

BIMM101: RECOMBINANT DNA Techniques

Winter Quarter, 2019

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Office Hours: Wed 4:30pm-5:30pm (Also available during/after lab; other office hours can be arranged as needed)

Lecture: SEQUO 147; Tue/Thu 12:30– 1:50 PM

Labs: Tue/Thu 2:00– 5:50 PM

AO1:YORK 3306

A02: YORK 3406

Required materials: Lab Manual (purchase at bookstore), carbonless copy lab notebook, knee-length laboratory coat, and UV-blocking safety glasses or goggles are required for the laboratory and are available at the bookstore. Long pants and closed-toe/heel shoes are required in lab. ****No ankles can be showing, bring extra socks if needed**.** Fine-tipped sharpie pen (dark color) for labelling tubes in the lab.

Computers: We will often use computers for data analysis and other exercises. We have access to some computers, however if you have your own laptop computer it is recommended you bring it to use on days when we have scheduled computer activities.

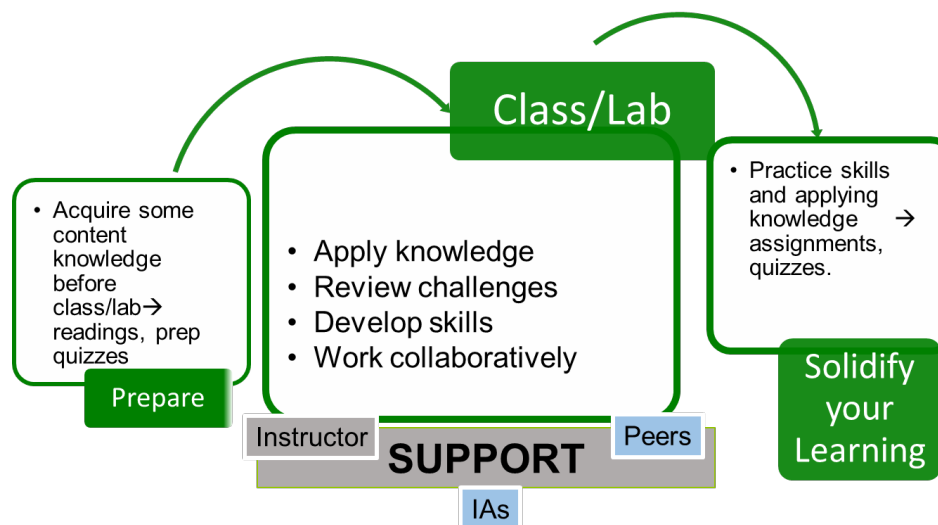
LEARNING GOALS

- Apply knowledge of molecular biology concepts and molecular techniques to plan experiments, explain and troubleshoot results
- Demonstrate proficiency at the basic molecular biology techniques used in the lab
- Explain the importance of proper controls in designing experiments and interpreting results
- Perform basic lab math skills, statistical analysis, and graphing

LEARNING IN THIS COURSE

This course is designed to be a **collaborative** environment for everyone to learn together and construct a shared understanding of the material. **Active participation** both in class and in lab is expected. Being able to **communicate** understanding, and confusion, is critical to success in any discipline, and is very useful for learning¹. We will use class time to work on applying our knowledge, troubleshooting difficult topics, and practice solving problems.

Instead of memorization, we will focus on developing an understanding of



fundamental concepts as they apply to different examples. Therefore, quizzes will include questions that are based on solving problems in new contexts.

1 Smith et al., 2009. <http://www.sciencemag.org/content/323/5910/122.short>

Absences: Lab attendance is required –

if you miss one lab with no excuse, you will lose 5% from your final grade. If you miss two labs, you will be asked to drop the course. If you are ill, you must get in touch with me, not your IA, and make up the lab in a way that we will determine. You must be on time for lab. Two late arrivals to lab will be counted as one absence.

Late and missed assignments and quizzes

Late assignments will be subject to a 10% deduction per day (note that assignments handed in after the first 10 minutes of lab are considered late) up to a maximum of 2 days late (after which you will receive a 0). There are no make-up quizzes offered except in the case of a documented medical or family emergency (in which case the instructor will decide how to go about the makeup testing).

ACADEMIC INTEGRITY

<https://students.ucsd.edu/academics/academic-integrity/index.html>

Integrity of scholarship is essential for an academic community. The University expects that both students and faculty will honor this principle and in so doing protect the validity of University intellectual work. For students, this means that all academic work will be done by the individual(s) to whom it is assigned, without unauthorized aid of any kind.

In this course, we need to establish a set of shared values. Following are values* adopted from the International Center for Academic Integrity (<http://www.academicintegrity.org/icaai/home.php>), which serve as the foundation for academic integrity.

