BIMM 101 Recombinant DNA Techniques

Summer II 2018

Dr. Goran Bozinovic Office hours: during the lab or by appointment gbozinovic@ucsd.edu

HSS 1145G

IA:	email	Section
Engelhart, Darcy Christine	dengelha@ucsd.edu	A01
Miaule. Alexandre Francois	amiaule@ucsd.edu	A02

 Lectures:
 Tue-Fri
 11AM-12:20PM
 YORK 4080A

 Labs:
 Tue-Fri
 1PM-4:50PM
 York 4318 / 4332

Course Website: http://tritoned.ucsd.edu

- Lectures will cover the theory behind the experiments performed in lab. The quizzes and exam will have questions on the material that will be covered during lectures.

Learning objectives:

- Learn the theory behind molecular techniques, and the applications of the methodologies in biological research
- Become proficient at basic molecular biology techniques
- Learn the importance of proper controls in designing experiments and interpreting results
- Improve lab math skills and ability to graph data correctly
- Learn to make logical conclusions from experimental data
- Become familiar with bioinformatics databases and applications
- Learn to find, read, and evaluate primary literature
- Become aware of the implications of the technology for society

Required texts:

BIMM 101 Lab Manual from University Readers

From Genes to Genomes by Dale (1st or 2^{cd} edition) on reserve at BML and electronic version available from UCSD computer http://onlinelibrary.wiley.com/book/10.1002/0470856912 Readings on tritonEd (tritoned.ucsd.edu)

Required Materials – needed by second day of class:

Labcoat (the bookstore has cheap ones)
UV blocking safety glasses (also at bookstore)
Lab notebook with carbon copies (bookstore or Grove general store)
Fine point Sharpie for labeling – get a dark color
A calculator

!!! Mandatory Lab Safety Training Prior to Lab Participation:

All students, enrolled or waitlisted, for the laboratory courses below are REQUIRED to demonstrate an understanding of general lab safety and UCSD Undergraduate Biology lab rules and policies. Completing the on-line Lab Safety Training and passing the Lab Safety Assessment fulfills this requirement.

Enrolled students who miss the first lab meeting, or do not pass the Lab Safety Assessment, will have their lab space revoked. It is the student's responsibility to drop the course or risk receiving

- a "W" or non-passing grade in the course.
- The Lab Safety Training and Assessment are available at any time, but the assessment will apply to a specific quarter.
- The assessment must be completed and passed BEFORE the first lab meeting. Plan!
- The self-study training and assessment should take approximately 1-hour to complete.
- The assessment portion is timed. Students will have a maximum of 30-minutes to complete the assessment.
- Students may take the assessment as many times as it takes to pass.
- Students will be certified once the assessment is passed. Certification information will be available to lab instructors for verification prior to the first class meeting.

Certification is valid for one quarter.

http://biology.ucsd.edu/education/undergrad/course/ug-labs.html

Course Requirements

Course Web Site:

Many of the course materials are available only through the course website on TritonEd (https://tritonEd.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. Use the Discussion Board to ask questions on material from lecture or lab. The instructor and IAs will check the Discussion Board daily to answer questions, but students are encouraged to answer questions as well. This is a handy resource for last minute questions that come up during late night studying for an exam.

1. Lab Attendance Policies:

Attendance at each lab session is mandatory. If you are more than 10 minutes late to lab, or you leave lab before your group is done, you will be counted as absent for the day. An unexcused absence will result in 10 points being deducted from the associated lab report. 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.

Also, everyone is expected to be an active participant in every experimental procedure. Failure to make a meaningful contribution towards completing the laboratory experiment/activity will result in points being deducted from the laboratory report score.

Lab Notebooks:

It is mandatory that you keep a complete lab notebook. The notebook must contain everything that you did in the lab, including:

- Any changes in the protocol
- All data/results
- All calculations done during experiments
- Observations

- There will be three random notebook checks starting the second week of class in order to maintain GLP notebook keeping. Each check is worth 5 pts.

