

Bio 332, Spring 2018

Advanced Molecular Biology Laboratory

Instructor: Dr. Michael C. Yu, Email = mcyu@buffalo.edu

Office hours: By appointment.

TA: Ansuman Sahoo (Mon/Wed), Guolei Zhao (Tues/Thurs)

Undergraduate TA: Shiva Pal (Tu/Th), Andrew Boze (M/W); Instructional Support: Eileen Sylves

Section I: Tue (1-4pm), Thurs (1-4pm), and Fri (1-2pm)

Section II: Mon (1-4pm), Wed (1-4pm), and Fri (1-2pm).

Class Location

Laboratory session: 231 Hochstetter

Recitation session: 231 Hochstetter (NOT at the location indicated on the Hub)

Course Description

This advanced laboratory course is designed to give upper-level undergraduates (mostly seniors and in some rare instances, juniors) an opportunity to learn modern-day techniques routinely used in molecular biology, using state-of-the-art reagents and equipment. To develop a sense of independence in carrying out research as you would in an academic or industry setting, you will work as individuals, rather than a team with a lab partner. Techniques covered will include: human DNA fingerprinting, agarose gel electrophoresis, polymerase chain reaction (PCR), epitope tagging and expression of a recombinant protein, immunoblotting, co-immunoprecipitation, tandem-affinity purification, silver staining, isolation and analysis of RNA, and synthesis of cDNA from RNA templates. The majority of experiments will be carried out using the budding yeast *Saccharomyces cerevisiae* as model organism. A detailed, meticulous notebook keeping (see the last section on how to do this) is a must for this course.

Prerequisites

Since Bio 332 is a fast-paced laboratory course that requires a strong sense of independence, it is highly recommended that perspective students have already received a solid understanding of molecular biology principles and exposure of basic laboratory techniques. Bio 302 (Introduction to Molecular Biology) and Bio 329 (Genetics Laboratory) are two courses that would fulfill this requirement. In some special circumstances, you may petition a request to the instructor for the approval to take this course.

Recitation

The recitation session provides a great opportunity for students to engage in the Q & A session on the materials taught throughout the week. It also serves as a source for review sessions for exams and opportunities to go over any additional concepts, as well as a preview for the following week's experiments. You should be prepared to ask questions before you come to the recitation – many exam questions are often derived from the asked questions and discussed concepts during the recitation.

Lab Reports

Lab reports should contain the following sections: a short paragraph on the objective of the lab, a concise, abbreviated version of the procedure performed, the results section (attach any original data, such as gel doc printouts, etc here), and the discussion section (where you discuss the outcome of your data and how it relates to the hypotheses of the laboratory exercise). Lab reports are always due at least ONE week after the completion of the lab – by that week's recitation on Friday (1pm of the day). All labs must be submitted printed on paper during the recitation section when they are due; all labs **MUST** also be submitted electronically through the UBLearn's plagiarism detection software. Lab reports that have not been submitted to UBLearn's will not be graded. Any late reports will have points deducted, based on TA's discretion. There will be **four lab reports** total for the course: Human DNA fingerprinting, Yeast Molecular Biology, Biochemical Purification using Tandem-Affinity Tagged Approach, and Preparation of Yeast RNA and Synthesis of cDNA.

Course grade breakdown:

30% = Exam I, 30% = Exam II, 30% = lab reports

10% = Discretionary points (lab citizenship, discussion participation, etc)

****Unexcused absences from laboratory session will result in a grade no better than a "C". You must email Dr. Yu in advance if you anticipate to be absent, unless it was an emergency.**

Week 1: Jan 29 – Feb 2 (NO RECITATION THIS WEEK):

1. Introduction, laboratory safety training, basic laboratory refresher
2. Making solutions (e.g. YEPD media for lab #2), make solutions using a pH meter

Week 2: Feb 5 – Feb 9

3. Human DNA Fingerprinting (Alu Insertion polymorphism), casting an agarose gel
4. Human DNA Fingerprinting (Alu Insertion polymorphism), agarose gel electrophoresis

Week 3: Feb 12 - Feb 16

5. Yeast molecular biology: epitope tagging of a protein (Primer design strategy & electrophoresis of PCR product)
6. Yeast molecular biology: epitope tagging of a protein- part 2. (yeast transformation)

