primers (oligonucleotides): two per reaction; we will use primers here at a concentration of 200 nM. Our primer stocks are at 10μM.
* buffer: here we will use a stock that is 10X more concentrated than our desired final concentration, which is 50 mM KCl, 10 mM Tris, pH 8.3, 1.5 mM MgCl2
* water is added to bring the reaction up to the desired final volume. In our case, we will set up reactions of 25 µL.

An example of how you will calculate the amount of a reagent to add to a reaction:

How much dNTPs should I add to a 50 µL PCR to give a final concentration of 200 µM?

Use the formula **civi = cfvf,** where ci = the initial concentration (i.e. the concentration of the stock solution), vi = the initial volume (the volume of the stock solution), cf = the final concentration (i.e. in the reaction tube), and vf = the final volume (of the reaction). We need to find vi, the volume of the stock solution to be added to the reaction.

Solving for vi: vi = cfvf/ci

Substituting in the known values: vi = (0.2mM)(50 µL)/10 mM = 1 µL

So 1 µL of 10mM dNTPs stock must be added to a 50 µL total reaction volume to give a final dNTPs concentration of 0.2mM

NOTE: Be careful that your units are consistent! You cannot mix millimolar and micromolar in the same calculation and expect to get the correct answer—convert all values to the same units, e.g. 0.2 mM = 200 µM.

In the table below, you will calculate how much of each concentrated stock reagent to add to make a reaction with a total volume of 25 μL, using the approach shown in the example above.

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **[Stock** **solution]** | **[Desired final]** | **Vol. of stock to add** **for one 25 μL reaction** |
| dNTPs | 10 mM | 200 µM |  |
| buffer | 10X | 1X |  |
| DNA template | -- | -- | 2 µL |
| Primers | 10 µM | 250 nM |  |
| Taq polymerase | -- | -- | 0.125 µL |
| water |  |  | To give a final volume of 25µL: \_\_\_\_\_\_\_\_\_\_ |

Table 15. Components of polymerase chain reactions.

Saving time with a master mix

If you are setting up many reactions that require the same reagents (as you are here), a time-saving approach is to make a “master mix” or “cocktail” containing all these reagents in the proper proportions, and then distribute the proper volume of the master mix into each reaction tube. This approach saves on pipet tips, time, and effort, and reduces errors.

Two useful tips when making a master mix:

* Calculate the volumes to add based on the number of tubes you need + a few additional: that way you have a little extra so you don’t run out for the last tube. So, if you need to set up 4 reactions, combine enough reagents for 6 reactions. If you find you still run out of reagents, ask the instructor to check your pipetting technique and your pipettors.
* Add the water first to the master mix. Sometimes adding together the concentrated reagents by themselves without water may lead to precipitation.

You can calculate the ingredients of a master mix in Table 16.

Note that you can only include in the master mix the ingredients that will be the same in each tube. Therefore, the template DNA is not usually part of the master mix. Be sure to add the Taq polymerase last to the master mix, and only after the reactions are otherwise all ready to go and the PCR machine is heated up.

|  |
| --- |
| **Master mix** |
| **Reagent** **(stock concentration)** | **Vol. of stock** **for 1 reaction** | **Vol. of stock to add to master mix (multiply previous column by # of reactions +2)** |
| water |  |  |
| 10mM dNTPs |  |  |
| 10X buffer |  |  |
| 10 μM primers |  |  |
| *Taq* polymerase |  |  |
| Sum: Vol. to add to each tube |  |  |

Table 16 Master mix for polymerase chain reactions.

 Polymerase Chain Reaction (PCR)

**Notes:** Wear clean gloves and do not breathe into your PCR tubes. It is critical that everything be kept on ice as much as possible while setting up the PCR reactions. Especially important is the Taq: do not hold the tube at the bottom because the warmth of your fingers will start to degrade the enzyme.

CAUTION: It is very important during this work:

* to change pipet tips when necessary (i.e. almost every time) to avoid any possibility of cross-contamination.
* to keep all reagents and tubes on ice as much as possible;
* however, you should remove the tubes from ice when pipetting out of them

**Procedure**

1). Label 4 tubes as

 1 – wild type

 2 - *sdhB*

 3 - *W501*

4 - control (add water instead of DNA)

2.) In a 1.5mL tube on ice, set up the following PCR Mastermix:

Water 113.25 µL

10X Buffer 15 µL

dNTPs 3 µL

Primer 3593F 3 µL

Primer 4528R 3 µL

Taq Polymerase 0.75 µL

Total 138µL

3). Flick the bottom of the tube to mix the components, then tap it on the benchtop to collect most of the contents at the bottom of the tube. Add 23 µL of the PCR mastermix to each of your 4 PCR tubes on ice.

4). Next add 2µL of DNA to each of tubes 1 and 2, and 2µL of water to tube 4. Write down which DNA sample was added to which tube below:

Tube: 1 – wild type

 2 - *sdhB*

 3 - *W501*

4 - control (add water instead of DNA)

5). Cap the tubes. Flick the bottom of the tubes to mix them, then spin them for a short time in the mini-centrifuge to collect the contents at the bottom of the tubes .Use the special microcentrifuge for small tubes located in the instructor’s table. Place the PCR tubes on ice until it is time to place them in the PCR machine.

6). The thermal cycling conditions will be as follows:

|  |  |  |  |
| --- | --- | --- | --- |
| **Step** | **Temp.** | **Time** | **Purpose** |
| 1 | 94 °C | 2 min. | initial denaturation |
| 2 | 94 °C | 30 sec. | denaturation |
| 3 | 54 °C | 30 sec. | annealing |
| 4 | 72 °C | 45 sec. | extension |
| Repeat **steps 2, 3, & 4** 29 more times (30 times total) |
| 5 | 72 °C | 10 min. | final elongation |

*Table 17. Thermal cycling conditions*

After this is complete, the PCR products will be stored at 4°C until the next lab.

Gel electrophoresis confirmation of the PCR reaction

Pre lab: Read through the instructions for this lab and complete the questions about the pretend gel.