You will be expected to keep a formal laboratory notebook for all the work you do in lab. The notebook should be bound (spiral bound or composition book style are acceptable) and should have numbered pages with a table of contents (it is okay to write these in). Lab notes must be written in ink. You will need to hand in either photocopies or carbon copies of your notebook pages for the experiments that are written up as lab reports. Notebook entries should be in chronological order, with each project or set of experiments together and easily referenced by the table of contents. Each page should have a brief title for the experiment and the date on which the work was performed. Refer to the course lab manual for some other tips on entering information into your notebook. Starting on the second day of lab, you will need to have the following entered in your notebook at the beginning of each lab session:

A. From the <u>previous day's experiment</u>: all your data entered in labeled spaces, and any analysis for that experiment completed. Analysis includes any calculations and graphs that may be required to analyze the data. Your IA will tell you what analysis needs to be done for each experiment. There should also be a summary (not more than a few sentences) of the experiment that states how well the procedure worked and any major conclusions from the data.

B. For the <u>current day's experiment</u>, a brief purpose explaining what you are doing that day (one or two sentences is fine), and appropriately labeled spaces and tables in which you will enter any data collected that day. Also, make sure that your table of contents and page numbering is up to date.

There will be four unannounced notebook checks, worth 5 points each, where your IA will inspect your lab notebook. The IA will most likely choose certain labs or analysis to focus on at each check.

Lab Manual and Pop Quizzes: It is important to carefully read the pertinent sections of the lab manual before coming to class. The experiments will proceed much more smoothly, and you will get a lot more out of them, if you have read through the procedure and understand why you are doing them. To encourage you to read the lab manual before class, there will be 3 scheduled quizzes during lab periods staring after April 13th, each worth 10 points. They will be given at the beginning of lab and collected 15 minutes later. If you arrive after the quiz has been handed out, you will not be able to make it up.

Homework:

- Dilutions and DNA Quantification: due in lab - Aug 14

- PCR Analysis: due in Lab – Aug 21

Lab Reports: One comprehensive lab report (100 points), and one mini Lab Report (40 pts) are to be submitted throughout the quarter; while you will be collecting and sharing data with a lab partner, and you are welcome to discuss your results with your classmates, you must hand in your own lab report, written in your own words. You will be penalized for copying another lab report or for handing in the same (or very similar, such as just a few words changed here and there) lab reports as your partner. Specific guidelines for each lab report are posted on the course website. Although the lab report will be submitted electronically, all carbons from the labs associated with a lab report must be handed in class the day the lab is due.

Turning in Lab Reports: We will be using the website Turnitin.com for lab reports in addition to turning in hard copies to your IA. Lab reports submitted to the Turnitin.com site do not need to

have graphs, tables, or attachments, but you may include them if it is easier. Lab reports must be submitted to Turnitin.com before midnight of the due date, and a hard copy of the report (including all text, plus all tables, graphs, attachments, or anything else called for in the lab report guidelines) must be given to your IA at the <u>beginning</u> of the due date lab session. Lab reports not turned in at the beginning of the lab session, or not submitted to Turnitin.com by the end of the day will be considered one-day late. Ten points will be deducted for each working day that the lab reports are late (hard copy and Turnitin.com). 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 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.

<u>Lab report Late policy.</u> Lab reports are due at the beginning of lecture on the assigned due date (must be electronically submitted via Turn-It-In before 11:59:59PM of the due date). Penalty for turning lab reports late:

- 5 points if handed in later the same date;

After the first late day, you lose 2 points/day, so

- -7 points if handed in anytime the next day
- -9 points if handed in the 3rd day etc.

Lab report due dates:

Labs to be included in the Lab Report	<u>Due date</u>
LR1 – Experiment 2 (2A-2T)	Sept 4
mini LR – Experiment 3	Sept 7

^{**} Please submit your Lab Reports at the beginning of the lab. Mimi lab report should be submitted before your start your Final Exam on Sept 7.

Make-up 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 exam will only be excused for medical reasons where documentation can be provided. At the instructor's discretion, a missed exam that is excused will either be dropped from the student's point total for the class or made up by an oral exam scheduled within one week of the original exam.

Academic Integrity

Cheating will not be tolerated. The administrative policy on Academic Dishonesty outlined by UCSD will be followed. Students caught cheating during an exam or quiz will be removed and given a "zero" for that exam or quiz. A report will also be filed with the Academic Integrity Coordinator. Cheating includes (but is not limited to) plagiarism, talking during tests, or making use of forbidden materials during the test. Students are permitted to bring only non-programmable calculators and writing implements to exams. Tampering with graded exams will result in a failing grade for that exam.

