CHE 385: Biochemistry Laboratory

Spring 2012: Thursdays 2:00-5:00. Hoyt 369

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Course overview:
Biochemistry laboratory is a course that is designed to train students in modern techniques of biochemistry. Students will gain experience working with DNA and protein molecules through investigation of *E. coli* alkaline phosphatase. Protein expression, purification and characterization will be addressed through this semester long biochemistry project. Biochemistry theory will be addressed in readings and in pre-lab lectures. Students will also write several formal lab reports as well as read research articles.

Assessable Objectives:

1. Students will exemplify knowledge in the areas of molecular biology, protein purification and enzyme characterization through three formal laboratory reports.
2. Students will master biochemistry concepts as shown through a final exam.
3. Through reading and pre-lab questions, students will come to lab prepared for their hands-on experiments.

Texts:

Lab Manual: Kevin M. Weeks and Lisa Benkowski. *Molecular Biology and Biochemistry of E. coli Alkaline Phosphatase*. Printed at the Westminster College book store.

Text: Rodney Boyer. *Biochemistry Laboratory: Modern Theory and Techniques*. 2nd edition. Prentice Hall. ISBN: 978-0-13-604302-7

Lab Notebook: You will be required to keep a contemporaneous lab notebook throughout the course of the semester. Your notebook should be bound with numbered pages, and should contain a carbon copy sheet. All data, calculations, and drawings should be entered directly into your notebook.

Expectations:
You will be responsible for reading and preparing for each lab before we meet. Preparation includes answering the prelab questions that precede each lab and outlining the experiments for the given lab period. You will turn in this work at the *beginning* of each laboratory period. Readings will be assigned out of the Boyer text to go along with each week of lab. The assigned readings will help you answer prelab questions and speed up your work in the lab. Read the assigned sections BEFORE coming to lab.

Grading System:

Grade Scale	Distribution of assignments	
A 90-100%	Pre-lab questions and reading assignments	20%
B+ 87-89%, B 83-86%, B- 80-82%	Lab report on DNA work	20%
C+ 77-79%, C 73-76%, C- 70-72%	Lab report on protein purification	20%
D+ 67-69%, D 63-66%, D- 60-62%	Lab report on protein characterization	20%
F < 60%	Final exam	20%

Attendance:
Attendance is mandatory. Due to the strict scheduling of the course and the progressive, building, nature of the techniques learned, you must be in lab at your assigned time. Please be punctual. Lab starts at 2:00 pm, so be there and be ready to participate. **Each unexcused absence will result in reduction of grade by 10%.**

Student Evaluation and Important Dates:

Pre-lab and reading assignments: This includes turning in your pre-lab questions at the **BEGINNING** of lab. It may also include pop quizzes on the pre-lab reading material.

Lab research reports: More details about the research reports will be forthcoming.

1. **DNA experiments**. This lab report will be based on the molecular biology experiments in section I and II of the lab manual. It is due by **5 pm on Monday, February 19th.**

2. **Protein purification lab report.** This lab report will be based on the protein purification up through running an SDS-PAGE gel for analysis. It is due by **5pm on Thursday, April 12th**.

3. **Protein characterization lab report.** This lab report will include your total protein assay and activity assays for your alkaline phosphatase protein. It is due by **5pm on Monday, May 7th.**

Final Exam: The final exam will include material from all of the readings, laboratory experiments, pre-lab questions and lab lectures. The final exam period is **Thursday May 10, 2012 from 8:00-10:30 am.**

Lab Reports:
Lab reports will be discussed in much more detail as the course progresses. Just a reminder, these written reports need to be your own individual work. You may consult with other students when collecting data and during the proofreading process, but when it is all said and done, the final product must come strictly from your brain. You may cite outside sources in your reports, but make sure to properly reference them. Additionally, I encourage you to take some time and visit either the Chemistry library or online journals and read some primary literature (*Biochemistry* or *The Journal of Molecular Biology* are good choices). The benefits are two-fold, not only will you get a feel for how research papers are written, but you will learn your way around the current literature which will expose you to how the techniques you are currently learning are actually used. Boyer, pages 5-9 provides useful information about the set-up of your laboratory reports.

Lateness penalty: There is a 10% lateness penalty per day late.

Safety:
During the course of this laboratory experience we will be working with potentially pathogenic organisms (*E. coli*), hazardous chemicals, and a variety of other hazards (fire, glassware, high voltage power supplies, etc.). Therefore, not only is it reasonable and prudent, but it is required by the chemistry department and college health and safety office that safety glasses be worn at all times. Please adhere strictly to the following:

- wear safety glasses at all times
- wear gloves when handling microorganisms and chemicals
- no open-toed shoes
- long pants are recommended
- familiarize yourself with the fire exits in the room
- note the location of the fire extinguisher
- if you don't know what a chemical is…ask before using it.
- no food or drink in lab

Tentative Schedule of Labs: Read lab manual and Boyer *PRIOR* to lab.

Date	Experiment	Lab Manual Pages	Reading in Boyer
Jan 19 Week 1	**Experiment 0: Pipetting and Sterile Technique** (Basic techniques you need to master.)	1-8	Ch1 1-22
Jan 26 Week 2	**Experiment Ia: Purification of Plasmid DNA** (A quick method to obtain many copies of DNA plasmid.)	10, Promega manual	Ch 9 267-278
Feb 2 Week 3	**Experiment IIa: PCR of Alkaline Phosphatase** (Cloning a native gene out of bacteria and verifying the plasmid)	23-25	Ch 10
Feb 9 Week 4	**Experiment IIa: PCR Clean-up and DNA Visualization** (Did your PCR reaction work?)	25-26, 17-19 Promega manual	Ch 6: 165-167, 177-180,191
Feb16 Week 5	**Experiment IIIb: Lysis of Large Bacterial Culture**[2] (Break open the bacteria to find your protein.)	52-55	Ch 11:307-320
Feb 23 Week 6	**Planning your purification and making buffers**		
March 1 Week 7	**Protein purification week 1**		
March 15 Week 8	**Protein purification week 2**		
March 22 Week 9	**Protein purification week 3**		
March 29 Week 10	**Experiment IVb: Visualization of Protein by SDS-PAGE** (Let's see the quality of protein throughout purification.)	100-109	Ch 6: 167-176, 188-190
April 12 Week 11	**Experiment IIIf (A-B): Analysis of Fractions for Total Protein** (Which fractions contain the most protein?)	78-82	Ch 3: B
April 19 Week 12	**Experiment IIIc: Activity Assay for Alkaline Phosphatase** (Make sure the assay works with wt AP before we use our AP.)	56-60	Ch 8: B
April 26 Week 13	**Experiment IIIf (C): Assay for AP Activity** (Which fractions contain active protein?)	82-86	Ch 8: B
May 3 Week 14	**Experiment IVc: Determination of K_m and V_{max} for AP** (Characterization of your enzyme activity through a kinetic assay.)	110-114	Ch 8: B

[1]During this lab, we will transform E. coli Origami-BL21 with the pET-Aphos plasmid *instead* of the NovaBlue cells with the experimental plasmid.
[2] Experiment IIIa will be performed by your lab TA or prof. You must still read about the procedure on pages 47-51.
[3]In addition, read M.M. Bradford, *Analytical Biochemistry* 72, 248-254 (1976).
Note: We will skip the following experiments: Ib, IIb, IId, IIe, IVa.