In this lab we will use agarose gel electrophoresis to determine if the PCR was successful, and to determine the size of the PCR products. Agarose gel electrophoresis is the standard approach used to analyze most types of experiments involving nucleic acids. Agarose, a branched polysaccharide purified from sea algae, is melted in a buffer solution, poured into a mold with a “comb’ to create holes, and then submerged in an electrophoresis chamber containing the same electrophoresis buffer. Samples, mixed with a loading dye, are pipetted into the holes (“wells”) in the gel, and then an electric field is applied across the gel. The negatively-charged nucleic acid fragments in the gel are attracted towards the positive electrode and are separated by size as they move through the gel. Smaller fragments can move more quickly through the gel and so migrate farther down the gel. At the end of the electrophoresis, the DNA fragments are visualized by staining with a dye called SYBR Safe that fluoresces under UV light. . A size standard consisting of DNA fragments ranging in size from 100 bp to 1000 bp, in 100 bp increments, plus 1200 and 1500 bp fragments, will be added to the first well of each row. The picture below shows an example of the results of DNA electrophoresis.

 Ladder 1 2 3 4 5 (-) control

In which direction did the DNA travel?

Label the end of the gel at positive pole of the gel box, and the end at the negative pole.

Label the sizes of the different fragments of the DNA ladder.

What are the sizes of the PCR fragments?

What would you conclude if you saw a band in the – control lane?

**Procedure**

Before lab, an agarose gel will have been prepared by mixing 1 g of agarose with 100 ml electrophoresis buffer, boiling the solution to dissolve the agarose, allowing the solution to cool to about 60°C, adding a DNA stain (SYBR Safe), and then pouring the solution into a mold with combs that will form wells in the final gel. As the agarose solution continues to cool, it polymerizes, forming a semi-solid slab with a matrix that will allow DNA to move through it. The combs are removed and the gel is placed in a box filled with electrophoresis buffer.

**Notes:** Wear gloves and safety glasses while loading and handling gels.

1). Separate half of the PCR products (12 μl) into a new vial. Add 2.4 μl of loading dye to each of your PCR tubes and mix them. Load this mix into a well in the gel. Record which well your products have been loaded in. After all of the products have been loaded, a 100V current will be applied to the box for approximately 1 hr, or until the dye front has neared the end of the gel. After starting the gel, check that bubbles begin to come off the electrodes and that the dye is moving in the proper direction. You should be able to see this within a few minutes!

Note the times of starting and stopping:

Voltage\_\_\_\_\_\_\_\_\_\_ Time started\_\_\_\_\_\_\_\_\_\_\_ Time stopped\_\_\_\_\_\_\_\_\_\_\_

While the gel is running you will prepare the Restriction Fragment Length Polymorphism (RFLP) (next page).

2). The gel will be placed on a UV box, which causes DNA stained with SYBR Safe to fluoresce. The gel will be photographed, and copies of the photograph will be distributed so we can analyze the results of the gel.

Picture of the gel

**Restriction Fragment Length Polymorphism (RFLP)**

Restriction enzymes are found naturally in bacteria and can be used to cut DNA fragments at specific sequences. Two DNA fragments with different sequences, even though they might have the same size, can be digested into different pieces. Previous work has determined that the fragment you amplified from *D. melanogaster* and *D. simulans* mitochondria have different sequences and will be differentially digested by the enzyme RsaI (Montooth et al., 2010). Therefore, when the resulting restricted fragments are separated by size using gel electrophoresis, the DNA fragment from *D. simulans* mitochondria will have a different pattern of bands than the DNA fragment amplified form *D. melanogaster* mitochondria.

Now you will digest the DNA that you amplified from *D. melanogaster* and *D. simulans* mitochondria with AluI and RsaI enzymes.

**Pre lab summaries:** Fill the master mix calculations on table 18

1) Take half the PCR product that was not run out on the first gel.

2) Each restriction digestion event will need the following quantities:

 PCR product: **12 μl (already in the tube)**

 10X Restriction enzyme buffer: **2** **μl**

 BSA 10 mg/ml: **0.2 μl**

Restriction enzyme: **0.2** **μl**

H2O: **5.6 μl**

This will give a total reaction volume of **20 μl**

Again you should make a “master mix” as described previously. Please read appendix B on how to calculate dilutions.

|  |
| --- |
| *master mix* |
| **Reagent** **(stock concentration)** | **Vol. of stock for 1 reaction** | **Vol. of stock to add to master mix (multiply previous column by # of reactions +2)** |
| water |  |  |
| 10X buffer |  |  |
| RsaI |  |  |
| *BSA 10mg/ml* |  |  |
| Sum: Vol. to add to each tube |  |  |

Table 18. RFLP maxter mix.

3) Add 8μl of the master mix to each tube.

4) Incubate at **37 ºC** for at least 11 hours, overnight would be better.

5) Heat inactivate at 65ºC for 20 minutes

Gel electrophoresis confirmation of the RFLP

As before, before lab the TAs have prepared a new agarose gel by mixing 1 g of agarose with 100 ml electrophoresis buffer, boiling the solution to dissolve the agarose, allowing the solution to cool to about 60°C, adding a DNA stain (SYBR Safe), and then pouring the solution into a mold with combs that will form wells in the final gel.

1) Add 4 μl of loading dye to each of your tubes and mix them. Load this mix into a well in the gel. Record which well your products have been loaded in. After all of the products have been loaded, a 100V current will be applied to the box for approximately 1 hr, or until the dye front has neared the end of the gel.

Picture of the gel

Compare the restriction patterns to control (wild type) to score the haplotype. How many mitochondrial haplotypes are in the strains?

 Which one is similar to the control?

How do your results compare to the information in Table 1?

**Independent Project**

For this part of the class, you will carry out a project of your own design using *D. melanogaster*.

You are welcome to use any of the strains of flies we used in previous experiments. Additionally, a list of fly mutant stocks available for you to use will be on Moodle. You are welcome to use any strain you want from that list.

When deciding on an independent project, try to find something that is interesting to you and that you think is feasible within the time we have. A strategy that might help you is to consider two different approaches to a research problem.

1-Find a biological process that you are interested in. For example, those that influence:

a) Morphological characteristics: some of the mutant strains that you can find in the list of fly mutant stocks affect morphological structures of the flies e.g. *Wrinkled* flies have abnormal shape of the wings. Some of these mutant phenotypes vary from one individual to another. You could investigate, for example, whether or not environmental conditions (such as temperature or food quality) or genetic background affect the expressivity of these traits.

b) Physiological characteristics: how flies perceive images, odors, etc.

c) Behavioral characteristics: courtship, mating choice, sleep (not recommended unless you want to stay all day long tapping a fly vial so that flies cannot sleep), etc.

d) Stress resistance: temperature stress (either by heat shock or by chill coma), nutrient stress (too much or too little-starvation- food), flight exhaustion, etc

e) Developmental/post developmental processes: differences in developmental time (time for egg to adult), aging (how long a fly will live. Please consider that flies live an average of 3 months without any stress), etc.

