# CHM 4300L – Laboratory in Biochemistry and Molecular Biology

#### **Instructor**

Alix Rexford

Zoom ID: 277-621-8481

Office: LEI 302

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# **Learning Assistants:**

Bella Chen Faythe Corr Ryan Mulcare

#### Dr. Rexford's Office hours:

Monday 12:00-1:00 PM Zoom only Tuesday 9:00-10:00 AM HyFlex

Or by appointment

See Canvas course page for Zoom link

# **Laboratory Manual** (available at Target Copy)

Characterization of TEM1 β-Lactamase and Discovery of Antibacterial Metabolites from Streptomyces

# **Class Meetings**

Pre-laboratory lecture: Tuesday 10:40 – 11:30 AM LIT 201

Laboratory: Meet in LEI 200

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Section	Day & time	Teaching Assistant
BL05 (TuPM)	Tuesday @ 9:35 - 12:35 PM	Peter Yang
BL01 (WedAM)	Wednesday @ 9:35 - 12:35 PM	Chen-Yu Li
BL02 (WedPM)	Wednesday @ 12:50 - 3:50 PM	Nupur Sehgal
BL03 (ThAM)	Thursday @ 9:35 - 12:35 PM	Priya Priya
BL04 (ThPM)	Thursday @ 12:50 - 3:50 PM	Sreyashi Das

# **Course Description**

This course provides a practical, hands-on understanding of modern, fundamental techniques relevant to molecular biology and biochemistry. The laboratory covers topics including DNA cloning and manipulation, protein overexpression and purification, and enzyme kinetic measurements as well as the discovery of enzyme inhibitors and antibiotics from natural sources.

Additionally, this course provides instruction and feedback to help students improve their scientific writing. At the conclusion of this course students will have composed a journal-quality manuscript regarding their experimental findings in the laboratory.

#### **Laboratory Attire**

Students should wear safety glasses, gloves, and closed toe shoes with hair pulled back at all times while in the laboratory. Shorts, loose clothing, or jewelry are prohibited. Failure to follow safety precautions and proper use of PPE will result in your removal from the laboratory.

Course materials will be provided to you with an excused absence, and you will be given a reasonable amount of time to make up work. Find more information in the university attendance policies.

#### **Honor Code**

expect each of you to follow the Student Honor Code, available on the web (https://sccr.dso.ufl.edu/policies/student-honor-code-student-conduct-code/) You are expected to:

- a. uphold the highest standards of academic integrity in the student's own work,
- b. refuse to tolerate violations of academic integrity in the University community
- c. foster a high sense of integrity and social responsibility on the part of the University community.

Violations of the Honor Code will be reported to the Dean of Students, and may result in failure of the assignment in question and/or the course.

## **Laboratory Participation**

Attendance is required for all lab sessions. **Please be on time!** Your performance grade depends on coming to lab on time, with proper safety attire, and having *read the experiment thoroughly in advanced*. All students will begin the semester with 50 laboratory participation points. Five (5) points will be deducted any time a student fails to clean up their station at the end of each laboratory. Five (5) points will be deducted for each tardy of ten minutes or more. Points may also be deducted for gross negligence in the lab or major errors in following protocols, at the discretion of the instructor.

There will be 1-4 short, pre-recorded lectures followed by questions to check your understanding which **MUST** be completed prior to attending lab for your own safety, as well as to ensure that everyone understands proper procedure. Proper preparation will allow you to work quickly to complete the lab in a timely manner.

Due to the continuity of the labs in the course, missed labs cannot be made up. If you miss a lab due to **an approved absence with appropriate documentation**, accommodations will be made. Missing a lab without an approved absence will result in a 10 point deduction from your course grade for the first absence, 40 point deduction for the second absence, and 100 point deduction for the third absence. Regardless of whether or not the absence is excused, you are still responsible for completing pre-lab quizzes and lab notebooks (using lab partner's data) by applicable deadlines.

## **Lecture Quizzes & Participation**

Attendance is required for all lecture meetings on Tuesdays, 10:40 - 11:30 am. During the **lecture period** we will review concepts from the previous week and discuss theoretical concepts and results, as well as potential problems that may arise in the following week. After a short class discussion, students will work together on editing their manuscripts, complete problem sets, and/or data analyses. Your active participation in these small group sessions will determine your lecture participation score.

All students will begin the semester with 50 lecture participation points. Three (3) points will be deducted for each tardy of five minutes or more. Five (5) points will be deducted for each unexcused absence after the first unexcused absence. Points may also be deducted for lack of participation and discussion with your group, at the discretion of the instructor.

#### **Assignments**

OneNote will be utilized for E- lab notebooks, which will be graded for accuracy and completeness. Set up your notebook by navigating to the Class Notebook tab and opening the OneNote notebook. Each lab will have a dedicated page with an included grading rubric. You MUST use these pages to submit all lab notebooks. The lab notebook grading rubric provides an overview of how to complete each page and upload a document to OneNote, if desired. Do not copy and paste any text or figures into OneNote.

