

# GENERAL INFORMATION

## Who, When, and Where

Section	01	02
<b>Days &amp; Times</b>	M 12:20 – 4:25 pm W 1:25 – 4:25 pm	Tu 12:20 – 4:25 pm Th 1:25 – 4:25 pm
<b>Professors</b>	<b>Sam Hazen</b> Assistant Professor, Biology 409A Morrill III hazen@bio.umass.edu	<b>Ned Young</b> nyoung33@gmail.com
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<b>Laboratory Coordinator</b>	<b>Katherine Dorfman</b> Laboratory Coordinator ISB 241C kdorfman@bio.umass.edu	
<b>Library Liaison</b>	<b>Maxine Schmidt</b> Integrated Sciences & Engineering Library mschmidt@library.umass.edu	
<b>Room</b>	<b>ISB 368/364</b>	
<b>Course website</b>	<a href="http://bcrb.bio.umass.edu/courses/spring2013/biol/biol388a/">http://bcrb.bio.umass.edu/courses/spring2013/biol/biol388a/</a>	
<b>Textbook</b>	In lieu of a textbook, a reading packet and lab manual will be available for purchase at Collective Copies.	
<b>Other materials:</b>	<ul style="list-style-type: none"> <li>• Bound lab notebook where you will keep your notes. (You can use the Student Lab Notebook with permanent binding from HM Publishing, available at the college bookstore, that you probably have left over from general chemistry.)</li> <li>• 1 GB USB flash drive or good working knowledge of the UDrive system.</li> <li>• PENCIL (preferably) or pen every day.</li> </ul>	

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## Introduction to the course

Molecular genetics and bioinformatics are powerful approaches to investigate the mechanisms of gene action. The aim of this course is to provide you with some basic practical knowledge and hands-on experience regarding some of the most common experimental methods used in molecular genetics and recombinant DNA research. *The course is intended for beginners*, and it is expected that students do not necessarily have basic familiarity with using micropipettes, operating laboratory equipment, or using bioinformatics software.

- Experiments will follow a logical sequence where the results from one experiment will be used in the next.
- **There will be times that you will be extremely busy and others not busy at all.** (The 'not busy' times are a good opportunity to get your notebook and wiki pages up to date.)
- The results of the planned experiments are unknown—you will be making actual discoveries in this class.
- Your success will depend on your ability to **come prepared**, listen carefully, pay attention to protocol details, and cooperate with others.
- You will at times be handling dangerous compounds and laboratory equipment; *think safety first* at all times (see page **Error! Bookmark not defined.**).
- The bulk of each day has been scheduled to perform experimental procedures, but times have been scheduled throughout for more general instruction and discussion.

### Preparation

All the reading and lab assignment pages for the each meeting have been made available on the course web site (<http://bcrc.bio.umass.edu/courses/spring2010/biol/biol397a>). It is your responsibility to go to the web site before every class to get your assignment for each day. It is furthermore your responsibility to read BOTH the lab manual and the assigned background reading.

You will get easy points by keeping up to date with your reading. I will be giving random quizzes on your reading assignments throughout the semester to check your

preparation. These quizzes will be open book/open notes, but will be timed. If you have prepared adequately, even if you do not remember something specific, you will be able to go rapidly to your notes and readings to find the answer. These quizzes will be graded, and the grades you receive will constitute 1/4 of your final grade.

## Participation

This is a small class, and it will be easy for the instructors to observe your class participation. Participation in this context means working efficiently and in cooperation with your lab partner. It makes sense to divide up laboratory duties between partners, and we do not expect that both members of a team will perform every duty entirely evenly. However, each student should try as much as possible to participate in all aspects of experiments. Efficiency means that you are prepared when you arrive in class—you've read the lab manual, and are ready to start right in. If it's clear that you and your partner are dividing up duties fairly evenly, and discussing your experiments and progress together, you're cooperating well. If you either hog all the action, or alternatively hang back all the time, that is not good cooperation, and your participation grade will suffer accordingly.

## Lab Notebook

Although it seems tedious at first, keeping accurate records is an invaluable skill for a scientist.

A notebook that accurately details the goals, methods, results, and conclusions of an experiment allows others to continue your work in your absence or replicate and expand upon your results. Such a notebook also makes it possible for you to review your experiments in preparation for writing a report, modify future ones as necessary, and, in the unlikely event of an accusation of misconduct, defend yourself against charges of fraud.

