

BIOL115L: Eukaryotic Molecular Biology
Laboratory Course Syllabus
Summer 2018

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Class Hours: Monday - Thursday 10am – 2pm: Thimann 215

*Note: In addition to the regular class meeting time, **students might be required to come into lab outside of class time** to set up and maintain experiments.*

Course Webpage: <https://canvas.ucsc.edu>

Course Description

A laboratory designed to provide students with direct training in basic molecular techniques. Each laboratory is a separate module, which together builds to allow for the understanding of the execution of molecular cloning, DNA/RNA isolation, gene expression, and identification of DNA sequences.

Students cannot receive credit for this course (115L) and either 187L or 287L. Students will be billed a materials fee for this course. Course is restricted to biological sciences/affiliated majors; biology minors, other majors by permission only.

Course Pre-requisite(s):

BIOL 100 or BioC 100A, and BIOL 101L, and a previous or concurrent enrollment in BIOL 115.

Course materials:

There is no textbook or reader/lab manual for this course that can be purchased ahead of time. The online course management program **Canvas** will be used, and all course materials (Power Point slides, lab protocols, handouts etc.) will be made available for download and *print* in **Canvas** (<https://canvas.ucsc.edu>) Students are expected to know how to use and navigate **Canvas**, as well as check it regularly.

Content and Goals of Course:

1. To develop a strong background in the principles/methods of molecular biology. Students will become familiar with bacterial cloning/screens, nucleic acid isolation and amplification, sequencing and computational analysis.
2. To gain/improve on the ability to plan experiments, solve problems, think analytically and interpret experimental results.
3. To understand the practice and importance of maintaining a laboratory notebook. Periodic notebook checks will ensure consistent and organized recordings of experiments.
4. To prepare students for more advanced course work in cell and molecular biology in a laboratory setting.
5. To improve scientific writing skills and gain experience with online databases.

Course Grading:

Assignment	%	Possible points
Solutions quiz	10%	40 points
Journal club	5%	20 points
Assignments (2)	10%	40 points
Oral presentation	10%	40 points
Final lab report	40%	160 points
Pre labs (4)	10%	40 points
Lab notebook	10%	40 points
Paper draft	5%	20 points

Grading scale:

A+	97.0 - 100%
A	93.0 – 96.9%
A-	90.0 – 92.9%
B+	87.0 – 89.9%
B	83.0 – 86.9%
B-	80.0 – 82.9%
C+	77.0 – 79.9%
C	70.0 – 76.9%
D	60.0 – 69.9%
F	0.00 – 59.9%

Solutions quiz (10%)

You will be given a quiz that will encompass many of the math skills needed to be successful in this lab including serial dilutions, track dye addition and making common reagent dilutions.

Journal club (5%)

A primary research article will be distributed and each set of lab partners will be assigned a figure from the paper. You will be responsible for creating a PowerPoint presentation of your figure and presenting the information to the entire class. The paper and guidelines necessary to be successful will be posted on Canvas.

Written Assignments (15%)

Throughout the quarter, you will be assigned **3** homework assignments. Each is worth 5% of your total grade. Assignment due dates are indicated on the syllabus calendar; each assignment will be posted on Canvas.

Oral Presentation (10%)

Oral presentations are a common laboratory practice in which the lab member prepares and presents a short update of ongoing research/results and stimulates a constructive discussion. You and your lab partner will give a 10-15 minute oral presentation of your research to the class. Detailed guidelines will be distributed later in the quarter and posted on Canvas.

Final Research Report (35%)

This assignment shall be a written report covering your (and your lab partners) collective research spanning weeks 3 through 9 of the quarter. Detailed guidelines will be distributed later in the quarter and posted on Canvas. This document will be uploaded to TURNITIN.com.

Pre labs (10%)

Pre labs will be assigned to ensure your preparedness regarding nucleotide extraction and purification and cDNA synthesis as well as qPCR.

Lab Notebooks (10%)

You are expected to read and summarize protocols for each lab session *before* the start of that session. Your lab notebooks will be collected and assessed throughout the quarter, so keep them up to date. Total points will be tallied at the end of the quarter.

Data rough draft (5%)

You will have two weeks following the writing workshop to turn in a draft of your introduction including two references (incorporated appropriately), a sample of the qPCR gel annotated figure including a figure legend and text describing your figure, and a sample of your cDNA synthesis and qPCR methods, as well as a sample of one reference you will use in your introduction. The draft will be read and feedback given in an attempt to allow your final paper to be exceptional.

Late Submission of Final paper and Assignments Assignments are due at the BEGINNING of class (**10:00 am sharp**) in hard copy (and on turnitin.com when specified by instructor). **Final paper will not be accepted late!** Assignments will earn penalties for being late and penalties are as follows:

- By midnight of due date: 5% total deduction
- Midnight the following day after due date: 15% total deduction
- Each 24-hour period thereafter: an additional 10% per day. This includes weekend days. Exceptions to these point deductions will only be made for valid, documented excuses approved by the instructor

All assignments that must be uploaded to Turnitin.com will receive a late penalty of 1% per day until uploaded. Also, late assignments will not be graded or handed back until properly uploaded.