Select **data analyses** will be turned in via Canvas and graded on a PASS/FAIL basis. You will have at least one (1) chance to correct your data analyses before grades are final, however, you MUST turn in completed data analyses on the initial due date. Failure to attempt ALL parts of the assignment will result in a score of zero (0).

To make the most of our class time, you will have 10-30 minutes of pre-lab videos to watch prior to lab, followed by a short quiz to check your understanding. During the lecture we will review concepts from the previous week and discuss issues and results, as well as potential problems that may arise in the following week. After a short class discussion, students will work together on editing their manuscripts.

Throughout the semester you will be preparing a **publication-quality manuscript** encompassing the semester's project. During lecture I will introduce a single section of a research paper and assign journal articles to each student. The following week, you will present the journal article to your lab partners and discuss components of that section of the paper. You will also prepare a rough draft of each section of your paper and submit it to Canvas by the due date indicated in the table below.

Manuscript Section	Labs to be	Rough Draft Due	Peer Reviews Due	Final Draft Due
	included	Date	Date	Date
Introduction	N/A	September 6	September 9	September 16
Methods	Labs 1-5	September 23	September 30	October 7
Results	Labs 1-8	October 11	October 14	October 21
Discussion	Labs 1-10	October 25	November 4	November 12
Final Poster	All Labs			December 2

Labs listed in the table above are REQUIRED to be included in your *final draft of the specified section*, although the most recent lab(s) may be excluded in the rough draft as you won't have completed it prior to the rough draft due date.

After submitting your rough draft on Canvas, you will be randomly assigned two (2) anonymous **peer reviews**. Instructors do not grade rough drafts. You must complete the peer reviews using the supplied rubric AND make a comment on the overall paper no later than the Monday following the rough draft due date to earn credit on rough drafts. A copy of your lab notebook will earn zero (0) points regardless of completion of peer reviews.

After completing peer reviews and discussion with your lab partners, you should edit your paper section according to your class discussions and turn in a **final draft** by the due date listed above. These will be graded according to the provided rubric. Turning in an identical copy of the rough draft will result in zero (0) points on the final draft.

You are expected to make the recommended alterations to each report section on future submissions. Each lab group will collaborate on a final poster which contains highlights from each report section and is **due December 2, 2024**. A participation survey will be utilized to determine effort put into the final group project and scores will be determined accordingly.

Tentative lecture schedule:

Date	Discussion Topics	Assignment	Due Date
August 27	Project overview  Introduction Section PowerPoint	Syllabus Quiz  Lab Overview Quiz	September 30 @ 11:59 pm
September 3	Jigsaw: Introduction section	Lab Overview Quiz	N/A
September 10		Problem Set: PCR primers  Data Analysis: PCR product quantitation	Pre-lab 3 September 13 @ 11:59 pm
September 17	Method Section PowerPoint	Problem Set: Restriction digest 1	N/A
September 23	Jigsaw: Methods section		N/A
October 1		Problem Set: Restriction digest 2	October 4 @ 11:59 pm
October 8	Results Section PowerPoint	Problem Set: Protein expression	Pre-lab 7
October 15		Data Analysis: Expression Conditions	October 18 @ 11:59 pm
October 22	Discussion Section PowerPoint	Data Analysis: Linear regions	October 28 @ 7:00 pm
October 29		Data Analysis: TEM1 Purification	November 29 @ 12:00 pm* *BL05, all other sections due @ 7:00 pm
November 5		Data Analysis: Kinetics of TEM1	Novembre 8 @ 11:59 pm
November 12	Poster Presentations		December 2 @ 11:59 pm
November 19	Optional: Poster Presentations		

# **Late Assignments**

Laboratory notebooks will be kept on OneNote and locked the no earlier than the Saturday one (1) week following the lab. Since it is not "turned in" it cannot be turned in late. Please keep up with your notebook weekly.

Pre-lab quizzes must be completed prior to the start of lab for your safety. There are no makeups for pre-lab quizzes.

Report section rough drafts are peer reviewed only. Instructors will not grade rough drafts. Full credit is earned on rough drafts by *turning it in on time* AND *completing peer reviews on time*. Rough drafts must be written in full sentences and follow the guidelines set forth in the powerpoint presentations and lab report grading rubric to earn ANY credit. A copy of your lab notebook will earn zero (0) points regardless of

completion of peer reviews. Failure to complete two (2) peer review grading rubrics prior to the lecture after rough drafts are due will result in zero (0) points for the rough draft. Late submissions will be accepted with the deductions described below, however, it is the student's responsibility to notify the instructor that the rough draft was late in order to be assigned peer reviews.