During laboratory sessions, student cooperation and collaboration is highly encouraged. This includes discussion of experimental data with fellow students during lab hours. After the laboratory session is over, however, you are required to work on your own. *Each student must hand in an independently written and independently thought-out data analysis for each lab*. If you are caught working with another student on your lab report, both of you will receive a "zero" for that lab report, and you might be reported to the Academic Integrity Coordinator.

It is NOT acceptable to use any old lab reports to assist you in any way. If you happen to be in possession of old copies of lab reports for this class, it is best that you do not even look at them, since they could unintentionally influence the way that you write your own report. If we discover that you have used an old lab report in any way, you will automatically receive a "zero" for that lab report, and you might be reported to the Academic Integrity Coordinator. While your lab reports will be returned to you, you are NOT permitted to share them with anyone for any reason. If we find that you have shared your lab report with anyone, you will be reported to the Academic Integrity Coordinator, even if you have already completed the class. You are required to read, understand, and sign the "BIBC103 Integrity Policy" which governs the way the academic work in this class is completed.

- **6. Lab performance:** There are no points for lab performance per se. However, your effort, attitude, and the success of your experiments will be considered when assigning the final grade, especially if you are on the borderline between two grades. If you miss one lab with no excuse, you will lose 5% from your final grade. If you miss two labs, you will receive an F for the course.
- **7. Exams**: There will be two exams Midterm (100 pts) on Friday, August 28th, and Final exam (150 pts) on Friday, Sept 7th. Final exam is cumulative. Depending on the performance of the class, the exam scores might be adjusted.
- The exam adjustment policy:
- 1) If any student receives a 100% on the exam it will NOT be curved;
- 2) If any student receives a score between 95 and 100%, that will be the new maximum score (for example if the highest grade is 96, everyone's score will increase by 4 points);
- 3) If the highest score is less than 95% then that student's score will be the new 95% (for example if the highest grade is 89, everyone's score will increase by 6 pts).

Grading:

HW #1	20
HW#2	20
Notebook Checks (3)	15
3 Quizzes (10 points each)	30
LR 1	100
Mini LR	40
Midterm Exam	100
Final Exam	150
Total possible points:	475

Please make sure you regularly check your scores in TritonEd to make sure no errors have occurred

Letter grades will be assigned as follows:

Grade Overall class percentage

A+, A, A- 98, 92, 90

B+, B, B-88, 82, 80

C+, C, C- 78, 72, 70

D+, D, D-68, 62, 60

F Below 60

Note: Just coming to lab does not ensure that you will get a passing grade in the class. You must hand in all assignments and get passing scores on those assignments (an average of 70) to get a C- in the class.

Policy on cheating: Anyone caught cheating (which includes but it is not limited to plagiarizing lab reports, cheating on a test or quiz, or changing an answer for a regrade) will be reported to the Academic Integrity Office.

BIMM 101 Summer 2018 Student contract:

1. I understand that if I am late for lab on a day a quiz is given, I will not be allowed to take the quiz and will receive a 0 score for that quiz.		
Name Date		
All lab reports for the class must be independently written, i.e., your own work in your		
own words. While discussion of data among lab partners is encouraged, each student on their		
own must complete all text, references, figures, graphs, and tables. The submission of reports		
by lab partners that contain shared work is forbidden and will result in points being deducted		
from both reports. The exception to this is when a figure is the raw data that is supplied to each		
member of the group (specifically absorption spectra and gel photographs). In this case the		
labeling of that figure must be done independently. If you have questions about the difference		
between discussing your work with others and unauthorized collaboration, please ask your		
instructor or I.A. for clarification.		
Because lab reports are to be your own work in your own words, you may not copy to		
any extent current or past laboratory reports that were written by other students. This is known		
as plagiarism, which is a direct attempt by the student to present the work of others as his/her		
own and is no different than cheating on an exam. Directly copying material from other sources		
without putting it in your own words is also plagiarism, even if the source is cited as a reference.		
Plagiarism in lab reports is rigorously sought out and penalized. Students are required to upload		
an electronic version of each lab report to Turnitin.com, where the report is screened with a		
plagiarism checker against all reports in the Turnitin database. All incidents of plagiarism will		
automatically be turned in to the Academic Integrity Coordinator. Following UCSD's Policy on		
Integrity of Scholarship (www-senate.ucsd.edu/manual/appendices/app2.htm), students found		
to have committed plagiarism or other academic misconduct will receive both an administrative		
(decided by the Council of Deans) and academic penalty (decided by the instructor).		
Furthermore, all submitted reports are retained in the Turnitin database. Similarity hits by the		
plagiarism checker will also reveal the name of the student who provided the plagiarized		
material. Giving one's own lab report to other students to allow them to copy material from that		
report is also academic dishonesty and will be pursued and penalized as rigorously as for the		
student committing the plagiarism.		
2. I understand that if I plagiarize a lab report and it is detected by tritonEd.com, the matter		
will go to the Academic Integrity Office on campus. I also understand that if I give a lab report		
to a student who takes the lab in a subsequent quarter, and he or she plagiarizes my lab		
report, I will also be subject to disciplining by the Academic Integrity Office.		
Name Date		

