BIMM 101 Recombinant DNA Techniques

Summer II 2016

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HSS 1145G

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Lectures: Tue-Fri 9:30-10:50 CENTR 222

Labs: Tue-Fri 11-3 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

Remember that lab attendance is required – if you miss two labs, you will be asked to drop the course. If you are ill, you must leave a message with your instructor, not your IA, and make up the lab in a way that we will determine. You must be on time for lab; the IAs go over the experiments at the beginning of lab, and also quizzes are administered then.

!!! 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 students 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 ahead!
- 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

- **1. Lab Notebook**: 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.
- **2. Take home assignments:** there will be two take-home assignments

HW #1: Dilutions and DNA Quantification- 30 pts – due Tue, Aug 9

HW #2: Statistical Analysis (Lab 10) - 30 pts - due Friday, Aug 19

- **3. Quizzes**: There will be 4 scheduled quizzes during lab periods staring July 5, 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.
- **4. Lab Reports**: Two lab reports, each worth 100 points, 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.

All lab reports should include:

- 1. Purpose of the experiment: this section should be BRIEF no more than a few sentences: simply state why you are doing the experiment.
- 2. Results should include the following:
- data or data analysis
- figures, gels (or representations thereof)
- any sample calculations
- a brief statement about what each result means
- 3. Discussion:
- Note any unusual observations
- Discuss success or failure of the experiment if there was a problem, discuss probable source.

Lab reports should have no more than 5 pages of text – figures can go on separate

pages. More specific description of the lab report can be found in the course TritonEd page. 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.

Lab report Late policy.

Lab reports are due at the beginning of lecture on the assigned due date (must be electronically submitted before 11 AM of the due date).

Penalty for turning lab reports late:

- 5 points if handed in later on 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	Due date	
LR1 - Labs: 4-11	Aug 23	
LR2 - Labs 16-18	Sept 2	

- ** Please submit your Lab Reports at the beginning of the lab. Lab reports 2 and 3 (submit them stapled together) should be submitted on the morning of the final exam on Friday, July 29. You may also submit your reports to your IAs earlier.
- **5. Lab attendance**: Attendance is taken within the first 15 minutes of every lecture session. If you are ill, please notify me (gbozinovic@ucsd.edu).
- **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. Exam**: There will be two exams Midterm (100 pts) on Tuesday, August 23rd, and Final exam (150 pts) on Friday, September 2nd. 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 that 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	30
HW#2	20
Notebook Checks (3)	15
4 Quizzes (10 points each)	40
LR 1	100
LR2	100
Midterm Exam	100
Final Exam	150

Total possible points: **555**

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 2016 Student contract:

Name

Date

Tentative Schedule, Summer 1, 2016

Week	Class	LAB
1	Tue,	LAB 1
	8.2	A. Pipetting
		B. Dilutions
		C. Calibration of a pipettemen D. Mol. Bio. Review
	Wed,	LAB 2
	8.3	A. Agarose gel electrophoresis on two DNA samples of unknown size and
		concentration (estimating using standard curve)
	Thu	LAB 3 *Computer Lab*: A. Image J analysis of gel electrophoresis results &
	8.4	graphing
	Fri	LAB 4
	8.5	A. Introduction to bioluminescence
		B. Cloning the <i>lux</i> A and <i>lux</i> B gene into <i>E.coli</i> : outline of experiments C. Part 1: Isolation of chromosomal DNA from <i>Vibrio fischeri</i>
2	Tue	LAB 5
	8.9	A. Finish purification of chromosomal DNA from Vibrio fischeri
		D. Computer Lab - Bioinformatics Part I: exploring the Lux operon on NCBI +
		primer design
	Wed	HW # 1 Due – Dilutions and DNA Quantification LAB 6
	8.10	A. Spectrophotometric analysis of <i>Vibrio</i> DNA
		B. Design and set up experiment to vary conditions of PCR (amplifying <i>V. fischeri</i>
		luxAB genes)
	Thu	LAB 7
	8.11	A. Checking the success of the PCR reaction by gel electrophoresis
		B. Computer Lab: Using Image J to analyze PCR results + make graph
		C. Time to repeat PCR if needed or expand upon variations from Lab 6
	Fri	Lab 8
	8.12	A. Run gel of repeats (if necessary)
		B. Clean up best <i>lux</i> AB PCR product from lab 6
		C. Restriction digest of <i>lux</i> AB PCR products and pGEM with <i>Xba</i> I and <i>Eco</i> RI
		D. Computer Lab: Bioinformatics Part II and III (restriction digestion) & Part IV Primer Design
3	Tue	LAB 9
	8.16	A. Clean up Xbal and EcoRI digest of pGEM
		B. Quantification of digests from gel
		C. Ligation of pGEM and <i>lux</i> AB inserts

	Wed 8.17	A. Transformation of competent cells with ligation products B. Practice statistical analysis: ANOVA and post-hoc test
	Thu	Lab 11
	8.18	A. Screening for clones containing $luxA$ by adding exogenous aldehyde (Assessing ligation efficiency (blue/white colony counting) \rightarrow Pool data from whole class to do statistical analysis of results).
		B. Plan Synthetic Biology project C. Start overnights of cultures containing plasmids with different promoters
	Fri	Lab 12
	8.19	A. Alkaline lysis miniprep: purification of plasmid DNA from overnight cultures
		B. Setting up digests of Biobrick plasmids HW #2 due – Statistical Analysis Due
4	Tue	Lab 13
	8.23	A. Removing the stuffer fragment from the plasmids containing the promoter sequences
		B. Gel purification of the DNA fragment containing the RFP sequence
		Ligating plasmids with promoter sequences and RFP sequence
		Midterm Exam in Lab; LR 1 due
	Wed	Lab 14
	8.24	A. Transformation of competent cells with RFP ligation products B. Begin PTC: Do PTC taste test, isolate cheek cells DNA and set up PCR
	Thu	Lab 15
	8.25	A. Analyze effect of promoters on RFP expression (fluorometer
		measurements). Pool class data for analysis.
		B. Pick one plasmid and set up overnights (will isolate and send for sequencing to confirm promoter-RFP ligation and reading frame)
		B. Digest PTC cheek cell PCR and run gel
	Fri	Lab 16
	8.26	A. Begin RNAi project: Set up <i>C. elegans</i> plates
		B. Isolate plasmid and run gel to estimate concentration, send for sequencing
5	Tue	Lab 17
	8.30	A. Observe worm phenotypes and isolate RNA
		B. Quantitate RNA and set up quantitative RTqPCR
		C. Computer lab: Analyze plasmid sequencing results (supp. hand-out)
	Wed 8.31	LAB 18 A. Computer Lab: Analyze results of RT-qPCR measurement of <i>unc</i> -22 mRNA
	Thu 9.1	LAB – Review/Paper discussion
	Fri 9.2	Final EXAM in Lab LR 2 due in lab
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