Week 4: Feb 19 – 23 (NO RECITATION THIS WEEK):

7. Yeast molecular biology: colony PCR to check for proper tagging, cast SDS-PAGE
8. No lab – reserved for overflow.

Week 5: Feb 26 – Mar 2

9. Yeast molecular biology: co-immunoprecipitation (Part 1) (lysate & coIP)
10. Yeast molecular biology: co-immunoprecipitation (Part 2) (SDS-PAGE & Transfer)

Week 6: Mar 5 – Mar 9

11. Yeast molecular biology: co-immunoprecipitation (Part 3) (Western blotting)
12. Yeast molecular biology: co-immunoprecipitation (Part 4) (Western blotting)

Week 7: Mar 12 - Mar 16

13. **Practical Exam I (Mar 13 for M/W and Mar 14 for Tu/Th)**
14. Preparation of Yeast RNA and synthesis of cDNA (Part 1) (RNA extraction)

UB Spring Break – Mar 19th to Mar 23th

Week 8: Mar 26 – Mar 30

15. Preparation of Yeast RNA and synthesis of cDNA (Part 2) (Reverse transcription)
16. Preparation of Yeast RNA and synthesis of cDNA (Part 3)

Week 9: Apr 2 - Apr 6

17. Preparation of Yeast RNA and synthesis of cDNA (Part 4) (Gel electrophoresis)
18. Preparation of Yeast RNA and synthesis of cDNA (Part 5) (qPCR/qPCR data analysis)

Week 10: Apr 9 – Apr 13

19. Reserved for lab overflow.
20. Biochemical purification using tandem-affinity tagged approach (Part 1) (cell harvest)

Week 11: Apr 16 – Apr 20

21. Biochemical purification using tandem-affinity tagged approach (Part 2) (lysate)
22. Biochemical purification using tandem-affinity tagged approach (Part 3) (IP/wash/cleave)

Week 12: Apr 23 – Apr 27

23. Biochemical purification using tandem-affinity tagged approach (Part 4) (2nd step/elu)
24. Biochemical purification using tandem-affinity tagged approach (Part 5) (SDS-PAGE/transfer)

Week 13: Apr 30 – May 4 (NO RECITATION THIS WEEK)

25. Biochemical purification using tandem-affinity tagged approach (Part 6) (western & silver stain)

26: Practical Exam II (May 2 for M/W and May 3 for Tu/Th)

No FINAL exam

How to Develop a Laboratory Notebook

One of the most important duty when working in a lab is keeping a detailed lab notebook. Since science is built upon the premise that results are reproducible, we must document detailed information so others can reproduce our work if they read our notes. For this class, you should use a 3-ring binder. Your notebook is the only source of information for all that you have done during the lab sessions. It should be an accurate account of exactly what you did, why you did it, when you did it (also the time it takes), what the results were, and what these results mean relative to the scientific question asked. Here are a few simple rules to keep in mind while developing your notebook.

1) Notebooks do not have to be "neat" but they should be legible (i.e. write neat enough for others to be able to read it). Record the date at the top of each page. Do not recopy your notes. NEVER!! By recopying your notes, you may filter out some information that seem insignificant at the time, but may be very valuable later. You could "re-organize" your thoughts and write these re-organized thoughts down, but it should not replace the original data recording/observations you made.

Also, a notebook is not designed to be a duplication of the lab manual. Just cite the lab manual pages, and then record any deviations from the published protocol.

Divide your notebook into 3 sections: a) copies of protocols you use; b) your notes and hard copies of your data; c) papers and reprints.

2) You may use either pen or pencil, but if you use pencil, make sure it is dark enough to be read easily.

3) Record all measurements and calculations you perform – including the equations used.

4) You should be able to understand and explain why you are performing a particular procedure. What is the purpose of the procedure/experiment and what is the outcome you are expecting to see? Is there more than one possible outcome for the procedure, for example?

5) Record the data in hard copy format and backup any electronic data. If the data is a dried-down gel (such as coomassie blue or silver stained), then tape the entire gel onto your notebook, label ALL the lanes as to which samples were loaded onto the gel, and date the gel appropriately. The date should reflect the day in which the experiment was performed. Leave a blank space in your notebook for the pending data.