Lab Schedule

Dates	Lab Exercises	Lab Manual Section
7-Aug	Calibration of a pipettemen	
	Pipetting	Lab 1
	Dilutions	Add. info "working in
		the lab" s. E, F, G
8-Aug	Agarose gel electrophoresis of two DNA samples of unknown size and	Experiment 1, 1A-1D
	concentration (estimating using standard curve)	
9-Aug	Computer Lab	
	Image Studio Lite Analysis of Agarose Gel	Appendix A
	Graphing	Appendix B, C
	Set-up liquid cultures of RFP and control promoter	Starting Experiment
		2, 2A
10-Aug	Extract plasmids	
	Check plasmids with AGE & nanodrop, HW1 posted	2B
14-Aug	Design and set up RFP PCR experiment; HW1 Due	Sub-experiment 2-1.
		2C
	Start computer lab - plasmid map, restriction enzymes, designing	Appendix D
45.4	primers	51.11.12.0
15-Aug	Run gel of PCRs, repeat if needed	Finish 2C
	Clean up PCR	2D
	Set up digest of Pro1 plasmid and RFP PCR product Finish Appendix D computer lab	2E
16-Aug	Clean stuffer from Pro1 - heat inactivate PCR digest	2F
10-Aug	Run gel of digest	2F
	Plan LIGATIONS	Sub-experiment 2-2:
	Tidil Elo/Micho	part of 2G
17-Aug	Set-up ligations & transform bacteria with ligations	2H
	Computer Lab: Design mutagenesis primers; HW2 posted	2K
21-Aug	Count colonies; HW2 due	21
	Plan how to analyze ligation data	start 2I
	Pick red colony from plate and start liquid culture	21
22-Aug	Purify recombinant Pro1-RFP plasmid and run gel	2J
	Set up mutagenesis PCR	2L
	Computer lab: analyze ligation data	plan previously
		developed
23-Aug	Gel of PCR mutagenesis, repeat PCR	2M
	Kinase/ligase/dpn treatment	2N
	Transform cells	2N
24-Aug	Check repeat PCRs, KLD and transformation if needed	
	Analyze transformations	20
	Computer lab: Bioinformatics Intro to GenBank (optional)	Appendix F
	Set-up liquid cultures: three colonies from mutagenesis	20
28-Aug	Streak cultures to maintain; Midterm Exam in Lab @ 9AM	2P
	Purify plasmids from 3 cultures and send for sequencing	2Q

	Check plasmids using AGE	2Q
29-Aug	Computers: Analyze Sequencing results (if available, or do next lab)	2R
	Plan how to analyze RFP data (optional: analysis as homework or analyze next lab)	Start 2T
30-Aug	Computers: Analyze Sequencing results if not done on Tues	2R
	Use streaked bacteria to measure RFP	2S
	Observe <i>C.elegans</i> and induce RNAi	Experiment 3. 3A
31-Aug	Computers: Analyze RFP data	
4-Sep	Observe worm phenotypes; Lab Report Due	3B
	Extract RNA and set up RT-qPCR	3C
5-Sep	Computer Lab: Analyze qPCR data	Brief instructions at
		end of Exp. 3.
6-Sep	Clean up and Review	