OR

2-Find a genetic interaction to study. Those can be divided in three categories

a) Gene-gene interaction. Interaction of mutant alleles from different genes.

b) Gene-environment interaction. Effect of environment on the phenotype of mutant strains.

c) Gene-gene-environment interaction (the experimental design in this category might get complicated). Effect of environment on the interaction between different mutant strains.

Once you know the biological problem and the genetic interaction you want to study, it is time to decide CAREFULLY the phenotype you want to measure and how to quantify it. After this, I encourage you to search for published work. Please, be creative and add your own original ideas. You might need to reconsider your hypothesis and/or modify part of your original design so that you can add something new to the field with your experiment.

You will be presenting your proposal to the rest of the class. Hopefully you will get feedback that will help you to consider whether your project is feasible.

Suggested steps for this research project

* Research in scientific literature.
* Develop hypothesis.
* Consult with instructor, and revise as necessary.
* Design experiments to test hypothesis (see below)
* Consult with instructor, and revise as necessary.
* Prepare presentation.
* Practice presentation.
* Give presentation.
* Start experiment(s).
* Collect data.
* Analyze results. Perform statistical analysis. Consult with instructor.
* Write a draft of the final lab report. Discuss draft with instructor
* Prepare presentation.
* Practice presentation.
* Revise lab report.
* Submit lab report
* Give oral presentation.

Feel free to consult with instructor as needed, even if not specifically indicated

## Experimental Design

1. Think about the controls that you will use:

A positive control shows that your experiment is capable of detecting the result that you expect to see. A frequent positive control is the wild type. However, it will depend on your particular research experiment.

A negative control shows that our experiment is not giving us a false result, perhaps due to contamination. An example of a negative control is a mutant strain that you already know is abnormal for what you want to test, e.g.: *sdhB* if you are measuring climbing ability.

## 2. Think about testing adequate numbers of individuals. You would not want to make a conclusion from looking at just one mutant and one wild-type individual, you need to know that a difference you see is reproducible and consistent. The number of individuals to test depends on the magnitude of the difference you see: if a difference in phenotype is very clear, you do not need to look at thousands of individuals to be confident that the difference is real, but a small but real difference may require large numbers of individuals to be tested to give convincing results. A good approach is to do a small-scale experiment initially, say with 10-20 individuals of each genotype, and then repeat with more individuals if it appears there may be a subtle effect.

## 3. Think about testing in a physiologically relevant way. In general, the most meaningful experiments are ones in which the conditions are close to natural conditions.

4. Think about designing a feasible experiment. While it might be interesting to know how your mutant behaves in zero gravity, the lead time for getting experiments on the International Space Station is too long to be practical for a one semester lab course. It might also be interesting to do a time course of your mutant’s response to, for example, gravity, measuring it every 2 minutes for 48 hours, but this would also present practical difficulties unless you can automate the data-collection process as some scientists have done.

## 5. You should perform appropriate statistical tests on your results to evaluate their significance.

Planning ahead for your experiments

If your experiments will require any special materials or supplies, you must inform the instructor as soon as possible so that these can be obtained or ordered. Be aware that ordering special items can take a while, so the earlier you let the instructor know the more likely it is that you will actually have the items in time for your experiments!

##### Planning for the remainder of the semester

From this point on in the semester, if the labs have gone more or less as originally intended, there will no longer be a set plan for each lab which all students will follow. Instead, you will pursue your independent project. Note that you are still required to come to the scheduled lab period at the beginning to get any announcements or discussion, but you may choose if necessary for your experiment (and if you can arrange it with the instructor and/or TA) to do some of your work at other times during the week.

This does NOT mean that you are done with lab work for the semester, only that you are now knowledgeable enough to plan your own work schedule. The instructor will expect to see you put progress, time, and effort into lab at least equivalent to that required in the first part of the semester. **Be sure to keep good notes on what you do each time you are in lab.**

You need to present as part of your lab notebook your notes for experiment 4. You can staple them after this page or you can provide an independent notebook. If so, don’t forget to write your name on it.

**This is a good time to clean out your freezer and refrigerator racks of any unneeded tubes: old PCR tubes where you have already analyzed the reactions (especially if they didn’t work), and tubes of reagents that you no longer trust.**

**Appendices**

1. **Use of Micropipettors**

Micropipettors are expensive precision instruments that must be used properly. Please pay attention during the instructor’s description of how to use them, and read the instructions below carefully!

Pipettors are named according to the largest volume that they can hold: a P10 (gray top button) can pipet no more than 10 µL, a P100 (yellow top button) no more than 100 µL, and a P1000 (blue top button) no more than 1000 µL (1 mL). Note that the different sized pipettors use different pipet tips. For the highest accuracy in pipetting, use the smallest size pipettor that can pipet the proper volume, e.g. 10 µL will be most accurately pipetted using a P10, not a P100, and 100 µL will be most accurately pipetted using a P100, not a P1000.

**•Never** rotate the volume adjuster above or below the limits of the pipettor (below 0 or above the upper volume range).

**•Never** immerse the barrel of the pipettor in liquid or use the pipettor without a disposable tip in place.

**•Never** let the plunger of the pipettor snap back after depressing it, as the plunger could be damaged and aerosols could enter the pipet barrel.

**•Never** lay down the pipettor with fluid in the attached tip, as fluid could enter the barrel and damage the pipettor.

**• Never** drop or flame the pipettor, as this will damage it.

**•** Be sure to **set the volume correctly**. Setting the volume incorrectly is a very common source of errors when pipetting! If you have questions about the proper setting, ask the instructor or TA.

**• Be sure** that you can see the liquid entering and exiting the pipet tip as you pipet. This precaution will help you to pipet correctly and accurately.

**•** If your pipettor is not working properly, or you have questions, consult the instructor or TA.

**Pipetting Directions**

1. Rotate the volume adjustor on the pipettor to the desired setting.

2. Firmly attach a proper-sized pipet tip on the end of the pipettor:

a. You will be provided with tips in dispenser boxes.

b. The proper way to remove a tip is not to pick it up with your fingers, which could contaminate the other tips in the box, but to insert the thin tip of the pipettor into the pipet tip as the tip sits in the dispenser box, give a little twist while pressing down to seat the pipet tip firmly on the pipettor, and then remove the tip from the box.

c. Once the pipet tip is out of the box, you can also give it an extra twist onto the pipettor with your hand if you are careful not to contaminate the pipet tip.

