

BIBC 103: Biochemical Techniques

Fall Quarter, 2015

Instructor: Aaron Coleman, Ph.D.
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Office Hours: Mondays 2:30 – 3:45 PM

Lecture: Center Hall 214; MWF 9 – 9:50 AM

Labs: First floor Bonner Hall; WF 10 – 2 PM or 3 – 7 PM

Course Objectives:

This course will introduce some of the experimental methods used in biochemistry and molecular biology, with an emphasis on those techniques used to study proteins. You will gain a conceptual understanding of, and some hands-on experience in, various protein purification techniques, expression and purification of recombinant proteins from bacterial cells, and methods for analyzing the different properties of proteins. The laboratory work will consist of three multi-day projects, as well as some smaller, single-day experiments. As this is an introductory lab course, all lab work will emphasize the learning of basic lab skills and good lab practices.

More importantly, this course is designed to give an appreciation of what science is and how it works. Science is not just a bunch of random facts...it is a process! It is easier to understand biology, or any field, when you understand why we know what we know about it. Understanding how information in biology is brought to light is just as important as the information itself. Through the laboratory projects we will develop the skills necessary to interpret data from experiments in order to answer questions about biological systems, and to design experiments to answer new questions. In keeping with this, the importance of good experimental design, including the use of appropriate controls, will be highlighted in all experiments. A complete list of the learning goals and expected outcomes for the course can be found on Ted.

Materials Required:

- 1) Biochemical Techniques Lab Manual, 2015/2016 Edition (available from the Bookstore)
- 2) Bound laboratory notebook
- 3) Safety glasses
- 4) Lab coat
- 5) Flash drive for recording data

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

Exams (100 pts. and 250 pts.)	350 points
Lab quizzes (4 at 35 pts. each)	140 points
LDH purification table analysis	75 points
Lab report on FGF signaling	260 points
Lab Notebook (five 20 pt. notebook checks plus 45 pts. for FP project check)	145 points
Lab practical and bioinformatics	30 points
Total	1000 points

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

910-1000	A	790-799	C+
900-909	A-	705-789	C
890-899	B+	695-704	C-
810-889	B	600-694	D
800-809	B-	0-589	F

Course Web Site:

Except for the lab manual, all course materials will be accessed through the course webpage on Ted (<https://ted.ucsd.edu>). Much of the data you generate in your experiments will be accessed through Ted, in addition to lab report guidelines and practice problem sets for quizzes and exams. Be sure to check Ted frequently for announcements and updates on assignments.

Lab Notebooks:

You will keep a formal laboratory notebook for all of 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. You will use the following format for your notebook, and will include the items listed below:

- a. The experiment date in the upper left-hand corner of each page. Make all entries in chronological order.
- b. Project title following the date (*e.g.*, LDH Purification and Analysis). Be sure to separate the three projects in your notebook.
- c. Experiment title underneath the project title. This should be one or two sentences describing the procedures performed.
- d. Briefly list any changes to the procedures from the lab manual. Other than that you do not need to write out procedures.
- e. Raw data and important observations. Enter numerical values in an organized table form. For large numbers of numerical values collected electronically, you may paste a printout of the Excel spreadsheet into the notebook. Also include any important observations (be brief). Look for prompts in the lab manual for what to include.
- f. 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 the graphing program if you are having trouble.
- g. All electrophoresis gel and Western blot images should clearly labeled with text, printed, and pasted into your notebook.
- h. Include a brief statement explaining anything that went wrong with that experiment—what would you do differently if you had to do the experiment again.

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 TA will perform unannounced lab notebook checks throughout the quarter.

Lab Quizzes and Exams:

The purpose of the lab quizzes is to be sure you are mastering the basic concepts behind your experiments as we go through the class. This includes understanding 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. The quiz dates are given in the lab schedule. Quizzes will be given at the beginning of lab, will take 15 – 30 minutes, and will consist of 5 to 7 questions. The quizzes are often scheduled on days where we introduce new lab projects in lecture, and will emphasize that information, including some information not covered in the lab manual. The topics that will be covered on each quiz will be posted in an announcement on Ted on the Monday prior to the quiz.