Late submissions for report sections and the final manuscript are accepted with the following grade penalties:

10 % deduction: < 24 hours after due date

25 % deduction: 24 – 48 hours after due date

50 % deduction: 48 – 72 hours after due date

Not accepted after 72 hours late

# **Grading**

Assignment	<b>Points</b>
Laboratory notebooks	200
Pre-lab quizzes	100 total
Lab performance	50
Lecture quizzes & participation	50
Lab report rough drafts	50 total
Lab report final drafts	100 total
Final manuscript	50

Note that extra credit is built into the course assignments

The following letter grades will be assigned based upon total points accrued for all course work:

**A:** > 540 pts

**B:** 539.9 – 480 pts **C:** 479.9 – 420 pts **D:** 419.9 – 360 pts

E: below 360 pts

## **Regrades and Grading Errors**

Mistakes happen and grading errors can be especially frustrating. I will do my best to make sure that you are always kept up to date with your performance in the course and post your grades in a timely manner. It is your responsibility to make sure that your grade on Canvas reflects the scores you receive on assignments and bring it to my attention ASAP when you notice a discrepancy. If you feel that a grading error was made on a particular assignment, you have *one week from the date the grade was posted to Canvas* to request a regrade. Regrades will include a rescore of the entire assignment and may result in a lower score. Scores that have been posted to Canvas for a minimum of one (1) week are final.

## **Student Responsibilities**

You are expected to come to class on time and behave in a manner that is respectful to the instructor and to fellow students. Opinions held by other students should be respected in discussion, and conversations that do not contribute to the discussion should be held at a minimum.

<sup>\*</sup>You almost never make up the points lost from turning it in late. I don't recommend doing it!

#### **Privacy Statement**

Our class sessions will be audio visually recorded for students in the class to refer back. Students who participate with their camera engaged or utilize a profile image are agreeing to have their video or image recorded. If you are unwilling to consent to have your profile or video image recorded, be sure to keep your camera off and do not use a profile image. Likewise, students who un-mute during class and participate orally are agreeing to have their voices recorded. If you are not willing to consent to have your voice recorded during class, you will need to keep your mute button activated and communicate exclusively using the "chat" feature, which allows students to type questions and comments live. The chat will not be recorded or shared. As in all courses, unauthorized recording and unauthorized sharing of recorded materials is prohibited. Recordings will not normally be made during breakout sessions, however those that are made are for the sole use of the instructor and will not be made available to students or others.

#### **Students with Disabilities**

Students requesting classroom accommodation must first register with the Dean of Students Office. The Dean of Students Office will provide documentation to the student who must then provide this documentation to the Instructor when requesting accommodation. Contact the Disability Resources Center (http://www.dso.ufl.edu/drc/) for information about available resources for students with disabilities.

#### **Course Evaluations**

Students are expected to provide professional and respectful feedback on the quality of instruction in this course by completing course evaluations online via GatorEvals. Guidance on how to give feedback in a professional and respectful manner is available at gatorevals.aa.ufl.edu/students/. Students will be notified when the evaluation period opens and can complete evaluations through the email they receive from GatorEvals, in their Canvas course menu under GatorEvals, or via ufl.bluera.com/ufl/. Summaries of course evaluation results are available to students at gatorevals.aa.ufl.edu/public-results/

#### U Matter, We Care

Your well-being is important to the University of Florida. The U Matter, We Care initiative is committed to creating a culture of care on our online campus by encouraging members of our community to look out for one another and to reach out for help if a member of our community is in need. If you or a friend is in distress, please contact <a href="mailto:umatter@ufl.edu">umatter@ufl.edu</a> so that the U Matter, We Care Team can reach out to the student in distress. A nighttime and weekend crisis counselor is available by phone at 352-392-1575. The U Matter, We Care Team can help connect students to the many other helping resources available including, but not limited to, Victim Advocates, Housing staff, and the Counseling and Wellness Center. Please remember that asking for help is a sign of strength. In case of emergency, call 911.

## **Tentative Laboratory Schedule (Dates subject to change)**

Date	Lab	DNA Molecular Biology/Protein Biochemistry	Streptomyces Microbiology
August 27 - 29	1	Polymerase Chain Reaction	Plate Soil Samples
September 3 - 5	2	DNA Agarose Gel, PCR Cleanup & Restriction Digest	Select Streptomyces

September 10 - 12	3	DNA Agarose Gel & Cleanup, DNA Quantitation	Re-streak Streptomyces
September 17 - 19	4	Ligate DNA; Transform TOP10 Cells with Ligation Reactions	Prepare <i>Streptomyces</i> plate for Antibacterial Test
September 24 - 26	5	Miniprep Plasmid DNA; Restriction Digestion	Spot Bacterial Test Strains for Antibacterial Test
October 1 - 3	6	Restriction Digest Analysis; Transform Bl21(dE3) Cells with Recombinant Plasmid	Start Streptomyces Liquid Culture
<b>October 8 -</b> 10	7	Pilot-Scale TEM1 Expression Trials	Isolate Resin from Liquid Culture
October 15 - 17	8	TEM-1 Purification from BL21(dE3) cells	Extract Metabolites from Resin
<b>October 2</b> 2 - 24	9	Purification tests	
<b>October 29 -</b> 31	10	Inhibition assays	Kinetics w/TEM-1 & Antibacterial test
November 5 - 7	Virtual	Exploring TEM1 structure with PyMol	N/A