GENERAL INFORMATION

**Time:** Mondays, 8:30AM – 12:20PM  
**Location:** Langley A148

**INSTRUCTORS:**  
Dr. Mark Rebeiz  
Life Sciences Annex 202A  
Office hours: By appointment, TBA  
Phone Numbers: 412-624-2261  
Email: rebeiz@pitt.edu

PREREQUISITE

*BioSci 0350 (Genetics Lecture)* is a *co-requisite* for this course.

INTRODUCTION

Genetics is a broad field dedicated to the study of inheritance. Its scope ranges from the subtle heritable differences in appearance (“traits”) between individuals to the molecular underpinnings of how the genome is copied, interpreted and leveraged to build an organism. At its core, genetics is the studied through the characterization of “genes”, a concept that has had many meanings over its 150-year history. One of the most popular and powerful model systems in genetics research is the fruitfly, *Drosophila melanogaster*. Flies are easy to culture, and thus simple to set up controlled matings. Furthermore they are genetically pliable, and boast a variety of very useful tools for genetics research, including a fully sequenced genome. This course will provide a first-hand experience of the field of genetics by performing a *genetic screen* using *Drosophila melanogaster* to find new genes that contribute to the development of a variety of *Drosophila* body parts. Specifically, we will be performing an *enhancer trap screen*, in which the green fluorescent protein will be used to detect random insertions of a “reporter gene” throughout the genome, and will “report” the activities of nearby gene regulatory sequences. Thus, insertion lines will produce fluorescent protein in a body part where a nearby gene is expressed. This lab will provide a hands-on perspective of Mendelian genetics, mutations, chromosomes, and DNA analysis using cutting edge technologies to solve a contemporary research problem.
LEARNING GOALS
• Understand basic Mendelian genetic crosses
• Learn how transposon induced mutations work and can be used for the purpose research in genetics
• Understand molecular techniques of DNA extraction, cloning, PCR, and sequence analysis
• Grasp how gene function can be tested through reverse genetics techniques such as RNAi
• Learn how to explore published genetics research, and critically evaluate your own data in light of previously published work

COURSE OBJECTIVES
• To achieve learning goals through active research activities
• Map genetic mutants to a particular chromosome and position in the genome
• To identify new genes that contribute to the development (and possible evolution) of adult structures in Drosophila
• Determine the likely expression patterns of identified genes using reporter systems
• Evaluate possible phenotypes upon disrupting gene function with RNA-interference

ORGANIZATION
Quizzes
The lab may begin with a short quiz that tests your preparedness for the events of the day in lab.

Lectures
Each lab begins with a short lecture to introduce the topic of the current day’s experiment, and provide background information.

Experiments
The lab is unique in that your entire semester will focus on accomplishing a unique project. This means your experimental success is essential on a weekly basis! Methods and supplementary information will be available on CourseWeb before the laboratory. It is your responsibility to print these handouts out and read them before class.

Cleanup
At the end of the lab session, it is your responsibility to clean up your station. Put away reagents (fly food, etc), organize your fly vials in the trays provided, throw away trash. Return dissection tools and fly brushes to TA or instructor, and replace microscopes on their racks in the scope closet. Wipe down your space with a damp paper towel. Your effort in leaving your station the way you found it will weigh on your performance grade.
GRADES
Within the laboratory, we expect you to:

1. Be prepared in advance by reading relevant protocols or background materials, as suggested (there will be quizzes, and preparedness will affect your performance grade)
2. Show effort and improvement in your lab techniques so you improve your skills of experimental analysis, experimental design and experimental interpretation (this will impact your performance grade)
3. Keep clear and concise records in your lab notebook (which makes up 30% of your grade)
4. Be prepared to interact verbally and respond to questions during lecture and lab (this will affect your participation grade).
5. Prepare for presentations, and improve your presenting skills based on our feedback
6. Demonstrate safe and responsible laboratory practices, and work well with others, and show proper laboratory etiquette (this is just a good idea)