3. Pick up the microcentrifuge tube out of which you will be pipetting. To reduce contamination, hold the body of the tube, not the lid. Hold the tube up nearly at eye level rather than leaving it in the rack; in this way you can properly position the tip in the liquid, and observe the changes in liquid level in the pipet tip. Also, do not have another person hold the tube for you.

4. Practice depressing the plunger on the pipettor.

a. You should be able to feel two stop positions: depressing to the first position measures the desired volume, depressing to the second position is used to “blow out” the last remaining drop of liquid.

b. Do not depress to the second stop when measuring the volume, or you will get a bigger volume of liquid than you intended.

5. To withdraw a sample from the tube:

a. Hold the pipettor nearly vertical;

b. Depress the plunger to the first stop and hold. Dip tip into the solution to be pipetted, and slowly raise the plunger to pull fluid into the tip. If you raise the plunger very quickly, you risk getting air bubbles into your sample (especially a problem when using the P1000) as well as creating aerosols, which with some samples could be hazardous.

c. Slide pipet tip out along inside wall of tube, to dislodge droplets stuck to the tip. Check that there is no air space at the end of the tube.

If something goes wrong along the way, and you have not touched the pipet tip to anything else, you can always put the liquid back in the tube and re-pipet it.

6. To expel the sample into a second tube:

a. Be sure you are holding the second tube so you can see it also!

b. Touch the pipet tip to the inside wall of the tube, to create a capillary effect, and slowly depress the plunger to the first stop to expel the sample. Depress the plunger to the second stop to blow out the last bit of liquid. Hold the plunger in the depressed position.

c. Slide the pipet out of the tube with the plunger depressed to avoid getting liquid back into tip.

d. Remove the tip manually, or eject it with the second (tip-ejection) button, into a container designated for that purpose, either a beaker or a plastic tub. Do not eject the tip into the sink.

7. To prevent cross-contamination of reagents, use a new tip for each transfer. The only exception is if you are adding the same reagent to a series of tubes in such a way that you will not contaminate your original stock solution. For example, if you are putting the same volume of water into a series of clean tubes that have nothing else in them, you can use the same tip for all the transfers.

1. **How to calculate dilutions**

Use this equation: civi = cfvf

c = concentration; v = volume; ci = initial concentration (i.e. of your stock solution);

cf = final concentration (i.e. that you want to obtain in your media)

You know the concentration of your stock solution (ci) and the final concentration you want (cf), and the final volume you want, i.e. how much media you want to make (vf). Now you need to solve for vi which is the volume of stock solution to add.

1. **The Chi-square(d) Test**

Introduction

The chi-squared test is a statistical test used to determine to what extent a set of data supports a particular hypothesis. It is especially widely used in genetics, where it provides answers to questions such as these:

* Does this trait behave as if it is controlled by a single gene?
* Could it be controlled by multiple genes?
* Are these two genes linked or do they segregate independently?

Note that the chi-squared test, like any statistical analysis, can show that your date support or fails to support an hypothesis, but cannot absolutely prove or disprove an hypothesis.

The results of chi-squared analysis should be presented in papers (and lab reports!) describing the genetic characterization of mutants.

Method

To apply the chi-squared test, follow these steps:

1. Based on the data and the type of cross, propose a genetic hypothesis that could explain the results. This hypothesis will serve as the basis for calculating the expected outcome of the cross based on Mendel’s laws. For example, if you are following a single trait in a cross of two heterozygotes and see 100 individuals with one form of the trait and 44 with the alternate form of the trait, you might consider this approximately a 3:1 segregation and therefore hypothesize that the trait is controlled by a single recessive allele. The null hypothesis would be that the trait is not controlled by a single recessive allele. If you are analyzing the F2 of a dihybrid cross for two traits, you might see a segregation ratio of 95: 33: 28: 8, and postulate that the two traits are controlled by two unlinked genes each with a dominant and recessive allele (giving a 9:3:3:1 ratio). When testing if two genes are linked, one usually tests the null hypothesis, that the two genes are not linked. In the F2 analysis of the enhancer, your hypothesis should include whether the enhancer is dominant or recessive and whether it is (by itself) auxin-resistant or auxin-sensitive.

2. Based on the hypothesis you proposed, and the total number of progeny in your data set, calculate the expected values for the different classes of progeny. For example, the hypothesis that a trait is controlled by a single recessive allele would lead to the prediction, for a cross of two heterozygotes, that 3/4 of the progeny will show the dominant trait and 1/4 will show the recessive trait. If you had scored 144 progeny, then the expected number of progeny with the dominant trait is (3/4 x 144) = 108, and the expected number of progeny with the recessive trait is (1/4 x 144) = 36 (see table below). Always use the actual total number of progeny that you observed to calculate the expected values; it is OK to have fractional values for the expected numbers, although it usually will not make a difference if you round off.

|  |  |  |  |
| --- | --- | --- | --- |
| Class | Observed (O) | Expected (E) | (O-E)2/E |
| Trait A | 100 | 108 | (100-108)2/108 = 0.59  |
| Trait a | 44 | 36 | (44-36)2/36 = 1.78 |
| Total | 144 | 144 | χ2 = 2.37 |

When calculating the expected values for two genes segregating in a single cross, be sure that you know what kind of cross was done! Two unlinked genes segregating in a test cross (AaBb x aabb) will give 1:1:1:1, whereas two unlinked genes segregating in a dihybrid cross (AaBb x AaBb) will give 9:3:3:1.

3. Apply the chi-squared formula: χ2 = Σ (O-E)2

E

This formula states that, for each class of progeny, subtract the expected from the observed, square the result, divide by the expected, and then add these values for all the classes of progeny. The sum is the chi-squared value. A straightforward way to calculate chi-squared values is with a table such as the one shown above.

4. Interpret the chi-squared value. The calculated chi-squared value and the degrees of freedom present in a cross are found in a chi-squared table, which gives a probability (*p*) value. This *p* value tells us the likelihood that the difference between the observed and expected values is due to chance. A low *p* value (less than 0.05) indicates that it is unlikely that the difference between observed and expected is due to chance, and therefore it is likely that this difference is meaningful.

The degrees of freedom (df) present in a cross equals the number of classes of progeny minus one. Essentially this tells us the number of categories that are independent of each other. If we have four classes of progeny and we know the numbers in three of the classes and the total number of progeny, then the number in the fourth class is determined. Thus, for four classes of progeny, the numbers in three of them are independent of each other, and the degrees of freedom is three.