Many labs insist on having their scientists use a bound notebook rather than a loose leaf or spiral-bound one to make the work transparent and to prevent accusations of dishonesty. If pages can't be removed, then it is easier to follow the actual course of events as recorded in the notebook, and for one scientist to carry on for another (who has retired, for example).

You should use the Student Lab Notebook with permanent binding from HM Publishing, available at the college bookstore. (You may have one left over from a chemistry lab.) Write in it with pen, and hand the copy to your instructor every Wednesday at the end of lab. See example notebook entry on page **Error! Bookmark not defined.**

Your notebook will be graded as follows:

- (5.0) Was it turned in on time, and are appropriate entries logged?
- (0.5) Is a purpose for each experiment/notebook entry stated?
- (1.0) Are the relevant calculations made?
- (0.5) Is a generalized procedure written?
- (1.0) Does it refer to data collected?

- (0.5) Are observations or thoughts written in the procedure
- (0.5) Does it refer to errors or specify that none were made?

## Grading

- 1/8 of your final grade will be based on your grades on in-class spot quizzes.
- 1/4 of your final grade will be based on the scores you receive on your lab notebook
- 1/8 of your final grade will be based on participation—the instructor’s observations of your performance during labs.
- 1/2 of your final grade will be based on the scores you receive on your lab reports and wiki project pages.

## Course Objectives

### First objective

#### DNA Basics: purification and quantification

- Concept:** DNA structure (review)
- Concept:** Gain a sense of the needle in a haystack aspect of the problem
- Skill:** Preparing genomic DNA
- Concept:** Learn how we observe DNA in the lab
- Concept:** Learn to quantify DNA
- Skill:** UV spectrophotometry and agarose gel electrophoresis

### Second objective

#### Make the connection between the letters on paper, and the DNA in the organism.

- Concept:** Central dogma and eukaryotic gene structure (review)
- Concept:** ORFs, exons, and introns
- Skill:** *In silico* translation
- Skill:** *In silico* ORF prediction
- Skill:** *In silico* intron/exon prediction
- Concept:** cDNA and ESTs
- Skill:** Using ESTs to map introns and exons of unknown genes.
- Concept:** Polymerase Chain Reaction (PCR)
- Skill:** Picking primers
- Skill:** Setting up PCR reactions
- Concept:** Restriction mapping
- Skill:** Making *in silico* maps of genes and choosing appropriate REs

**Skill:** Carrying out restriction digestions

**Skill:** Plotting standard curves to determine molecular weights of gel bands

**Skill:** Using imaging software to determine molecular weights of gel bands

## Third objective

### Predict function based on similarity to known genes or proteins.

**Concept:** Sequence similarity can be used to predict aspects of gene function.

**Concept:** Domain structure of proteins.

**Concept:** Basic Local Alignment Tool (BLAST) creates pairwise alignments

**Concept:** Nucleotide similarity and amino acid similarity reveal different levels of relatedness

**Skill:** Using NCBI BLASTN and BLASTP

**Concept:** Gene families: multiple similar genes that occur in a single genome

**Concept:** Evolutionary conservation of sequences.

**Skill:** Using multiple resources (bioinformatics and library) to define putative gene functions

## Fourth objective

### Think about expression patterns

**Concept:** Microarrays

**Concept:** Publicly available microarray data

**Concept:** Experimental validation of gene expression using RT-PCR

**Skill:** Basic statistical analysis of array data

**Skill:** Preparing RNA

**Skill:** Designing RT-PCR primers

**Skill:** Quantifying RT-PCR product amounts

**Skill:** Normalizing reactions to housekeeping gene

## Fifth objective

### Identify gene knockouts to uncover physiological functions

**Concept:** Mutation as a means of determining gene function

**Concept:** Random versus site-specific mutation

**Concept:** Insertional mutagenesis

**Concept:** T-DNA

**Concept:** SALK gene tagging project

**Skill:** Performing searches to identify T-DNA knock-outs

**Skill:** Predicting consequences of T-DNA insertions based on location

**Concept:** Think through basic Mendelian genetics pertaining to T-DNA lines

**Concept:** Allele-specific PCR to distinguish mutant versus WT alleles

**Skill:** Growing *Arabidopsis*

- Skill:** Make tiny DNA preps (leaf squishes) for molecular screening
- Skill:** Perform PCR to distinguish WT and mutant alleles
- Skill:** Assess results to identify homozygous mutant plants
- Skill:** Observe homozygotes during the rest of semester to determine phenotypes