The total points possible in this class are distributed as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Lab Performance</td>
<td>15%</td>
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<tr>
<td>Class Participation</td>
<td>20%</td>
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<tr>
<td>Lab Quizzes</td>
<td>15%</td>
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<tr>
<td>Mid-term presentation</td>
<td>5%</td>
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<tr>
<td>Final Lab Presentation</td>
<td>15%</td>
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<tr>
<td>Lab Notebook</td>
<td>30%</td>
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</tbody>
</table>

ATTENDANCE
Attendance is mandatory for all lab and lecture sessions. Your grade for the day missed will be zero (Participation/Performance, Quizzes, and Lab Notebook entries). All absences must be excused directly by the instructors prior to the class meeting. Email is the best method. All absences must be justified, and we reserve the right to require documentation. When students are unable to attend, we will try to make the lab material and experiments available at alternative times, but this may not always be feasible.

OFFICE HOURS
We will normally have time during class to deal with most of the issues that will come up. However, meetings can be scheduled at other times. E-mail Instructor or TA to arrange (both Instructor and TA’s labs are in an annex of the biology complex which is always locked so you need to make an appointment). Questions about anything can be e-mailed at any time to either Instructor or TA.
RECOMMENDED TEXT
There is no textbook for the course. However, background material for our laboratory
practices are well covered in the following text available through Amazon.com, & we will have
a copy in lab for your perusal:

Laboratory Press; 2nd edition (July 1, 2004)

I will draw material for this course from primary research papers (peer reviewed, publications)
and I will expect you to read them and draw relevant information from them. Such materials
will be provided to you.

You will need to use computers (in the lab & elsewhere) to look up web-based material to
perform your lab work. You will also be expected to 1) examine DNA sequences, 2) use data
management programs, such as Excel, to plot your data & results, & 3) use imaging programs
to label digital images you will produce.

LABORATORY NOTEBOOK
Your laboratory notebook holds the record of everything you did in the lab. We strongly recommend that the
lab notebook take the form of a three ring binder filled with loose leaf paper. During the course of the
semester you will be working on several projects in parallel (see the horizontal columns in the overall lab
schedule on the last page of this document). It will help to make separate sections of your lab notebook for
each project: Chromosomal segregation mapping of enhancer trap lines; inverse PCR, detection of
expression patterns. This organization will facilitate in the grading of notebook content. We want to teach you
how to keep records and generate notebooks as you would in a research lab. While this varies from lab to lab,
here are some common principles to follow:

1) Lab notebooks must be “real-time” documents of your actions and observations in the lab, like an airplane
“blackbox”. Notes must be written in permanent ink. Notes must not be copied over late in the semester, as it
represents a source of error. So, do not copy your notebook to make it neater. However, your notebook must be
readable and well organized. An important result is virtually worthless if the procedure and reagents are not
recorded accurately so the result is repeatable.

2) Your lab notebook must have as much of your primary data as you can gather during your experiment. This
includes the numbers on the print-out from the spectrophotometer, the hand-written values (and the check
marks beside the numbers) for the separate reagent volumes added to a reaction, or a photograph of your gel.
Nothing is as valuable as your primary data. Primary data might not always be available in hard copy form, such
as the temperature shown on the thermometer in the water bath, but primary data should be saved when
possible. Keeping the primary data in your notebook allows later third-party validation of your calculations from
the original data.

3) The form – at the top, every page should have a date, a concise heading, and a page number. Each
experiment should have at least four sections:

- Experimental goal – a brief statement concerning the objective of the day’s work,
- Procedure – an outline of the protocol or a reference to the printed protocol used, including any
deviations from the cited protocol,
- Results – primary data and observations should be recorded neatly and necessary notations made of
intermediate results, and
• Conclusions – the number(s) you calculated and the calculations including relevant units, or a clear and concise statement of your conclusion and the rationale behind this interpretation. Make conclusions easy to find by boxing or highlighting them. In bound notebooks, conclusion sections could be several pages away from the others. Refer from one to the other with page numbers.

4) Include a Table of Contents at the beginning of your notebook that guides the reader to individual pages with dates and descriptions of each experiment.

DISTRIBUTION OF INFORMATION

Almost all course material will be distributed to you via CourseWeb. **We will only communicate to you through your pitt.edu email account.** We will never respond to other email addresses for you (not yahoo, not gmail, etc.).

