BIOSci 1830: BIOCHEMISTRY LABORATORY

Lecture and Lab: Tuesday and Thursday, 12:00 - 4:50 PM.
A146-A148 Langley Hall

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UTAs: Alexandra LaMonaca
Office Hours are available by appointment.
Raven Brower
Office Hours are available by appointment.

PREREQUISITE
The one prerequisite for this class is BioSci 1810. You should have earned a C or better in BioSci 1810. The following courses passed with a C or better also provide essential background: General Chemistry I & II, Organic Chemistry I & II, and Calculus I & II.

INTRODUCTION & COURSE OBJECTIVES
This course is an advanced introduction to the experimental practice of biochemistry. We make proteins and then fiddle with them to learn what they do and how they work! It’s an upper-level laboratory course designed to challenge you with high level undergraduate – low level graduate student experiments and concepts. To succeed in this course, you’ll draw upon your experience from other laboratory science courses and use concepts from lecture courses. This course involves substantial effort; you will earn three credits, so you should expect to invest about 4-6 hours of work outside of class, in addition to the ten hours of laboratory and lecture each week. Work hard, keep your brain switched on, and maintain a sense of humor along the way. You’ll be able to use this knowledge and the logic you learn to build a solid foundation for your future career, whether science or non-science related. Importantly, this course will teach you problem solving and to be more self-reliant in the lab or any setting. Instead of asking others how to do something, you’ll learn to think for yourself, dissect a problem and calmly apply logic to solve it. You’ll move from an information consumer to a information producer, from a dependent to a provider.

ATTENDANCE
Attendance is mandatory for all lab and lecture sessions. All absences must be excused directly by the instructors prior to the class meeting. Email is the best method. All absences must be justified, and we
reserve the right to require documentation. When students are unable to attend, we will try to make the lab material and experiments available at alternative times. Missing more than 25% of the labs is grounds for failure.

**Techniques Used**

<table>
<thead>
<tr>
<th>Laboratory Safety*</th>
<th>Plasmids as cloning &amp; expression vectors</th>
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<tbody>
<tr>
<td>Accurate &amp; Precise Pipetting*</td>
<td>Protein Over-Production &amp; Purification</td>
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<tr>
<td>Using Biochemical Buffers for pH control*</td>
<td>Affinity Chromatography</td>
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<tr>
<td>Aseptic Technique*</td>
<td>Ion Exchange Chromatography</td>
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<tr>
<td>Centrifugation (Ultracentrifuge*, Sorvall &amp; Microcentrifuge)</td>
<td>Conductance</td>
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<tr>
<td>Protein Quantitation (Bradford Assay)</td>
<td>Enzyme Assays</td>
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<tr>
<td>Spectrophotometry</td>
<td>Enzyme Kinetics</td>
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<tr>
<td>Bacterial Growth &amp; Quantitation</td>
<td>Protein Complex Characterization</td>
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<td>Plasmid Strain Growth &amp; DNA Extraction</td>
<td>SDS-PAGE</td>
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<tr>
<td>Agarose Gel Preparation</td>
<td>Native Protein Gels (Agarose &amp; PAGE)</td>
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<tr>
<td>DNA Electrophoresis</td>
<td>Gel Analysis &amp; Photography</td>
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<tr>
<td>Restriction Endonuclease Digests &amp; Mapping*</td>
<td>Gel Band Quantitation</td>
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[= Techniques briefly reviewed]

**Course Requirements**

**Format and Time Expectations:** The course will consist of a two, 5 hour sessions on Tuesday and Thursday from noon to 5 PM. This time will begin with a 15-60 minute lecture concerning an overview of the experiments to be done and the physical and chemical laws impacting these experiments. You should expect to spend time outside of class preparing for the lab; READING PROTOCOLS BEFORE COMING TO THE LAB, doing homework, reading background material, preparing presentations, and studying for quizzes. There will be homework assignments and one presentation (one poster and one PowerPoint), and a notebook requirement.

**Text:** There is no textbook for the course. We’ll draw material for this course from primary research papers, reviews, product literature, and third party research protocols. If you want background information for general laboratory practices we have the following text (also available through Amazon.com): L. A. Seidman & C. J. Moore, *Basic Laboratory Methods for Biotechnology, Textbook & Lab Reference* (2000) Prentice-Hall.

**Computers:** You’ll need to use computers to look up web-based material to perform your lab work (available in the lab or the HHMI Computing Labs). You’ll also need to use data management programs, (Excel, etc), and use imaging programs to create professional quality figures (PowerPoint, Photoshop, Illustrator, etc).