Inclusion and accessibility (<http://disabilities.ucsd.edu>)

Any student with a disability is welcome to contact us early in the quarter to work out reasonable accommodations to support your success in this course. Students requesting accommodations for this course due to a disability must provide a current Authorization for Accommodation (AFA) letter issued by the Office for Students with Disabilities (OSD), which is located in University Center 202 behind Center Hall. Students are required to present their AFA letters to faculty and to the OSD Liaison in the Division of Biological Sciences in advance so that accommodations may be arranged. For further information, contact the OSD at 858-534-4382 or osd@ucsd.edu.

Course Requirements and Grading: Your final grade for the class will be calculated using the following criteria:

Final Exam	180 points
Lab Quizzes (3 at 40 pts. each)	120 points
Lab Notebook (3 X 20 pt notebook checks)	60 points
PCR minireport	30 points
Ligation minireport	50 points
RFP Report	110 points
RNAi Report (in final notebook)	50 points
Total	600 points

Point Cutoffs for Grade Assignments: (Cutoffs may be lowered at the instructor's discretion.)

99%	A+	79%	C+
90%-98%	A	70%-78%	C
89%	B+	60%-69%	D
80%-88%	B	<60%	F

In addition: Extra credit pts are available

Greater than 80% CAPE response: 5 pts

Course Web Site:

Many of the course materials are available only through the course website on TritonLink Education, or Ted (<https://ted.ucsd.edu>). All students will need to be able to access this site. Once you are enrolled in the class you will have access to the site using your ACS username and password. Be sure to check the course website frequently for announcements and updates on assignments. Items such as lab report guidelines and image files of gels and other data will be provided through the website. The Additional Materials folder contains additional background material for some of the experiments.

Lab Notebooks:

You will keep a formal laboratory notebook for a lot your work in the class. A well-kept lab notebook serves as a portfolio of the experiments and techniques you have performed, something that can be useful when interviewing for research internships and laboratory jobs. Your notebook needs to be bound (no loose pages), but composition books and spiral-bound notebooks are both okay. The notebook does not need to have carbon copy pages, you will not have to turn in copies of notebook pages. Your notebook should be kept up to date as you carry out each lab. Analysis (including plots and gel images) must be completed and added to the notebook by the lab period following collection of the data. Your IA will perform unannounced lab notebook checks throughout the quarter.

NOTE: YOUR IA WILL GIVE YOU SPECIFICS ON WHAT THEY ARE LOOKING FOR.

HOWEVER, THESE BASIC GUIDELINES WILL BE THE GUIDE

- Write the **experiment date** in the upper left-hand corner of **each page**. Make all entries in chronological order. You do not need page numbers or a table of contents—you will index your entries by the experiment date.
- Project title** following the date on each page (*e.g.*, Site-Directed Mutagenesis in the P1_RFP plasmid). Be sure to separate the three projects in your notebook.
- Brief introduction stating overall purpose/overview of lab**
- Experiment title** underneath the project title on each page. This should be a single sentence indicating the specific procedure that was performed.
- Briefly list any changes to the procedures from the lab manual. Other than that, you do not need to write out procedures.
- Raw data and important observations: Enter numerical values in an organized table. For large numbers of numerical values collected electronically, you may paste printer tapes or a printout of the Excel spreadsheet into the notebook. These must be permanently fixed; you will not get credit for items loosely tucked into the pages. Also include any important observations (be brief). Look for prompts in the lab manual for what to include.
- Data analysis: Include any calculations, statistics, or graphs immediately following the raw data. This should be done for any and all data you collect (with the exception of the exercises in Lab 1). Graphs and plots should be done using Excel (or another graphing package) and should be labeled in text. They need to be printed and pasted into your notebook. Be sure they look professional!—ask for help with graphing in Excel if you are having trouble.
- All electrophoresis gel and Western blot images should clearly labeled with text, printed, and pasted into your notebook.

- i. Include a brief statement of the conclusions from the experiment. This may be a single sentence to simply verify that you successfully concluded that procedure on days where you don't collect any data, to a short paragraph describing the results of a multi-day experiment. You should also succinctly describe anything that went wrong with that experiment. What would you do differently if you had to do the experiment again?
- j. Your lab notebook should not contain lecture notes!

