BIBC 103: Biochemical Techniques

Fall Quarter, 2018

Instructor: Aaron Coleman, Ph.D.
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Office Hours: York Hall 3080A (my office) and York 2300 (conference room); we will move to the conference room to accommodate larger numbers.
Mondays 3:30 – 4:30 PM

Lecture: Peterson 104; Mon/Weds/Fri 2 – 2:50 PM
Labs: First floor Bonner Hall; Weds/Fri 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. 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 ask 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 TritonEd.

Materials Required:
1) Biochemical Techniques Lab Manual, 2018/2019 Edition (available from the Bookstore)
2) Bound laboratory notebook
3) Safety glasses
4) Lab coat

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 in NIH 3T3 cells	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 TritonEd (https://tritoned.ucsd.edu/webapps/login/). Much of the data you generate in your experiments will be accessed through TritonEd, in addition to lab report guidelines and practice problem sets for quizzes and exams. Be sure to check TritonEd 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. See page *ix – x* in the lab manual for how to format your notebook and what information it should contain. Pay particular attention to the following:

a. Write the **experiment date** in the upper left-hand corner of **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.
b. **Project title** following the date on **each page** (*e.g.*, LDH Purification and Analysis). Be sure to separate the three projects in your notebook.
c. **Experiment title** underneath the project title on **each page**. This should be a single sentence indicating the specific procedure that was 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. 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). 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 graphing in Excel 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 of the conclusions from the experiment. This may be a single sentence to simply verify that you successfully concluded that 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?

i. 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.

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 TritonEd 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 TritonEd 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 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.**

Turning in the Lab Report:
Lab reports are due at the beginning of lab (or lecture) 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 report must also be submitted to Turnitin.com, which is accessed through TritonEd. The report must be submitted to Turnitin 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 report is 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 reference database solely for the purpose of detecting plagiarism of such papers. Use of the Turnitin service is subject to the terms of use agreement posted on the Turnitin site.

Lab Report Grading and Regrade Policy: Your lab report will be graded by your IA, based on the lab report guidelines. 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 lab report, discuss this with your IA 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.

Schedule

	Dates	Experiment/Activity	Lab Manual Chapter
Wk 0	Sept. 28	Organization/safety; **I**ntroduction to micropipettes and pipetting exercises	Lab 1
Wk 1	Oct. 3	Making a pH buffer; Quantitative measurements	Lab 2 and pp. 1 – 10
	Oct. 5	LDH 1: Initial purification of LDH from crude homogenate: centrifugation, ammonium sulfate precipitations; prepare size exclusion column	Lab 3
Wk 2	Oct. 10	LDH 2: Affinity chromatography	Lab 4
	Oct. 12	LDH 3: Size exclusion chromatography **Quiz 1**	Lab 5
Wk 3	Oct. 17	LDH 4: Activity assays; Bradford protein assays	Lab 6
	Oct. 19	LDH 5: SDS-PAGE of LDH purification fractions	Lab 8
Wk 4	Oct. 24	LDH 6: Examine SDS-PAGE gels; Native gel electrophoresis of LDH with activity stain	Lab 7
	Oct. 26	FGF 1: FGF Signaling: Develop hypotheses to explain data in lab manual and design experiments to test **Quiz 2**	Lab 9B
Wk 5	Oct. 29	Exam 1 during Monday lecture	
	Oct. 31	FGF 2: Prepare Samples for Western blot and ELISA **LDH Purification table analysis due at beginning of lab**	Lab 9B
	Nov. 2	FGF 3: MAPK Western blot—SDS PAGE and electroblotting	Lab 10
Wk 6	Nov. 7	FGF 4: MAPK Western blot—Immunodetection	Lab 11
	Nov. 9	FGF 5 ELISA for phospholipase C activity; Bioinformatics 1	Lab 12 Lab 19 (part A only)
Wk 7	Nov. 14	Work up ELISA data; FP 1: Fluorescent proteins (FP): Make competent cells and transform with plasmid **Quiz 3**	Lab 13 pp. 163-171 only Lab 15
	Nov. 16	FP 2: Purification and analysis of fluorescent proteins	Lab 16
Wk 8	Nov. 21	Bioinformatics 2; Set up lysozyme crystallization	Lab 19 (parts C – E) Lab 20
		Lab report on FGF signaling in NIH 3T3 cells due at beginning of lab	
	Nov. 23	Thanksgiving Holiday—no lab	
Wk 9	Nov. 28	FP 3: SDS-PAGE of fluorescent proteins	Lab 17
	Nov. 30	FP 4: Examine SDS-PAGE gels **Quiz 4**	Lab 18
Wk 10	Dec. 5	Examine lysozyme crystals	Lab 20
	Dec. 7	Exam 2 in lab; last lab notebook check on FP project	