PLB/ZOO 438
Plant and Animal Molecular Genetics Laboratory - SYLLABUS Spring 2012

This course consists of twice weekly, two-hour laboratory experiments covering topics in classical (transmission) and molecular genetics. We will cover the theory pertaining to each lab in a one hour lecture at the beginning of the week. The course carries 3 credits.

Instructors:	Matt Geisler (PLB)
When:	Spring semester Tuesday morning – one hour theory class (both instructors are flexible on time) Tuesdays and Thursdays – 1-3 pm or 2-4 pm – lab (consult with Chairs)
Pre-requisites	Biology 305 or equivalent
Lab fee	$30.00
Textbook	No
Location	LSII, Room 0457
Enrollment limit	24

Week	Lecture Topic	Laboratory Experiments
1	Introduction, laboratory safety, lab books, record keeping (Matt).	(1) Safety, autoclaving, molarities, other measures of mass, volume, concentrations. Using analytical balance. (2) Use pH meter and pipettes; make reagents.

Objectives: students will become familiar with lab safety and keeping records. Computation of different measures of concentration, sterilization, use and care of pH meters, making up stocks of common lab reagents will be introduced.

2	Introduction to the biology of fruit flies & Arabidopsis; overview of phenotypic variation in both (Kamal).	(3) Drosophila culturing; use of dissecting microscopes; sexing adult fruit flies; identification of marker phenotypes. (4) Growing Arabidopsis; identification and analysis of phenotypic variants.

Objectives: students will be able to culture fruit flies, grow Arabidopsis, and identify the phenotypic variations to be used in crosses that illustrate Mendel's laws (week 3).

3	Mendel's laws of segregation and independent assortment deviations from Mendel's laws (Kamal).	(5) Arabidopsis crosses. Drosophila crosses. (6) Analysis of dihyrbrid data using pre-ordered segregating F_2 populations of drosophila and Arabidopsis (Carolina, Inc.)

Objectives: Students will be able to set up fruit fly crossing experiments, remove stamens and cross pollinate Arabidopsis flowers; recognize and interpret *Drosophila* and Arabidopsis F_2 data that illustrate Mendel's law of segregation and independent assortment; recognize and interpret dihybrid F_2 data that illustrate genetic interactions.

4	Three point linkage mapping; Sex linked traits in drosophila	(7) Transmission probabilities – problem solving; Hardy Weinberg principle - problem solving

	(Kamal).	(8) Map distance - problem solving
5	Test 1	Test 1
6	DNA Extraction – chemistry (Matt)	(9) from mouth swap using kit; DNA quantification (10) using CTAB (Arabidopsis); DNA quantification

Objectives: Understand the chemistry of DNA extraction protocols. Be able to extract DNA from human mouth swap and Arabidopsis tissue. Quantify DNA using fluorometer.

7	PCR (Kamal)	(11) Amplification of Arabidopsis DNA using primers for known genes; also degenerate primers. (12) Amplifying human using *Alu* insert primers. Gel electrophoresis of amplified DNA

Objectives: Understand the principles of polymerase chain reaction; amplification using locus specific primers.

8	Bacterial transformation (Matt)	(13) Bacteria growth media; bacteria transformation (14) Plasmid preps; colony picking; colony PCR

Objectives: Learn basic microbiological techniques relevant to molecular cloning; sterile technique, genetic manipulation of bacteria using plasmids, antibiotic resistance markers, and detection of modified genes.

9	RNA extraction; RT-PCR (Matt)	(15) RNA extraction (16) RT-PCR

Objectives: Understand the difference between DNA and RNA handling, creation of cDNA, the concept of gene expression as measured by RT-PCR. RNA will be extracted from 2 different tissues and 2 treatments of Arabidopsis and the differential expression of 2 genes will be measured.

10	Molecular genetic variation: RAPD in grasses; *Alu* insert in humans (Matt / Kamal)	(17) Randomly amplified polymorphic DNA (RAPD) in grasses. (18) Variation at PV92, a human specific *Alu* insert.

Objectives: Understand the concept of genetic variation within a population of individuals. Learn two tools for identifying and quantifying these variations.

11	Data Analysis (Kamal)	(19) Analyses of RAPD data (20) Analysis of *Alu* insert data

Objectives: Be able to analyze single locus and RAPD marker variation in a population. Make use of RAPD marker analysis software to identify genotypes.

12	Plant transformation (Matt)	(21) Bacteria based transformation (22) Screening transformed plants

Objectives: Students will use prepared strains of Agrobacterium to transform Arabidopsis plants, and screen previously prepared T1 populations using BASTA herbicide spray and Kanamycin antibiotic resistance. Students will examine transformed plants for mutant phenotypes in an activation tagged Arabidopsis seed collection.

13	DNA sequencing (Kamal)	(23) mtDNA sequencing reactions (24) Introduction to bioinformatics

Objectives: Understand the chemistry of cycle-sequencing and high-throughput 3 gen sequencing; be able to setup cycle sequencing reactions.

14	Analysis of sequence data; bioinformatics (Matt)	(25) Sequence alignment; GenBank searches (26) Bioinformatics

Objectives: Using previously generated sequence data from dideoxy sequencing, students will learn base calling, contig building and sequence data storage and retrieval. Students will understand different sequence types (coding, introns, UTR, intergenic, non-protein genes), BLAST searches, multiple sequence alignment, functional domain detection, sequence annotation and become familiar with 4 online genome databases for Humans, Arabidopsis and Drosophila (Ensembl, TAIR, Flybase, and NCBI).

15	Final exam	