**Communication:** Distribution of Information: Almost all course material will be distributed to you via CourseWeb. We will only communicate to you through your pitt.edu email account. We will never respond to other email addresses. This requires that you maintain a functional “pitt.edu” account. If you send us attached documents you MUST follow the 4 underlined rules below;

1) **File Name**
   a) You must include your last name or first and last initials, followed by the assignment name. This is a valuable habit for now and your future because it is; a) courteous and b) insurance that your document is properly credited to you.
   b) Never make a file name too long, as some email programs do crop names or choke on them.
   c) Never include unusual characters, (especially “/” and “&”) such as the following: "!, @, #, $, %, ^, &, *, (,),++, ?, <, >, "". Transfers from Mac to Windows do not tolerate these characters.
d) In the document you must place your name in the document (not just in the document name, or header).

2) When emailing a document;
   a) We strongly recommend that you copy yourself when sending a document. This way you can check if it was sent, and you can check that the document was indeed attached and openable after its electronic journey.
   b) Consider pasting the text from the Word document in the "body" of the email, as well as attaching it. This makes the process almost bomb proof.

Work Load: We will assign homework, have one or two quizzes, a poster, and a 20 minute presentation. You also will be graded on the quality of your lab notebook. As stated before, you should expect to spend as much time outside of class preparing for the lab, doing homework, reading background material, preparing presentations, and studying for exams.

_________________________________________________________________________________

Notebook: Your notebook will represent a large portion of your grade because it is such an essential tool in a lab. Experiments are commonly repeated. If one records the essential information in their notebook than the notebook allows an experiment to be reproducible. Lab notebooks must be “real-time” documents of your actions and observations in the lab, like an airplane “black box”. Notes must be written in permanent ink. Notes must not be copied over late in the semester. Your lab notebook must have as much of your primary data as you can gather during your experiment. The form – bound or loose-leaf – of your lab notebook is less important to us than being well-organized. Follow this outline form of essential features of your lab notebook:

1) Write in real-time or within hours after completing your experiment, while your thoughts and memories remain clear. Do not copy lab notebooks by hand.
2) Save as much primary data as possible, attaching it by staples or tape if necessary.
3) Notebooks should be conceptually and/or historically ordered.
4) Every page should have a date, a concise title, and a page number.
5) Reserve the first two or three pages for your Table of Contents for the notebook.
6) Organize each experiment with the headings: a) Goal, b) Procedure, c) Results, and d) Conclusions (or some reasonable equivalent).
7) Affix and label data appropriately. For gel photographs and spectrophotometer printouts, label them clearly and affix them in your notebook where they make sense. Unlabeled gel lanes with no loading guide are lost data. Unattached data are doomed. Data affixed many pages away from the relevant experimental description are difficult to find and very hard to be sure of their value. Your printouts have dates, so this should help.
8) Produce good quality gel photographs and other outputs. Recognize that clear data is respected data. Photos that are too dark, poorly aligned, or too small are less valuable than large, properly framed, correctly exposed photos.
9) Include your actions, your observations, your rationale, your thoughts, and your interpretations in the record. Do not include printed protocols that we gave you, although you may choose wisely to outline these protocols in your notebook.

Grading Policy

Lab experimental techniques and performance, lab data notebook, homework, quizzes, and a final exam will all be components of the final grade. Within the laboratory, we expect you to:

1) Be prepared in advance by reading relevant protocols or background materials, as suggested,
2) Show effort and improvement in your lab techniques so you improve your skills of experimental analysis, experimental design and experimental interpretation,
3) Demonstrate safe and responsible laboratory practices,
4) Work well with others, most importantly, with your lab partner, and show proper laboratory etiquette,
5) Be prepared to interact verbally and respond to questions during lecture and lab.

The total points possible in this class are distributed as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Lab Attendance, Performance, Homework (4)</td>
<td>40%</td>
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<tr>
<td>Quiz (1)</td>
<td></td>
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<tr>
<td>Presentations (2: 1 Poster and 1 Powerpoint)</td>
<td>30%</td>
</tr>
<tr>
<td>Lab Notebook</td>
<td>30%</td>
</tr>
</tbody>
</table>

**Policy on the Export or Import of Lab Materials**

We do not permit any student to take any teaching lab samples, reagents or other materials to their research lab or back from their research lab to the Langley Teaching Lab. This policy is directed to biological reagents and samples, and it does not include electronic or paper documents. When students take experiments back to another lab space, or carry supplies from their lab to the teaching labs, we cannot control the experiment or personal safety, which it is our responsibility to do.

