

Recombinant DNA Techniques
BIMM 101
Winter Quarter 2018

Instructor: Swarna Mohan, Ph.D.

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Lecture: Tues/Thurs 12:30 – 1:50pm, York 3010

Lab: Tues/Thurs 2:00 – 5:50pm York 4318 (F01) and York 4332 (F02)

Office Hours: Thursdays 10 – 11am in H&SS 1145LB or by appointment (24 hours' notice required)

Course Materials:

1. BIMM 101 lab manual
2. Carbonless copy paper lab notebook
3. Safety glasses
4. Lab coat (knee length)
5. Proper attire (full-length pants, socks that cover ankles, close-toe/heel shoes)
6. Fine-tipped sharpie pen for labeling tubes.

Course Objectives:

- Apply molecular biology concepts and techniques to understand experimental results and troubleshoot problems.
- Develop basic molecular biology lab skills.
- Learn how to design experiments, include proper controls, interpret results and draw logical conclusions from data.
- Learn how to use basic bioinformatics databases and applications.
- Perform basic lab calculations, statistical analysis, and graphing.
- Learn to find, understand, and evaluate primary literature.

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 there will be an issue. Only the instructor can excuse an absence. Two unexcused absences will result in the student failing the course.

Triton Ed: We will be using Triton Ed (TEd) (<https://tritoned.ucsd.edu/webapps/login/>) as the course web site. Except for the lab manual, all course materials will be accessed through the course webpage on TritonEd. In addition to lab report guidelines, much of the data you generate in your experiments will be accessed through TritonEd. Be sure to check the TritonEd frequently for announcements and updates.

Quizzes, Exam, and Assignments:

Your final grade for the class will be calculated using the following criteria:

Cumulative Final Exam	150 points
Weekly quizzes (Week 2 -9; lowest quiz grade will be dropped)	105 points
Lab Notebook Checks	50 points
5 mini lab reports and 2-3 assignments	195
Total	500 points

Grade cutoffs:

450-500	A	390-394	C+
445-449	A-	345-389	C
442-444	B+	340-344	C-
400-441	B	295-339	D
395-399	B-	0-294	F

The grade cutoffs may be adjusted downward at the instructor's discretion.

Quizzes and Exam:

The purpose of the lab quizzes is to ensure that you understand the purpose of the lab projects and how each experiment fits into this, the basic concepts underlying the procedures, and simple mathematical and analytical skills based on what you have actually done in lab. Starting week 2, weekly quizzes will be given at the beginning of lecture every Tuesday. Quizzes will take 10 – 15 minutes, and will consist of 5 to 7 questions. Make up quizzes will not be given.

A cumulative final exam will be held on the last day of lab. The exam will be problem solving-based and will include some basic questions on the concepts we have covered, but will have an emphasize on taking the information you have learned and extrapolating to solve problems you have not seen before. The graded exam and the exam key will be available to you for review as soon as the exams are graded. You are not permitted to take the exam with you. Any exam not returned will be subject to a 20% penalty.

Submitting mini lab reports and lab assignments:

Lab reports and assignments 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 lab reports and assignments must be submitted to Turnitin.com, which is accessed through TEd, before the hard copy is turned in. Assignments and lab reports not turned in at the beginning of the lab session on the due date will be considered one-day late. Ten percent of the total points will be deducted for each working day that the assignment or report is late. Assignments and lab reports will not be accepted after three working days past their due date.

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.

Regrade policy:

All assignments and lab reports will be graded by your IA, based on the guidelines provided by me. 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 assignment, you may submit the assignment to me for a regrade within one week of receiving the graded assignment. Along with your assignment, you must submit a written explanation of why you are submitting it for a regrade. Your assignment will not be regraded without a written explanation. Submission of an assignment for regrade does not guarantee a score change for that assignment. Quizzes cannot be submitted for a regrade.

Note: Unless the regrade request is due to an addition error, all regrades will be done to the entire assignment.

Lab Notebooks:

You will keep a formal laboratory notebook for all of your work in the class. Your notebook needs to be the carbonless copy lab notebook (available at the book store). See pages 8 and 9 in the lab manual for how to format your notebook and what information it should contain. Pay particular attention to the following:

1. Write the experiment date on 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.
2. Include the **Experiment and Project title** on each page. Be sure to separate the different projects in your notebook. The experiment title should be a single sentence indicating the specific procedure that was performed.
3. Briefly list any changes to the procedures from the lab manual. Other than that you do not need to write out procedures.
4. **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).
5. **Data analysis:** Include any calculations, statistics, or graphs immediately following the raw data. This should be done for any and all data you collect. 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.
6. All electrophoresis gel images should clearly labeled with text, printed, and pasted into your notebook.
7. Include a brief statement of the conclusions from the experiment. This may be a single sentence

to simply verify that you successfully concluded the 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?

8. Your lab notebook should **not** contain lecture notes!

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.