DISABILITIES

If you have a disability for which you are or may be requesting an accommodation, you are encouraged to contact both your instructor and Disability Resources and Services, 216 William Pitt Union, [412-648-7890/412-383-7355(TTY)] as early as possible in the term. DRS will verify your disability and determine reasonable accommodations for this course.
<table>
<thead>
<tr>
<th>LAB</th>
<th>Date</th>
<th>Lecture Topic</th>
<th>Lab Activity</th>
<th>Basic Drosophila Husbandry</th>
<th>Chromosomal segregation of enhancer trap lines</th>
<th>Mapping insertions by Inverse PCR</th>
<th>Characterization of enhancer trap line expression</th>
<th>RNA Knockdown of new genes</th>
<th>Lab Notebook Grading</th>
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<tbody>
<tr>
<td>1</td>
<td>1/4/16</td>
<td>Introduction to the course, lab safety, fly husbandry</td>
<td>Working with flies: visualizing phenotypes, setting up crosses</td>
<td>Set up yw cross #1</td>
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<td>2</td>
<td>1/11/16</td>
<td>Balancer chromosomes, transposons, genetic screens</td>
<td>Insertion site mapping cross #1</td>
<td>Check on cross #1</td>
<td>Segregation mapping cross #1</td>
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<tr>
<td>3</td>
<td>1/18/16</td>
<td>Dr. Martin Luther King Day, No Class</td>
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<td>4</td>
<td>1/25/16</td>
<td>Gene regulation, inverse PCR</td>
<td>Inverse PCR</td>
<td>Score Progeny</td>
<td>Check on segregation cross #1</td>
<td>DNA Isolation, PCR setup</td>
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<td>5</td>
<td>2/1/16</td>
<td>Gel electrophoresis, DNA sequencing</td>
<td>Insertion site mapping Cross #2</td>
<td>Check on mapping cross #2 and balancing cross</td>
<td>Cross enhancer trap lines to UAS-GFP</td>
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<td>6</td>
<td>2/8/16</td>
<td>Green Fluorescent Protein (GFP) reporter systems</td>
<td>Set up GFP reporter cross, finish inverse PCR</td>
<td>Check on mapping cross #2 and balancing cross</td>
<td>Cross enhancer trap lines to UAS-GFP</td>
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<td>7</td>
<td>2/15/16</td>
<td>DNA sequence analysis, and Drosophila genome tools</td>
<td>Computer lab</td>
<td>Score mapping cross proegy and BALC cross</td>
<td>Cross enhancer trap lines to UAS-GFP</td>
<td>DNA sequence analysis</td>
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<td>8</td>
<td>2/22/16</td>
<td>Student Presentations and Discussion of genes found in class</td>
<td>Scoring proegy (mapping cross)</td>
<td>Score mapping cross proegy and BALC cross</td>
<td>Cross enhancer trap lines to UAS-GFP</td>
<td>Order RNAi lines</td>
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<td>9</td>
<td>2/29/16</td>
<td>Larval morphology, dissection, microscopy</td>
<td>Searching for expression patterns (larvae/pupae)</td>
<td>Catchup / Propagate Balanced Stock</td>
<td>Characterizing larval GFP expression</td>
<td>Cross RNAi lines to GAL4 drivers</td>
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<td>10</td>
<td>3/7/16</td>
<td>Spring Recess, no class</td>
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<td>11</td>
<td>3/14/16</td>
<td>Drosophila pupal anatomy</td>
<td>Searching for new expression patterns in new inserts</td>
<td>Catchup / Propagate Balanced Stock</td>
<td>Characterizing pupal GFP expression</td>
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<td>12</td>
<td>3/21/16</td>
<td>Allelic strength and phenotypic consequences</td>
<td>Determining lethality and phenotype</td>
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<td>13</td>
<td>3/28/16</td>
<td>Presenting Scientific Results</td>
<td>Organizing stocks, final preparations for student presentations</td>
<td>Submit balanced, characterized stocks</td>
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<tr>
<td>14</td>
<td>4/4/16</td>
<td>None</td>
<td>Student Presentations</td>
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<td>15</td>
<td>4/11/16</td>
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<tr>
<td>16</td>
<td>4/18/16</td>
<td>Final Exam Period</td>
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* Schedule is subject to modification