Knowing the degrees of freedom, we find where our calculated chi-squared value will fall in the table. In the case of this example, there are only two classes of progeny, and so df = 1. Therefore we look in the first line of the table for the chi-squared value, and see that our chi-squared value of 2.37 falls between 0.016 and 2.706, the chi-squared values in the columns headed by 0.90 and 0.10 respectively. The column headings are the *p* values; therefore we can say that our p value falls between 0.90 and 0.10, which is written 0.90>*p*>0.10, or simply *p*>0.1. As the *p* value is not less than 0.05, these data do not show a significant deviation from that expected by chance from a 3:1 ratio, and the data therefore do support the hypothesis that a single recessive allele is controlling this trait.

**Additional notes**

1. Be cautious in your interpretation! Remember that a *p* value can support but not prove your hypothesis.

2. You can test multiple hypotheses with the same set of data; just be sure to state the specific hypothesis that you are testing in a given case.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **↓df** | **0.90** | **0.50** | **0.25** | **0.10** | **0.05** | **0.025** | **0.01** | **0.005** |
| **1** | 0.016 | 0.455 | 1.32 | 2.706 | 3.841 | 5.024 | 6.635 | 7.879 |
| **2** | 0.211 | 1.386 | 2.77 | 4.605 | 5.991 | 7.378 | 9.210 | 10.597 |
| **3** | 0.584 | 2.366 | 4.11 | 6.251 | 7.815 | 9.348 | 11.345 | 12.838 |
| **4** | 1.064 | 3.357 | 5.39 | 7.779 | 9.488 | 11.143 | 13.277 | 14.860 |
| **5** | 1.610 | 4.351 | 6.63 | 9.236 | 11.070 | 12.833 | 15.086 | 16.750 |

The top row is the *p* values, the left-hand column is the degrees of freedom, and the numbers in the body of the table are the chi-squared values that correspond to a given degrees of freedom and *p* value.

##### **Prelab summaries**

Why are prelab summaries mandatory and will be part of your final grade?

The first part of the genetics project lab will set the foundations for you to be independent when doing your own research project. You will successfully reach this independence when you will be able to answer, why are you doing your research, what are you researching on, how are you performing it and what are your results suggesting? However, without previous experience doing your own research, answering those questions might be less intuitive than it appears. The pre lab preparation will force you to read and understand the why, what and how of each experiment and it will help you to do the same in your independent project.

In addition to this, because many of the steps of the protocols you will use in the genetics project are very sensitive to handling factors such as temperature and time, the pre lab preparation will help you to be ready to promptly execute each day’s procedure, increasing your chances of experimental success. Experimental success *per se* will not be part your grade (you are not responsible if your flies don’t want to breed… well not the majority of the times anyhow☺). However, attitude is a very important factor in this class, and you will do better if you get into the experiment and do more than just "follow instructions".

1. **Article Reponses**

During the semester the instructor will upload in Moodle scientific articles for you to read. As part of your grade, you will write a paragraph summarizing the main discoveries of the paper and a question related to the research (this is the ”article written response”).

For the article oral response, you will briefly (8 minutes) explain a scientific paper to the rest of the class. This paper should be your own selection.

Both exercises will help you to analyze and criticize published research and apply those skills to your own research.

1. **Independent project proposal and Literature search strategy**

Proposal

Before carrying out any research, each pair will make a presentation (10 minutes), with Powerpoint slides, to the class about your proposed research. This should include background information, including references from the published literature (at least 4), relating to the strains you plan to use and/or the area you plan to investigate. It should lay out details of your planned research, including numbers of flies and dates for activities. It should also describe the statistics you will use to analyze your data and the potential broader impact of your research. Read Guidelines for Oral Communication in Biology below.

Literature Search Strategy:

Before the due date for your proposal you should send the instructor a literature search strategy explaining how you found the published papers for the background information of your proposal. You will also explain what information you got from them. Use the Information Literacy guidelines in Biology described below to find relevant literature.

1. **Independent Project Oral Presentation**

At the end of the semester, you will present the results of your project in a power point presentation to the class (10 minutes). You will present with your lab partner. However, I strongly encourage you to practice INDIVIDUALLY with the instructor before. Please see oral presentation guidelines below.

1. **Lab reports**

During the Genetics Project you will write two lab reports. The first lab report will describe one of the guided experiments. The instructor will decide the experiment based on the success of each experiment.

The final lab report will be a presentation of the results of your independent project. As before, please follow the required format explained in the appendix (general directions for lab reports and Biology Department references formatting).

1. **Guidelines for Information Literacy in Biology**

Information Literacy is the ability to find, evaluate, and use sources of information when information is needed for some purpose. In the field of biology, information literacy is often used to do background literature research to support a novel research project.

Information literacy is the ability to do the following four things.

I. Identify what information is needed

In planning a project, you need to develop a research question with a working hypothesis that you will be testing. Before starting your project, you need to carry out literature research for the following purposes:

1. To find out if the project you are considering has already been done;

2. To find as much background information as possible about the subjects you will be investigating;

3. To learn about methodological approaches that have been used in the past, and that you might use for your project.

II. Access information

You need to determine the best and most appropriate sources of information to refer to in preparing for your project. To be able to do this, you must be able to differentiate between several types of sources:

1. Peer-reviewed primary research articles;

2. Peer-reviewed research review articles;

3. Peer-reviewed books, such as textbooks;

4. Peer-reviewed governmental or institutional reports;

5. Popular science books and articles;

6. Articles in news periodicals;

7. Websites other than databases or data analysis tools.

For scientific research articles, it is generally only acceptable to cite sources that have undergone prepublication peer-review (categories 1-4). How can you know if something has gone through this process?

Reading about research in popular science books and magazines, news periodicals, and blogs or other websites is an excellent way to get informed and enthusiastic about a wide range of topics.  **However, these are not appropriate resources to cite in a research article.** If you have come across such a resource and found it useful, you should find and read the original peer-reviewed article being discussed in the popular article, and cite the peer-reviewed article.

Next you must design and carry out a search for articles and other appropriate sources. The most efficient way to do this is to search a bibliographic database. Adelphi has a subscription to Scopus, a database that contains citations to articles from a very wide range of academic disciplines. Another database is PubMed, which is a free resource from the National Institutes of Health, covering biological research relevant to medical fields.

You must decide on appropriate search terms. If your terms are too broad, you will be swamped by a pile of irrelevant results. If they are too narrow, you will miss important results, and may get none at all. The best way to find a balance is to carry out a series of searches, with different combinations of search terms. Most databases (including Scopus and PubMed) also support the use of Boolean operators (‘AND’, ‘OR’, ‘NOT’), which can also help target your search. The most important thing for a literature search is to plan to spend a fair amount of time on it.