Lab Attendance Policies:

Attendance at each lab session is mandatory. An unexcused absence will result in 10 points being deducted. If you know that you need to miss a lab session, discuss this with the instructor (not the IA, they are not authorized to give you permission) to see if it will be possible to make up the lab session or excuse you from the lab with no consequences. Please bring this to the instructor's attention as soon as you know that it will be an issue. **Only the instructor can excuse an absence. Two unexcused absences will result in the student failing the course.**

Turning in Lab Reports:

Lab reports are due at the beginning of lab on due date listed in the lab schedule. In addition to the hard copy turned in to your lab IA, an electronic copy of the report must also be submitted to Turnitin.com, which is accessed through Ted. The report must be submitted to Turnitin.com before the hard copy is turned in, and the hard copy must contain the Turnitin.com submission receipt in the appendix. Lab reports not turned in at the beginning of the lab session on the due date will be considered one-day late. 10% will be deducted for each day late. Students agree that by taking this course all required papers will be subject to review for textual similarity by Turnitin.com for the detection of plagiarism. All submitted papers will be included as source documents in the Turnitin.com reference database solely for the purpose of detecting plagiarism of such papers. Use of the Turnitin.com service is subject to the terms of use agreement posted on the Turnitin.com site.

Re-Grade Policy on Lab Reports: Your lab reports will be graded by your IA, based on the same lab report guidelines (general and specific) that you receive. Note that I work closely with all the IAs to ensure that the grading is accurate and equivalent between sections. If you disagree with the grading of your lab report, discuss this with your IA first to get clarification on why points were deducted.

Making Up Quizzes and Exams:

Please note that it is extremely burdensome for the instructor and IAs to have to prepare and proctor make-up exams. Missing a scheduled quiz or exam will only be excused for medical reasons where documentation can be provided.

LECTURES WILL CORRESPOND TO LAB TOPICS AND

Blmm101 Winter 19 SCHEDULE

	<u>Dates</u>	<u>Experiment/Activity</u>	<u>Lab Manual Section</u>
<u>Wk 1</u>	Jan. 8	Pipetting,Dilutions,Calibration of a pipetteman	Lab 1
	Jan 10	Agarose gel electrophoresis	Experiment 1, 1A-1D
Wk 2	Jan 15	Computer lab: Image Studio Lite analysis of agarose gel Graphing Set-up liquid cultures of RFP and P1 control promoter	Appendix A Appendix B,C Experiment 2A
	Jan 17	Extract plasmids, run on AGE, quantify with nanodrop	Experiment 2B

Wk 3	Jan 22 <u>Lab Quiz #1</u>	Design and set up RFP PCR experiment Computer lab-plasmid map, restriction enzymes, primer design	Sub exp 2-1, 2C Appendix D
	Jan 24	Run gel of PCRs, Clean up PCR; Set up digest of Pro 1 plasmid and RFP PCR product Finish Appendix D computer lab if needed	2C-2E
Wk 4	Jan 29	Clean stuffer from Pro 1-heat inactivate PCR digest Run gel of digest Plan and set up ligation	2F 2G
	Jan 31	Transform bacteria with ligation Computer lab: Design mutagenesis primers	2H 2K
Wk 5	Feb 5 <u>PCR report due</u>	Count colonies Plan how to analyze ligation data Pick red colony from plate and start liquid culture	2I
	Feb 7	Purify recombinant Pro1-RFP plasmid; run AGE Set up mutagenesis PCR Computer lab: analyze ligation data	2J 2L
Wk 6	Feb 12 <u>Lab quiz #2</u>	Run gel of PCR mutagenesis; repeat PCR Kinase/ligase/dpn treatment Transform cells	2M 2N 2N
	Feb 14	Check repeat PCRs, KLD and transformations if needed	
Wk 7	Feb 19 <u>Ligation report due</u>	Computer lab: Bioinformatics Intro to Genbank Analyze transformations Set up liquid cultures: three colonies from mutagenesis	Appendix F 2O
	Feb 21	Streak cultures to maintain Purify plasmids from 3 cultures Check plasmids using AGE & send for sequencing	2P 2Q 2Q
Wk 8	Feb 26 <u>Lab quiz #3</u>	Computer lab: analyze sequencing results Use streaked bacteria to measure RFP Plan how to analyze RFP data	2R 2S 2T
	Feb 28	Observe <i>C. elegans</i> and induce RNAi Computer lab: Analyze RFP data	3A 2T
Wk 9	March 5	Observe worm phenotypes Extract RNA and set up RT-qPCR	3B 3C
	March 7 <u>RFP Paper due</u>	PTC extraction and PCR Computer lab: Analyze qPCR data	4A Instruction at end of Exp.3
Wk 10	March 12	Digest PTC PCRs, check with agarose gel, PTC taste-test (phenotype) Computer lab: Analyze PTC data	4B 4B
	March 14	<u>Clean up; Final Exam;; final lab notebook check including RNAi minireport due</u>	