**Disabilities Resource Services**

If you have a disability for which you are or may be requesting an accommodation, you are encouraged to contact both your instructor and Disability Resources and Services, 216 William Pitt Union, [412-648-7890/412-383-7355(TTY)] as early as possible in the term. DRS will verify your disability and determine reasonable accommodations for this course.

**Academic Integrity Policy**

Cheating/plagiarism will not be tolerated. Students suspected of violating the University of Pittsburgh Policy on Academic Integrity, noted below (**), will be required to participate in the outlined procedural process as initiated by the instructor. A minimum sanction of a zero score for a quiz, exam, or paper will be imposed.

** The integrity of the academic process requires fair and impartial evaluation on the part of faculty and honest academic conduct on the part of students. To this end, students are expected to conduct themselves at a high level of responsibility in the fulfillment of the course of their study. It is the corresponding responsibility of faculty to make clear to students those standards by which students will be evaluated, and the resources permissible for use by students during the course of their study and evaluation. The educational process is perceived as a joint faculty-student enterprise which will perforce involve professional judgment by faculty and may involve—without penalty—reasoned exception by students to the data or views offered by faculty. Senate Committee on Tenure and Academic Freedom, February 1974

**Office Hours**

For the Instructor, office hours will be set or by appointment. The Teaching Assistant will have office hours. All relevant contact information (Office location, phone number, and email addresses) will be provided to the class in the front end of the syllabus.

**References Drawn From - Available in Office**


**Biosci 1830 Schedule for Spring of 2015**

<table>
<thead>
<tr>
<th>Week of (Tues-Thurs)</th>
<th>Experiments / Concepts</th>
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| Jan 6-8              | **Introduction Lecture:** Syllabus, Lab Safety, Orientation, Review of Basic Lab Skill Sets, Protein Production & Purification, Spectrophotometry.  
**Project I:** Beta-galactosidase: Protein Expression and Purification – Day 1 |
| Jan 13-15            | **Project I:** Beta-galactosidase: Protein Expression and Purification – Day 2  
**Project I:** Beta-galactosidase: Protein Quantitation & Characterization by Bradford Assays & SDS-PAGE |
| Jan 20-22            | **Project I:** Beta-galactosidase: Miller (Beta-galactosidase Activity) Assays, plotting column profiles & Native Agarose Gels  
**Project I:** Beta-galactosidase: Kinetics – Day 1 |
| Jan 27-29            | **Project I:** Beta-galactosidase: Kinetics – Day 2  
**Project I:** Beta-galactosidase: Kinetics – Day 3 |
| Feb 3-5              | **Project I:** Beta-galactosidase: Kinetics – Day 4  
**Project I:** Beta-galactosidase: Kinetics – Day 5 |
| Feb 10-12            | **Project II:** Production & Purification of Wildtype and mutant HK97 Bacteriophage Prohead II: Differential Centrifugation |
| Feb 17-19            | **Project II:** Quantitation and characterization of HK97 Proheads: Bradford assays, Native Agarose, SDS-PAGE |
| Feb 24-26            | **Project II:** Manipulation of protein complexes: Glucose Dissociation & Native Acrylamide Gels. |
| Mar 3-5              | **Project II:** Manipulation of protein complexes: NaSCN Dissociation: Is Prohead II constituted of hexamers, pentamers, or both. |
| **Mar 10-12**        | **SPRING BREAK (March 3-11)** |
| Mar 17-19            | **Project III:** Production and Purification of Wildtype and Mutant Prohead I Complexes |
| Mar 24-26            | **Project III:** Screening for new Delta domain localization mutations |
| Mar 31-April 2       | **Project III:** Screening for new Delta domain localization mutations |
| April 7-9            | **Project III:** Screening for new Delta domain localization mutations |
| April 14-16          | Pot Luck and Lab Presentations. Lab Clean-up. |
| April 21-23          | FINALS WEEK (no Final) |
Langley Teaching Lab Safety

The 3 Main Goals are for you to....

1. ...Learn substantial concepts and techniques,
2. ...Keep safe from any injury,
3. ...Do all this while not breaking OUR stuff.

