

CHM 4300L – Laboratory in Biochemistry and Molecular Biology

Instructor

Alix Rexford
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Office Hours

Monday 10:00 – 11:30 am
Tuesday 11:00 – 12:00 pm
Friday 10:30 – 11:30 am
Or by appointment

Laboratory Manual

Characterization of TEM1 β -Lactamase and Discovery of Inhibitors from Streptomyces
Available at Target Copy Center

Class Meetings

Pre-laboratory lecture: Tuesday 9:35 – 10:25 AM; FLI 109
Laboratory: Section 11008 Wednesday 9:35 – 12:35 PM; Leigh Hall 200
Section 11007 Wednesday 12:50 – 3:50 PM; Leigh Hall 200

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, basic bioinformatic analyses, protein overexpression and purification, along with enzyme kinetic measurements. Additionally, this course covers the discovery of enzyme inhibitors and antibiotics from natural sources.

Laboratory Attire

Safety glasses, closed toe shoes, hair pulled back. No shorts, loose clothing or jewelry.

Honor Code

I 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:

- uphold the highest standards of academic integrity in the student's own work,
- refuse to tolerate violations of academic integrity in the University community
- 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.

Class Attendance

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. 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.

Assignments

Notebooks will be graded throughout the semester for accuracy and completeness. **Laboratory notebooks do not leave the lab.** You will have one week to perform any necessary data analysis, including printing out pictures from the lab, before the notebook is graded. Everything should be recorded in your notebook

Laboratory Reports will be due ~2 weeks after the last experiment is performed.

Lab report 1 will cover labs 1 – 4 and 5 (parts 1 – 3)

Lab report 2 will cover labs 5 (part 4) and 6 – 9

Lab report 3 will cover labs 10 – 12

Guidelines for lab reports will be given 2 weeks before the due dates.

due Feb. 25

due March 31

due April 20

Quizzes will be given periodically during pre-lab lecture to cover basic principles and concepts related to the labs. There are **no makeup quizzes**. If you miss a quiz to due to **an approved absence with appropriate documentation**, accommodations will be made.

The experiments in this course are arranged in a series, and the product from one week serves as the starting material for the next. If you have problems, you will be provided with intermediate materials with no grade penalty; however, you will be expected to analyze critically where the problem(s) lay in your lab report, and this analysis will be grade.

Grading

Laboratory notebooks	200 pts
Lab Report 1 (due Feb. 25)	100 pts
Lab Report 2 (due March 31)	100 pts
Lab Report 3 (due April 20)	100 pts
Quizzes	50 pts
Lab Performance	50 pts

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

A: 600 – 540 pts

B: 539.9 – 480 pts

C: 479.9 – 420 pts

D: 419.9 – 360 pts

F: below 360 pts

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/

Laboratory Schedule

Date	Lab - Title
Jan. 8	1 – Isolation of <i>Streptomyces</i> strains from soil
Jan. 15	2 – PCR amplification of <i>tem1</i> Picking candidate <i>Streptomyces</i> colonies
Jan. 22	3 – Estimating the concentration of PCR products by gel electrophoresis PCR clean up, digestion of the expression vector and PCR product Restreaking of <i>Streptomyces</i> strain
Jan. 29	4 – Purification of DNA by preparative gel electrophoresis Purification of DNA from agarose gels DNA quantification of purified <i>tem1</i> DNA and pET28a vector DNA
Feb. 5	5 – Ligation of <i>tem1</i> DNA to pET28a vector DNA and transformation into TOP10 cells
Feb. 12	6 – Screening pET28a recombinants for <i>tem1</i> insertion Streaking of <i>Streptomyces</i> strains for antibiotic tests
Feb. 19	7 – Analysis of digested ligation products Performing antibiotic tests
Feb. 26	8 – Pilot expression of recombinant BL21(dE3) pET28a- <i>tem1</i> Extraction of <i>Streptomyces</i> cultures
Mar. 11	9 – SDS-PAGE analysis of the TEM1 pilot-scale induction experiments
Mar. 18	10 – Expression and purification of TEM1 from recombinant BL21(dE3) pET281- <i>tem1</i>
Mar. 25	11 – Analysis of TEM1 purification fractions by SDS-PAGE Constructing a purification table for TEM1 purification
Apr. 1	12 – Kinetic and inhibition assays for TEM1