Attendance

Each unexcused absence from lab will result in a deduction of 5% of the total points for the course- **NO EXCEPTIONS**. **Absences must be approved by the instructor (not the TA)** to be excused and to incur no deduction. To have an absence considered for approval as an excused absence, you must do the following: 1) contact the instructor within 24 hours of absence **AND** 2) provide valid written documentation to instructor and as soon as possible (e.g. copy of family death certificate, police report, note from professor, etc.) **NO EXCEPTIONS**. Tardiness to lab will result in a deduction of 1% of the grade for every 15 minutes the student is late. If students are 1 hour late they will be given an absence and not be allowed to participate in the lab for that day. Please be on time and prepared for lab, this will ensure your safety and allow for an optimal experience.

Lab notebook

Students should obtain a bound composition notebook for use as a laboratory notebook, this will be graded **WEEKLY** once we start the RNA extraction experiment. Students are required to download and **PRINT** protocols, when necessary, each week prior to arriving in lab as well as filling in lab notebooks with all pertinent information (see lab notebook guidelines). **No electronic devices allowed in lab (THIS INCLUDES PHONES)** for viewing the lab manual/protocol (hard copy lab manual/protocol only) while experiments are being performed.

Computers will be allowed ONLY after experiments to work on lab assignments and receive help working with data.

Attire: Long pants and closed toed shoes are required for the lab, no skirts, short dresses, shorts, sandals or slippers will be allowed in the lab. You will be provided a lab coat for the duration of the class. **If you arrive dressed inappropriately you will be asked to acquire the proper clothing before being allowed to participate in lab.**

Academic Integrity

All assignments you submit must be *entirely* your own work. This means you and your lab partner are not allowed to submit copies of the same graphs and tables even if you collected the same data together. Also, you are not allowed to collaborate with another student when actually writing your papers, unless explicitly allowed by the instructor. You may not use any other student's paper as a template for your own. You may not fabricate data under any circumstances, and you may not use anyone else's data. Any sharing of data or papers is allowed only with prior approval of the instructor. Sources of information used in your paper must be cited appropriately. Everything you turn in must be stated in your own words even if you cite the source. *Changing just a few words or rearranging the words in a sentence from a book, journal article, or internet website is not saying it in your own words; that constitutes plagiarism. If you break any of these rules, you will automatically receive a grade of 0 for the entire assignment, will automatically earn a grade of "F" in the course, and will be subject to disciplinary action by your college provost.* If you allow another student to copy your work or you help another student write his/her paper, you will also be violating the University's academic integrity policy and will be subject to disciplinary action, which may include a grade of "F" in the course. These penalties will apply even if you say you did not understand the rules, so *be sure to ask Dr. Shanks if you have any questions about this policy.*

To assist in enforcing this policy, and to check that you aren't using papers from other students (or yours from previous quarters), from other sources on the web, or from any other sources, you will be submitting your reports through the plagiarism checker **Turnitin.com**.

Turnitin.com: Your final lab report will be submitted electronically to turnitin.com, a web site that screens papers for plagiarism. Turnitin is used not only to check for plagiarism, but also to check that you are citing your references properly. This is a tool used by me when grading. **You will not receive credit for a paper unless it has been submitted to turnitin.com. Any paper not uploaded to turnitin.com by MIDNIGHT the day it is due will incur a 1% late penalty per day until it is uploaded.** Instructions for submitting papers will be provided.

Also, please note: you will not receive credit for ideas that are **quoted directly from any source**, even if you place the passage in quotation marks and cite the source. While this is not committing plagiarism and is not a violation of academic integrity rules, direct quotes are used only when a passage is highly unique or profound, or there is no other possible way to say the same thing. In general, if you are not able to state a concept in your own words, you don't understand it, and therefore cannot receive credit for that passage (though, in this case, no *disciplinary* action will apply, so long as the quote is cited correctly.)

Students with Disabilities

Students who need accommodations should contact the instructor **IMMEDIATELY** and provide written documentation from the DRC by the end of **WEEK ONE**. Additional information about support and accommodations for students with disabilities and learning differences may be obtained from the UCSC Disability Resource Center (DRC)
DRC contact: 146 Hahn, 459-2089 (voice), 459-4806 (TTY), drc.ucsc.edu

Flow chart of Cry1 experiments:



Schedule:

Week	Day Date	Topics	Lab	Resources
Investigation of CytochromeB Gene Insertion				
1	Mon 6/25	Introduction, Lab Notebook Guidelines, Written Assignments and general class info <i>Plate E. coli</i> dilutions Pre lab #1 and Solutions practice quiz available on Canvas	Check-in Equipment, orientation and lab etiquette Dilutions of bacteria culture O.D. of bacteria culture Plating of dilutions of bacteria	Plasmid DNA manual and Promega Wizard® Mini-Prep Manual (PDF) sections 3.A pg. 4, 4.B pgs. 8-9 We will use commercial protocols, all documents necessary to do pre-labs can be found in this column.
	Tue 6/26	Plasmid DNAs Restriction Endonuclease Digestion of purified DNA Assignment #1 is available on Canvas Pre-lab #1 due (printed copy)	Count colonies Plasmid DNA isolation – Miniprep Quantitation of plasmid DNA by Spectrophotometric analysis Restriction Digestions of Plasmid with EcoRI-HF (NEB)	The URL for EcoRI is below: https://www.neb.com/products/r3101-ecori-hf#Product%20Information Quantitation: “Plasmid DNA” manual pgs. 16-20
	Wed 6/27	Gel Electrophoresis Polymerase chain reaction (PCR)	Agarose gel preparation and run of plasmid prep and plasmid digestions Plasmid DNA PCR with Taq DNA Polymerase (NEB) using M13 and CytB primers	Agarose Gels: “Plasmid DNA” manual pages 11-14 PCR Protocol for Taq DNA Polymerase
	Thu 6/28	Overview of gene expression experiment Assignment #1 due (printed copy) Pre-lab #2 is available on Canvas Solutions quiz in class (45 min)	Agarose gel electrophoresis of pDrive PCR	Agarose Gels: “Plasmid DNA” manual pages 11-14. Quick-Load® 100 bp DNA Ladder (NEB) image provided on Canvas https://www.neb.com/products/n0467-quick-load-100-bp-dna-ladder#Product%20Information “Qiagen RNeasy Kit” manual: pages 51-53, 65-67 and for Formaldehyde Agarose gel pages 65-67
Determining Cry1 Gene Expression				
2	Mon 7/2	RNA extraction/purification Journal club groups chosen and article assignment available on Canvas Pre-lab #2 is due (printed copy) Pre-lab #3 is available on Canvas	Isolation and Quantitation of total Plant RNAs (Photoperiod) RNA analysis: Denaturing gel	Reverse Transcription (RT) reactions in “Quanta Biosciences qScript cDNA synthesis kit” Q-PCR in “iQ SYBR Supermix” Manual pages 6-8
	Tues 7/3	Reverse Transcription and qPCR of Cry1 cDNA Pre-lab #3 is due (printed copy)	Reverse Transcription of isolated RNA into cDNA Set up qPCR of all cDNA templates (Cry1 primers and EF1 α internal control primer pairs)	
	Wed 7/4	HOLIDAY!!!		
	Thu 7/5	Strategies for Cloning into Plasmids Assignment #2 is available on Canvas Pre-lab #4 is available on Canvas	Agarose gel analysis of PCR reactions with different annealing temperatures Large-scale PCR with Cry1 and EF1 α primer pairs	
No lab work Monday and Tuesday, we will meet in Biomed 200 @ 11am				
3	Mon 7/9	Writing Workshop		Biomed 200 Starts @ 11am
	Tues 7/10	Journal Club		Biomed 200 Starts @ 11am Beel et. al., 2012
Cry1 Cloning and Sequence Analysis				
3	Wed 7/11	Molecular cloning (ligation) Pre-lab #4 is due (printed copy)	Preparation of large scale Cry1 and EF1 α PCR products for cloning (QIAquick kit) Ligation of Cry1 and EF1 α PCR products into pDrive cloning vector	QiaQuick Spin Handbook: pgs. 11-16, 19-20 Qiagen pDrive PCR cloning: pgs. 6-9, 12-13
	Thu 7/12	Bacterial competent cells	Competent <i>E. coli</i> cell preparation	Chemical Competent Cells preparation guidelines provided on Canvas
4	Mon 7/16	Transformation Paper draft due (printed copy)	Transformation of <i>E. coli</i>	
	Tues 7/17	Colony screen	Colony PCR with M13 primers	

		Streak plating	Streak select sequencing candidate colonies	
	Wed 7/18	Agarose gel photo analysis Bacterial overnight cultures	Agarose gel analysis of colony PCRs Grow Bacterial O/N cultures	
	Thu 7/19	Restriction digest Automated Sequencing Plan to be in lab late, we <u>MUST</u> send DNA to sequencing	Plasmid DNA isolation – Miniprep Plasmid DNA analysis and Quantitation Restriction digestion and Spectrophotometry Agarose gel analysis of digests Prepare clones for sequencing	Promega Wizard® Mini-Prep Manual (PDF) sections 3.A pg. 4, 4.B pgs. 8-9 DNA + Quantitation: “Plasmid DNA” manual pgs. 16-20
5	Mon 7/23	Computer analysis of sequences		Instructions provided on Canvas
	Tues 7/24	No wet lab, will meet to work on papers/presentations		Bring your computers to lab
	Wed 7/25	Lab meeting style presentations		Room TBD Starts @ 10am Guidelines will be given
	Thu 7/26	Research paper due at 2pm Drop off to be determined (printed copy and uploaded to Turnitin.com)		Guidelines will be given