General Rules of the Laboratory

1. Know where the Room Exits, Eye Wash Stations, Shower, and First Aid Kits are located know how to use them. (Lab Layout drawing is the last page of this document.)
2. Hazardous Materials (acids, bases, toxins, etc.): Do not use them unless you have been instructed of their proper use - know where they are - after use, put them away where they came from.
3. In the event of fire or certain Hazardous Material spills, your instructor(s) will direct you to evacuate the lab – check and make sure your lab mate makes it out. Exit through Stair Well 1D (to the right) - if egress is blocked, exit to the Loading Dock.
   a. Glass Waste Bins: glass only, not gloves, plastic, tissues, etc.
   b. Biohazard Waste Bins : experimental plates only....
   c. Ethidium Waste Bin: Gels only, not gloves and paper towels
5. Gloves: always wear gloves in the lab in the lab, except when using computer keyboards.
6. Wear appropriate clothing: Lab coats are available.
7. Never use equipment without proper instruction: particularly centrifuges.
8. Goggles/Eyeglasses are recommended and available. 
   a. Contact wearers must wear eye protection when handling hazardous materials.
9. No food or drink in the labs.
10. No cell phones use in the lab (includes text).
11. Be a conscientious citizen – inform lab personnel of concerns or potential hazards, such as spills of unknown origin & do your part to keep the lab clean & organized.
12. Wash Hands Always: whenever you leave the lab.

Three Good Reasons for Proper Instrument Use & Care

1) You get BETTER DATA if you use our equipment properly, and this usually means a BETTER GRADE.
2) Proper use and care for our equipment IS SAFER.
3) Our instruments are expensive so proper reduces costs and lower lab fees.

Centrifuge Safety

There are two general types of centrifuges in the Langley Teaching Labs:

- **Table Top centrifuges (the only ones your class uses):**
  - for quick spins of 2 ml or less which do not exceed 20,000 rpm
  - 12 Eppendorf 5418, one Hermle LabNet
  - potentially dangerous when tubes with hazardous substances break

- **Sorvall & Allegra Centrifuges:**
  - referred to as “Floor model” centrifuges that look like washing machines
  - handle large volume samples (greater than 2 ml to 250 ml)
  - exceed 20,000 rpm
  - potentially dangerous & very expensive - usage requires training, (see below)
Table Top Centrifuges: What you must do:

- **Balance Tubes:** When loading tubes into *any* centrifuge rotor, the mass *must* be distributed *evenly*. Unbalanced rotors become stressed, as does the spindle (think axle). Over time, this stress leads to failure of the metal rotor or spindle, which is potentially dangerous and costly. To balance correctly, see the strategies to balance an eight-position rotor diagramed below.

- **Keep it Clean:** If a tube leaks or breaks, or if any solution wets the rotor or the inside, notify lab personal. If you know the liquid spilled is not dangerous, clean it up *and* inform lab personnel. Solutions in rotors cause micro-etching of the metal, from which stress fractures occur leading to failure of the metal rotor.

Sorvalls Centrifuges: What you must do:

- **You must be trained in use the Sorvalls.** Do not use a Sorvall until you have been trained to do so.

- **You must fasten the lid of the rotor completely.** There is no wiggle room for this. Not fastening a rotor lid destroys the rotor.

- **Carefully balance your centrifuge bottles with their lids** on the “trip balance”, for which you will be trained.

- **Never overfill a centrifuge bottle.** At speed the liquid will assume a vertical position that will leak out of overfilled containers. A 500 ml bottle can only safely hold 250 ml.

- **Always stay with the Sorvall as it goes to speed.** If there is a problem such as an imbalance, they can be detected early and the machine *MUST* be shut off immediately to minimize the danger and the damage.

- **Keep it Clean:** If a tube leaks or breaks, or if any solution wets the rotor or the inside, attempt to clean it or notify lab personal. If you know the liquid spilled is not dangerous, attempt to clean it up *and* inform lab personnel

**The two most common causes of centrifuge malfunctions are:**

1) **Operator Error (100% preventable)**
   a. Failure to place the lid on the rotor. (Sorvall only)
   b. Improper balancing of centrifuge tubes.
   c. Failure to properly secure the rotor to the drive.
   d. Overloading the rotor’s maximum mass.

2) **Metal Fatigue of the rotor. (partially preventable)**

   ➢ **IF A CENTRIFUGE MALFUNCTIONS**
   ➢ **Turn off the centrifuge IMMEDIATELY & do open!**
   ➢ **Tell your instructor or lab staff.**
Lab Layout Schematic:
Note the location of Exits and of Safety Equipment (EyeWash Stations, Emergency Showers, First Aid Kits). Other essential equipment is listed.