6) It is very important to include hard copies of your data followed by interpretations of the results. For example, what are the meanings for all these bands observed in your gel relative to the overall purpose of the experiment? Refer to the hard copy of your data and write detailed explanations of what your data meant to you and what you would need to do next if they were unclear/did not support your anticipated results.

7) At the end of each laboratory session, you should make a note in your notebook if you needed to repeat an experiment, or if you were ready for the next step as described in your lab manual. In other words, your stopping point of the day.

8) Record any observation that you think might be significant. If you deviated your procedure from the stated protocol, you should write down EXACTLY what that deviation was. E.g. "3 ml was used instead", or "10 mins incubation was done instead of 30 mins".

9) It is often a good idea to record the time when you started or stopped within a procedure. This will allow you to gauge your pace/speed in performing a certain procedure and make any modification to your preparations if needed.

Learning Objectives for Bio 332: in all assessments, 50% mastery equals objectives achieved.

1. Provide breadth of knowledge of basic principles and concepts, specifically, and be able to present experimental results in appropriate graphical formats :
 - a) be able to present experimental results in appropriate graphical formats -- this will be assessed in lab practical exams 1 and 2, using multiple-choice questions and in the practical section. Additionally, the results section in lab report #1 serves as a mean to determine whether a student understood how present experimental results in appropriate graphical formats
 - b) Explain how experimental results relate to hypotheses -- this will be assessed each of the lab reports.
2. Provide depth within specialized areas, specifically:
 - c) learn to perform common molecular biological techniques and manipulations - this will be assessed in lab practical exams 1 and 2, using multiple-choice questions and in the practical section. Additionally, the procedure section in each of the lab reports serves as a mean to determine whether a student understood how to perform these experimental techniques.
 - d) Relate results of experiments to hypotheses - this will be assessed in all lab reports, under the results and the discussion section.
 - e) Understand the reasoning for using particular methods – this will be assessed by the lab reports, under the discussion section.
 - f) Assess whether a particular method will address a particular question – this will be assessed by the lab reports under the discussion section, as well as both exams using multiple-choice questions and short-answer questions.
 - g) Troubleshoot experiments that given unexpected outcomes – this will be assessed in all the lab reports under the discussion section, as well as during the practical section of both exams.
 - h) Participate in a series of experiments similar to an actual research project – this will be assessed in all of the procedural section of the lab reports and actual attendance of the students in each lab section.
 - i) Understand laboratory safety – this will be assessed in exam 1, using 2-3 multiple-choice type questions.
3. Provide an understanding of experimental design and methodology, specifically:
 - j) Learn to write clear lab reports that critically evaluate experimental data - this will be assessed in discussion section in each of the lab reports.
 - k) Demonstrate the ability to summarize experimental data and explain how the data address experimental hypotheses – this will be assessed in the discussion section in each of the lab reports.
4. Develop approaches for integration of information, specifically:

- l) Learn to troubleshoot experiments - this will be assessed and evaluated by the instructor during the lab session, as well as in the discussion section of their lab reports.
 - m) Assess whether a particular method of address a particular question – this will be assessed in the discussion section in each of the lab reports.
 - n) Engage independently and collaboratively in the scientific process – this will be assessed and evaluated by the instructor during the lab session.
 - o) Critically evaluate data to determine its relationship to experimental hypotheses: this will be assessed and evaluated by the discussion section in each of the lab reports.
5. Encourage critical thinking and hypothesis building specifically:
- p) Communicate results of scientific investigations - this will be assessed by short-answer questions that simulate an experimental situation/results, as well as by the interactions between the instructor and students during the laboratory section.
 - q) Retrieve information from databases – this will be assessed by lab report #1
6. Provide skills in scientific communication, specifically:
- r) Communicate results of scientific investigations - this will be assessed in discussion section in each of the lab reports.
7. Encourage appreciation of scientific values, specifically:
- s) Demonstrate advanced knowledge in experimental techniques in molecular biology - this will be assessed by the observations of the instructor during the laboratory sessions, as well as in the practical section of both exams.

**The depth of learning objectives are all 2's, except for points "a", "o", and "q" where they are rated 1.