Once you have a number of citations, you must access them. Some articles can be accessed electronically; either they are freely available to everyone or they are in a journal to which Adelphi has an online subscription. Other articles will not be available electronically, but can be found physically in the library. Still others will not be available at Adelphi, but can be accessed through interlibrary loan (ILL); the link to request such articles is available on the Adelphi library Web site. It may be tempting to ignore articles that you cannot access electronically. Do not fall into this trap! You might miss that perfect article that gives you a critical idea for your project.

Finally, and most importantly, you must read your articles. It is not acceptable to cite an article based only on someone else’s citation, or having only read the database entry. You must read an article yourself before you can cite it.

III. Evaluate information

By making peer-reviewed sources the focus of your literature search, you have gone a long way in avoiding unreliable or weak sources of information. Nevertheless, just because an article underwent peer-review before publication does not guarantee it is right. You should read all articles critically, and evaluate for yourself whether you find them convincing (some would say that the real peer review begins after an article is published). Specifically, consider the following questions for each article:

1. Is there sufficient background information in the introduction to understand the research, and does the background information support the value of the research described?

2. Is the research novel?

3. Is the experimental design appropriate?

4. Are the results clearly described?

5. Do the authors’ conclusions follow from the results?

You also need to evaluate whether each article is relevant to your project. Does the article contribute important background information for the subject you are studying? Does it provide methods that you might use? Does it provide possible explanations for experimental results that you have gathered?

IV. Use information appropriately and ethically

Your literature sources will be an important part of the Introduction and Discussion sections of your research paper. In the Introduction, relevant material from your sources will be used to provide the background for your project. What information does the reader need to understand your project? What information demonstrates the current state of knowledge on your subject? What information demonstrates the value of your project?

In the Discussion, you need to put your research results in the context of your literature sources. Are your results what you expected based on the existing literature, or are they surprising? Can existing sources explain your results, or can your conclusions explain results published elsewhere? How do your conclusions expand the base of knowledge that you found in your literature search?

Of course, it is critical that you cite your sources appropriately. The citation format will vary depending on the venue, so you will need to find a formatting guide (the format for citations in BIO 224 will be given to you as a separate document). **However, one general rule for scientific papers is that you should not include direct quotes from your sources**. Instead, you must restate the relevant information in your own words, and follow it with an appropriately formatted citation to the source.

1. **Guidelines for Oral Communication in Biology**

Oral communication is an important aspect of participation in the field of biology. Short presentations accompanied by projected slides are an important way to communicate research results to colleagues at professional conferences. This guide will describe many of the conventions and expectations for these presentations.

**Structure of the Presentation**

You should start with a greeting to the audience (“Good morning” or whatever is appropriate) and introducing yourself and the title of your talk. Projected on the screen will be a slide with the title, your name(s), institutional affiliation (which is Adelphi University), and the date you are giving the presentation.

The title of your talk should describe your research project with some specificity. A title that conveys the main conclusion can be especially effective, as long as it is not too long. Here are some example titles, and how I would rate them:

“*Drosophila* Genetics Project”: so vague it doesn’t convey anything useful.

“Effect of Temperature on *Drosophila*“: still too vague; what is being affected?

“The Effect of Temperature on the Antennapedia Trait of *Drosophila melanogaster*”: this is a pretty good title.

“High Temperature during Development Increases Expressivity of the Antennapedia Trait of *Drosophila melanogaster*”: this one is my favorite.

If you have a catchy title you can use it, but be sure that it is relevant and that your overall title still describes the project.

The body of your talk should follow the same structure that you would use in a scientific paper: introduction, methods, results, and discussion. You should not place these headings on the slides. Instead, you will need to signal to the audience verbally when you are transitioning from one slide to another.

Introduction

Here you will provide the background information your audience needs to understand your project. You should emphasize justification: why is your project worth carrying out? Finish with a statement of the hypothesis that you are testing, or the goal of your project.

Methods

In this section, you must provide a concise description of the methods that you used in your project. It is especially important to specify the experimental design: how many replicates did you carry out? What different treatments were used?

Results

Here you must effectively summarize your data and the statistical analysis that you carried out.

Discussion/Conclusions

In this section you will interpret your data and explore what you conclude. Return to the justification for the project: how does your project expand knowledge of the subject you were exploring?

Acknowledgements

Your final slide should say Acknowledgements at the top, and should list any people who gave you any assistance in your project. If you received funding for your project, you should say what these funding sources were.

Questions

At the end of the talk, there will be a few minutes for questions from the audience.

**Preparing Your Slides**

Keep the following considerations in mind as you are preparing your slides.

Text

There is a limit to how much the audience will be able to read on the screen. If your text is too small, or if there is too much of it on a slide, the audience will be lost. Font sizes of 44 point for titles and 32 point for other text will work well. Using these text sizes will have the added benefit of limiting the amount of text you put on a slide. The text on a slide should hit main points in a very succinct way; you will expand on the words on screen verbally.

Pictures and Graphics

Pictures and graphics are generally more effective on the screen at imparting concepts to the audience than text is. However, you must make sure that any pictures or graphics are effective and relevant. A figure with extraneous information will confuse the audience. Again, you do not want your slides to be overly crowded.

If you use a picture or graphic from an outside source, you must cite that source. It is much better to make your own pictures and graphics. I would much rather see a presentation with visuals produced by the author, even if they are somewhat less polished, than one with professional-quality visuals that have clearly just been copied from somewhere on the internet.

If you plan on using some picture or graphic design as a background for your slides, make sure that it will not be a distraction to whatever will be in the foreground.

Tables and Figures

In presenting your data, figures are much more effective than tables. Try to design figures so that they can be quickly understood by the audience with a minimum of explanation (you will need to provide some explanation, but keep in mind that you have limited time to do so). You also need to find ways to convey the results of statistical analyses on your figures. Again, visual representations are more effective than text.

Another consideration to keep in mind in preparing figures: approximately 7% of males have some form of red-green colorblindness, meaning they have difficulty distinguishing red hues from green. To avoid losing this part of your audience, try to avoid color schemes that require distinguishing red from green in order to understand the figures.

Citing Published Literature

Oral presentations will contain fewer references to published literature sources than written papers will. Nevertheless, there generally will be some. These sources must be cited, although there is no general consensus on how to cite literature in presentations. Placing references at the end of the presentation (where they would be in a paper) distracts the audience from questions they may want to ask about the presentation.