The two exams are cumulative and will be problem solving-based. They may include some basic questions on the concepts we have covered, but will emphasize taking the information you have learned and extrapolating to solve problems you have not seen before. Practice questions will be given on Ted to help you prepare for the exams.

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 TA, 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 the Lab Report:

The lab reports is 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 TA, 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. Ten points will be deducted for each working day that the lab reports are 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 report will be graded by your TA, based on the same lab report guidelines that you. Note that I work closely with all the TAs 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 TA first to get clarification on why points were deducted. If you still disagree with the grading you may submit the report to me for a re-grade. This must be done within one week of receiving the graded report. I will re-grade the entire report and give you a new score, and this is the score that will be recorded. Note that it is possible your score may go down. This policy is not intended to scare students off from submitting re-grades; if you believe your report was not fairly graded based on the guidelines posted on Ted then I encourage you to submit it for a regrade.

	Dates	Experiments, Activities, and Due Dates	Lab Manual Chapter
Wk 0	Sept. 25	Organization/safety; Introduction to micropipettes and pipetting exercises	Lab 1
Wk 1	Sept. 30	Making a pH buffer; Quantitative measurements	Lab 2 and pages 1 – 10
	Oct. 2	LDH 1: Initial purification of LDH from crude homogenate: centrifugation, ammonium sulfate precipitations; prepare size exclusion column	Lab 3
Wk 2	Oct. 7	LDH 2: Affinity chromatography	Lab 4
	Oct. 9	LDH 3: Size exclusion chromatography Quiz 1	Lab 5
Wk 3	Oct. 14	LDH 4: Activity assays; Bradford protein assays	Lab 6
	Oct. 16	LDH 5: Native gel electrophoresis of LDH with activity stain	Lab 7
Wk 4	Oct. 19	Exam 1 during Monday lecture	
	Oct. 21	LDH 6: SDS-PAGE of LDH purification fractions; FGF 1: Fibroblast Growth Factor (FGF) Signaling—Interpret NIH 3T3 data in lab manual	Lab 8 Lab 9B parts A and B only
	Oct. 23	Examine SDS-PAGE gels; FGF 2: Develop hypotheses to explain data in lab manual and design experiments to test Quiz 2	Lab 9B parts C and D
Wk 5	Oct. 26	LDH purification table analysis due at Monday lecture	
	Oct. 28	FGF 3: Prepare Samples for Western blot and ELISA	Lab 9B part E
	Oct. 30	FGF 4: Erk Western blots—SDS PAGE and electroblotting	Lab 10
Wk 6	Nov. 4	FGF 5: Erk Western blots—Immunodetection Quiz 3	Lab 11
	Nov. 6	FGF 6: ELISA for phospholipase C activity	Lab 12
Wk 7	Nov. 11	Veterans Day Holiday—no lab	
	Nov. 13	Sterile technique; Work up ELISA data; Bioinformatics tutorial	Lab 14F Lab 19
Wk 8	Nov. 18	Fluorescent proteins (FP): Make competent cells and transform with plasmid; Set up lysozyme crystallization FGF Lab Report Due	Lab 15, Lab 13 A – B Lab 20
	Nov. 20	FP: Purification and analysis of fluorescent proteins	Lab 16
Wk 9	Nov. 25	FP: SDS-PAGE of fluorescent proteins Quiz 4	Lab 17
	Nov. 27	Thanksgiving Holiday—no lab	
Wk 10	Dec. 2	FP: Examine SDS-PAGE gels; Examine lysozyme crystals	Lab 18 Lab 20
	Dec. 4	Exam 2 in lab Lab notebook check on FP project—bring lab notebooks!	