Academic Integrity: As a UCSD student enrolled in this course you are expected to abide by the Policy of Integrity of Scholarship at UCSD (<http://academicintegrity.ucsd.edu/process/policy.html>). Cheating, and/or plagiarism will not be tolerated and the consequences for such behaviors can result in receiving a failing grade in the course and disciplinary sanctions such as suspension or dismissal from the University.

While you will work in groups during the lab sessions, each student must work on the assignments and lab reports independently. Working on assignments and lab reports with other students will be considered plagiarism and you will receive a zero for that assignment. Additionally, you are not permitted to use old lab reports for this class. If you are caught using old reports or assignments to assist you in any way, you will receive a zero for that assignment and additional disciplinary actions might be taken.

No student communication of any form is allowed during the quizzes and the exam. You are only allowed to use blue or black pen and a scientific calculator during the quizzes and the exam. You may not take the quiz or exam in pencil. During the exam you may **not** leave the room and come back to resume the exam. If you leave the room, your exam will be collected and graded as is. If you will need to leave the room for medical or psychological reasons, be sure to provide me with documentation from the Office for Students with Disabilities.

Disability access: 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 (please make arrangements to contact me privately) and to the OSD Liaison in the department in advance so that accommodations may be arranged.

Contact the OSD for further information:

858.534.4382 (phone)

osd@ucsd.edu (email)

<http://disabilities.ucsd.edu> (website)

Tentative Lab Schedule

	Dates	Lab Exercises	Lab Manual Section	Quizzes and Tentative due dates
1	Jan 9	Calibration of a pipetmen Pipetting Dilutions	Lab 1 Additional info "working in the lab" sections E, F, G	
	Jan 11	Agarose gel electrophoresis on two DNA samples of unknown size and concentration (estimating using standard curve)	Experiment 1, 1A-1D	
2	Jan 16	Computer Lab Image Studio Lite Analysis of Agarose Gel Graphing Set-up liquid cultures of RFP and control promoter	Appendix A Appendix B, C Starting Experiment 2, 2A	Quiz 1 (in lecture)
	Jan 18	Extract plasmids Check plasmids with AGE & nanodrop Computer Labs available if needed	2B	
3	Jan 23	Design and set up RFP PCR experiment Start computer lab - plasmid map, restriction enzymes, designing primers	Sub-experiment 2-1. 2C Appendix D	Quiz 2 (in lecture) Agarose Gel mini report due in lab.
	Jan 25	Run gel of PCRs, repeat if needed Clean up PCR Set up digest of Pro1 plasmid and RFP PCR product Finish Appendix D computer lab	Finish 2C 2D 2E	
4	Jan 30	Clean stuffer from Pro1 - heat inactivate PCR digest Run gel of digest Plan ligations	2F 2F Sub-experiment 2-2: part of 2G	Quiz 3 (in lecture)
	Feb 1	Set-up ligations & transform bacteria with ligations Computer Lab: Design mutagenesis primers	2H 2K	PCR mini report due in lab.
5	Feb 6	Count colonies Plan how to analyze ligation data Pick red colony from plate and start liquid culture	2I start 2I 2I	Quiz 4 (in lecture)
	Feb 8	Purify recombinant Pro1-RFP plasmid and run gel Set up mutagenesis PCR Computer lab: analyze ligation data	2J 2L plan previously developed	

6	Feb 13	Gel of PCR mutagenesis, repeat PCR Kinase/ligase/dpn treatment Transform cells	2M 2N 2N	Quiz 5 (in lecture)
	Feb 15	Check repeat PCRs, KLD and transformation if needed Analyze transformations Computer lab: Bioinformatics Intro to GenBank	2O Appendix F	Ligation mini report due in lab.
7	Feb 20	Set-up liquid cultures: three colonies from mutagenesis	2O	Quiz 6 (in lecture)
	Feb 22	Streak cultures to maintain Purify plasmids from 3 cultures and send for sequencing Check plasmids using AGE	2P 2Q 2Q	
8	Feb 27	Computer lab: analyze sequencing results Use streaked bacteria to measure RFP Plan how to analyze RFP data (optional: analysis as homework or analyze next lab)	2R 2S start 2T	Quiz 7 (in lecture)
	March 1	Observe <i>C.elegans</i> and induce RNAi Computer Lab: Analyze RFP data	Experiment 3. 3A 2T	
9	March 6	Observe worm phenotypes Extract RNA and set up RT-qPCR	3B 3C	Quiz 8 (in lecture)
	March 8	PTC extraction & PCR Analyze qPCR data *need own computers*	Experiment 4. 4A Brief instructions at end of Exp. 3.	Mutagenesis lab report due in lab.
10	March 13	Digest PTC PCRs, check with agarose gel, PTC taste-test (phenotyping) Pool genotype/phenotype data Optional: Analyze data	4B 4B	RNAi analysis due in lab.
	March 15	Final Exam in Lab		PTC assignment due in lab