My advice, and what I will expect for this class, is that a full reference to a paper (in the same format required for the References section of written papers) should appear on the slide that is referring to it. This reference should be located at the bottom of the slide, in a relatively small but still readable font size (24 to 28). An interested audience member can then make a quick note of the paper, or ask to return to it later.

Number of slides

Your talk should contain approximately as many slides as there are minutes for the talk (that is, ten slides for a ten-minute talk). This doesn’t mean you should try to spend one minute per slide; some slides will require less time and others will take more. However, ten slides for a ten-minute talk tends to work out pretty well.

**Giving Your Talk**

The best thing you can do to make you feel more confident in giving your talk is to practice. It is especially helpful to practice in front of someone who can time you. This person can also give you feedback on your delivery.

Some important things to work on in delivering your talk:

1. Speak up! Talking to a group requires you to speak louder than you normally would in a conversation, and this can take some getting used to for some people.

2. Slow down! Nervousness often leads to talking too fast. You need to give the audience time to absorb what you are saying. It can even be helpful to pause for a few beats before advancing to the next slide to allow things to sink in.

3. As much as possible face the audience and make eye contact. It makes you seem more confident, and it helps you keep the audience’s attention. At times, you will need to face the screen to point out something important, but try to keep the time looking at the screen to a minimum, and DO NOT simply read the text off the screen.

4. Similarly, consult notes if you feel it will help, but DO NOT simply read from a pre-written script.

Finally, be attentive in listening to the other talks. It can be very unnerving to talk to a group of people if none of them appear to be paying attention. Listen attentively to the other speakers, and they will return the favor. This makes the whole session more interesting and enjoyable for everyone!

A power point with [Guidelines for delivering a scientific presentation](https://moodle.adelphi.edu/mod/resource/view.php?id=551598) document will also be posted on Moodle.

#### Guidelines for Lab Reports

**Honesty**- Forms the basis of society and academics including the sciences. It is important for you to understand the concept of plagiarism. Information (regardless of the source) that is directly utilized by you must be attributed to the source; otherwise it could be considered plagiarism, a serious form of cheating. The information may be verbal, it also must be cited [e.g. Mary Jones (pers. communication.)]. Written work can be considered your own if it is original, or substantially different from a source (not just a few words rearranged!). Citation of work used is easy to do and should be employed (even when in doubt) to protect yourself from problems. See the student handbook regarding the university policy on honesty. Violations may result in serious academic consequences.

**Style-** Scientific writing, unlike prose, is highly formal and style restrictive. A reader’s time is limited, and words cost money for publishers. If a word is not essential in transmitting information, leave it out. Consequently, scientific writing is somewhat ‘telegraphic’ and generally not very entertaining, stressing information content over pleasurable reading. Be precise and descriptive so that the reader is not left to interpret your meaning. Do not change verb tenses back and forth; past tense is generally used by scientific authors. Avoid the overuse of personal pronouns [e.g. ..I then added the solution, and I mixed it with the remainder; rather… the solution was added and mixed with the remainder]. The report should be logically organized, not chronologically organized. The *hypothesis to be tested (if one is being tested) must be clearly and concisely stated in the introduction.*

**Requirements-**

1. Title page including the experiment title, date of the report and lab partner’s name
2. Must be on plain (unlined) paper, typed (12pt. font.), double spaced (about 12 words/line, 25 lines/page); pages numbered; reports should be concise. It is recommended to not exceed 10 pages (not including title page).
3. Reports will be evaluated based upon: clarity, style, spelling and syntax, organization, appearance, effort, understanding of subject material (please see project paper evaluation form).
4. Reports must be submitted on time. Late reports will not be accepted. Work on them ahead of time so that “my computer crashed” excuses are not used (to no avail of course).

Before writing the paper, you should draft an outline that will help you to think how to organize your manuscript. Use Figure 1 as potential recommendation for how to present your research.

**Format (and order) of the Report [use section headings below]**

**1. Title page-** Experiment name, course title, instructor, lab partner, date

**2. Abstract-** a very short (<100 words) paragraph describing the rationale of the project, results and conclusions.

**3. Introduction-** A page describing the background and importance of the subject; reason for the experiment; hypotheses being tested; scientific application or value.

**4. Materials & Methods-** Give an outline of the procedures followed and describe the main steps in the procedures. However, you do not need to describe in detail experimental procedures that were given in the lab handout. Instead, you can state “according to the procedures in the lab manual”. Do not include superficial materials (e.g. rubber bands, fly food, markers, etc.).

**5.** **Results-** Present and describe the results you obtained. You should describe the key points of the results in words (**only figures and tables is not enough**), and include statistical analysis. Present your results in a clear, concise and logical manner. Results are important as these data serve to ultimately statistically test the validity of the hypothesis; statistical analysis should be included here; data can be presented in many ways (e.g. tables, figures-graphs, etc.). Each table or figure must have a consecutive number, and a legend explaining each figure. Graph axis must be labeled, numbers must have abbreviations (e.g. kg., ml., etc.) so they are clear. Do not discuss results here, this should be in the discussion/conclusion section.

**6.** **Discussion-** This and the next section are crucial for a report as everything is drawn together and examined critically. Do not oversimplify or shortchange this section as in …”this was an interesting experiment demonstrating a number of important principles….” Generalities and clichés are of little importance to the experiment. Interpret and explain your results: What do they mean? Do they support or fail to support the hypotheses you proposed at the end of your Introduction? According to the statistical analysis, how confident can you be of your conclusions? How do they compare with previously published studies? How might the experiment been changed to show improved results? What were the possible sources of error? Could they have been better controlled?

**7.** **References -** There are two main styles for in-text citations in science, the “citation-sequence” and the “name-year” styles. Please use the “name-year” style. Detailed information about how to use this citation style, and also about how to cite on-line sources in your references, is given below.

Footnotes and quotations **should be avoided** (but may be used if there is a clear reason). Citing the reference in the body of the report **is necessary**. Remember, we don’t quote in science, we explain what others did in our own words.

**8.** **Acknowledgements-** Include any person (e.g. lab partner, etc.) that collaborated with you or gave you information used in your report. “I wish to thank Jane Jones, Adelphi University, for her assistance in analysis of my data.”

**Caveat-** *Although you work in groups of two, your report must be a sole product of your writing. Data are often shared, but written work is not. Besides being dishonest, you will not learn much from using someone’s work. The AU honor code will be strictly enforced, and violations are a serious matter.*



Figure 1. Focus of sections of a research paper. The introduction should start out very broad and narrow to the specific details of the project. The methods and results sections should stay narrowly focused on the details of the project, and the discussion should become more broad again. Note that the legend of a figure is placed below the figure.

1. **Bio Department – Project, Paper, and Lab Report Reference Formatting**

All references should follow the Council of Science Editors’ name-year format, as described below. This format is similar to APA format, but not identical.

The citation of newspaper or popular magazine articles, internet-only resources (unless they are affiliated with a peer-reviewed journal), or encyclopedias, is strongly discouraged, as these are rarely cited in scientific journals.

**In-text references**

* In-text references will generally be placed in parentheses at the end of a sentence. “The *Xyz* gene is located on chromosome 2 (Smith 2007).”
* Less commonly, the author will be mentioned outside of parentheses, with the year following in parentheses. “Smith (2007) found that the *Xyz* gene is located on chromosome 2.”
* Direct quotations from other sources are very rarely found in scientific journal articles, so you should avoid them.

Citation with one author

(Aguade 1998)

Two authors

(Foster and Walker 2009)

Three or more authors

(Lin et al. 2008)

Multiple sources cited for the same information (arrange in chronological order)

(Aguade 1998, Sabeti et al. 2002, Foster and Walker 2009)

**Reference list at the end of the paper**

* References should be arranged alphabetically by the first author’s last name.
* The list should have a hanging indent (the first line of each reference should be at the left margin, and any subsequent lines should be indented).
* Each author’s name should be listed as the last name followed by first and middle initials, without periods (that is, John B. Smith would be listed as Smith JB).
* For article titles, only the first word should be capitalized (with the exception of proper nouns).
* For journal titles and book titles, all words should be capitalized (with the exception of minor words such as “and”, “the”, and so on).
* Journal articles accessed online should be cited with the same format as print articles, with volume and page numbers. The URLs where articles were accessed should not be included (unless specified below), nor should Digital Object Identifiers (DOIs).

Journal article with one author

Aguade M. 1998. Different forces drive the evolution of the *Acp26Aa* and *Acp26Ab* accessory gland genes in the *Drosophila melanogaster* species complex. Genetics 150 (3): 1079-1089.

Author. Year. Article title. Journal volume (issue): pages.

Journal article with two to ten authors

Lin S, Coutinho-Mansfield G, Wang D, Pandit S, Fu X. 2008. The splicing factor SC35 has an active role in transcriptional elongation. Nature Structural & Molecular Biology 15 (8): 819-826.

Authors. Year. Article title. Journal volume (issue): pages.

Journal article with more than ten authors

Sabeti PC, Reich DE, Higgins JM, Levine HZ, Richter DJ, Schaffner SF, Gabriel SB, Platko JV, Patterson NJ, McDonald GJ et al. 2002. Detecting recent positive selection in the human genome from haplotype structure. Nature 419 (6909): 832-837.

First ten authors et al. Year. Article title. Journal volume (issue): pages.

Book

Brooker RJ. 2011. Genetics: Analysis & Principles. 4th ed. New York (NY): McGraw-Hill.

Author(s). Year. Book Title. Edition. City (State or Country): Publisher.

Article or chapter in an edited book

Foster WA, Walker ED. 2009. Mosquitoes (Culicidae). In: Mullen GR, Durden LA, editors. Medical and Veterinary Entomology. 2nd ed. pp. 201-253. Burlington (MA): Academic Press.

Author(s). Year. Article title. In: Editor(s). Book Title. Pages. City (State or Country): Publisher.

Thesis or dissertation

Frenke KB. 1972. Distribution of fecal coliforms in sediments of the New York Bight [thesis]. Garden City (NY): Adelphi University.

Author. Year. Title [thesis or dissertation]. City (State or Country): Institution.

Institutional report

World Health Organization. 2010. World Malaria Report 2010. Geneva (Switzerland): Author.

Organization. Year. Title. City (State or Country): Publisher.

(If the report is self-published by the organization, refer to the publisher as Author.)

Article on a professional website

Brody TB. 2011. Drosophila behavioral programs [Internet]. c1995, 1996. Society for Developmental Biology web server, The Interactive Fly website. [cited 2011 August 12]. Available from: http://www.sdbonline.org/fly/aimain/6behavior.htm.

Author(s). Year of last update (at bottom of web page, if given). Article title - usually seen at top panel of browser [Internet]. Place of publication (if known): Organization Name. [cited date]. Available from: URL.

(Note that if author is not given, substitute organization name. If this is not available, substitute article title (and do not repeat it after year).)

1. **Rubrics**

|  |  |
| --- | --- |
| Oral presentation | Poor---Poor ----------Excellent |
| Introduction of the topic, its importance and broader relevance | 1 2 3 4 5 6 7 8 9 10 |
| Roadmap or outline (Was it obvious where the talk was going?) | 1 2 3 4 5 6 7 8 9 10 |
| Understanding description and use of relevant literature | 1 2 3 4 5 6 7 8 9 10 |
| Organization, transitions between ideas and general flow | 1 2 3 4 5 6 7 8 9 10 |
| Clarity and persuasiveness of arguments and conclusions (supported by data presented, place in a broader context, take-home messages | 1 2 3 4 5 6 7 8 9 10 |
| Innovation and originality, synthesis of ideas or fields of study | 1 2 3 4 5 6 7 8 9 10 |
| Interaction with audience (eye contact, voice projection, enthusiasm, etc.) | 1 2 3 4 5 6 7 8 9 10 |
| Mannerisms (use of pointer, avoided distraction pauses or gestures, etc) | 1 2 3 4 5 6 7 8 9 10 |
| Visual aids: PowerPoint (quality, clarity, color scheme, etc) | 1 2 3 4 5 6 7 8 9 10 |
| Responses to questions and ability to foster discussion | 1 2 3 4 5 6 7 8 9 10 |
| Total | 100 |

|  |  |
| --- | --- |
| Title | /5 |
| Abstract | /5 |
| Introductiona) Places research in broad contextb) Background informationc) Rationale for doing projectd) Statement of hypotheses and goals | /20 |
| Materials and Methodsa) Complete but concise description of proceduresb) Description of statisticsc) Appropriate style | /20 |
| Resultsa) Complete but concise description of datab) Appropriate statistical analysis | /15 |
| Discussiona) Interpretation of resultsb) Problems encounteredc) Possible future directions for researchd) Return to broad context for paper | /20 |
| Referencesa) Correct formatb) Correctly cited in text | /5 |
| Tables and Figuresa) Correct formatb) Complete description in legendc) Correctly cited in text | /5 |
| Overalla) Clear writing free of language errorsb) Solid understanding of subject material | /5 |
| Total | /100 |

Lab reports points