CHM4300L, Laboratory in Biochemistry and Molecular Biology
Spring 2024

Instructor: Prof. Rebecca Butcher, butcher@chem.ufl.edu
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Laboratory manual: *Characterization of TEM1 β-Lactamase and Discovery of Inhibitors from* Streptomyces (available in print from Target Copy).

Lecture: FLINT 0109, Tuesdays, 10:40-11:30am
Laboratory: Leigh 200, Wednesdays, 9:35am-12:35pm (Section BLW3) and 12:50-3:50pm (Section BLW6)
Office hours: SFH 302B, Fridays 3-3:50pm, and by appointment

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.

Lab Attire, Safety Precautions, and Illness:

- Students should wear goggles, gloves (provided by lab), and closed toe shoes with hair pulled back at all times while in the laboratory. No shorts, loose clothing, or jewelry are allowed.
- If you are feeling ill for any reason, I encourage you not to come to class. Please notify me in advance of class that you will not be able to attend. In the event of an excused absence, course materials will be provided to you (e.g., the TAs or your lab partner can process your samples, or you can be provided with previously acquired data), and you will be given a reasonable amount of time to make up work.

Course grading:	Laboratory notebooks	30%
	Laboratory reports (2)	45% (20% Report 1 and 25% Report 2)
	Virtual lab 11 assignment	5%
	Lab performance	10%
	Quizzes	10%

Course grades will be assigned on a curve with the following percentages used for guidance: A: 90-100%, A^-: 86-89%, B^+: 82-85, B: 78-81%, B^-: 74-77%, C^+: 70-73, C: 66-69%, C^-: 62-65%, D^+: 58-61%, D: 54-57% D^-: 51-53%, E≤50%. For information on UF's grading policy, see: https://catalog.ufl.edu/ugrad/current/regulations/info/grades.aspx.

Assignments:
Laboratory notebooks will be graded at three times during the semester for accuracy and completeness. Please maintain a physical notebook that you keep in lab and update during lab time. Guidelines for the notebooks will be posted on Canvas. A simple composition notebook with cardboard cover is recommended for easy recycling at the end of the semester.

- Notebook will be physically turned in and **labs 1-4** will be graded on **2/14**.
- Notebook will be physically turned in and **labs 5-7** will be graded on **3/6**.
- Notebook will be physically turned in and **labs 8-10** will be graded on **4/17**.

Virtual Lab 11 assignment is due on **4/5** @ **5pm** on Canvas.

Lab Reports 1 and 2 are due **3/1** & **4/17**, respectively, @ **5pm** on Canvas. Guidelines for the reports will be posted on Canvas.

- **Lab Report 1** will cover **labs 1-5** and **lab 6-part 1**.
- **Lab Report 2** will cover **lab 6-parts 2-5** and **labs 7-11**.

Lab Report 2 Calculations (completed by filling out two Excel files) are due with Lab Report 2 and must be turned in on **4/17** @ **5pm** on Canvas. I am happy to check over your calculations in the two Excel files in advance; however, you must e-mail them to me no later than **4/10** @ **5pm**, no exceptions.

~**10 pre-lab quizzes** will be given on Canvas. The quizzes **will be available after lecture and must be completed before lab**. Quizzes will cover basic principles and concepts covered in lecture, as well as procedures that will be carried out in the upcoming lab. There are no makeup quizzes. If you miss a quiz 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 graded.

Attendance and Lab performance: Attendance is required for all lab sessions. Please be on time! Your lab performance grade depends on you coming to lab on time with proper safety attire, having read the experiment thoroughly in advance, and having completed the pre-lab quiz. If you are prepared, you will be able to get to work quickly and to complete the lab efficiently. 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. However, you must notify me in advance if possible.

Schedule:

Date	Lab	Molecular Biology and Protein Biochemistry	Microbiology and Antibiotics
1/17	**1**	PCR Amplification of *tem1*	Isolation of *Streptomyces* Bacteria from Soil
1/24	**2**	Analysis and Purification of PCR Product; Digestion of the pET28a Vector DNA and *tem1* insert DNA for Ligation	Picking Candidate *Streptomyces* Colonies
1/31	**3**	Purification and Quantitation of Restriction Digested DNA	Streaking Pure Cultures of *Streptomyces spp.*
2/7	**4**	Ligation of the *tem1* Insert with the pET28a Vector DNA; Transformation of Ligation Products into TOP10 Cells	Starting Antibiotic Assay
2/14	**5**	Culturing Transformed *E. coli* TOP10 Cells; Isolating and Purifying Plasmids from Transformants	Completing Antibiotic Assay
2/21	**6**	Analysis of Digested Plasmids to Determine Ligation Results	Starting a *Streptomyces* Liquid Culture and Creating a *Streptomyces* Frozen Stock

2/28	**7**	Pilot Expression of Recombinant BL21(DE3); SDS-PAGE Analysis of TEM1 Pilot-Scale Induction	Collection of Resin from *Streptomyces* Culture for Metabolite Extraction
3/6	**8**	Expression and Purification of TEM1 from Recombinant BL21(dE3) pET28a-*tem1*	Extraction of Metabolites from Resin
3/20	**9**	Analysis of TEM1 Purification	
3/27	**10**	Inhibition Assays for TEM1	Testing of Extracts for TEM1 Inhibition and for Antibiotic Activity
4/3	**11**	Virtual Lab: Pymol	

Academic honesty: 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:

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.

Accommodations for 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 https://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 https://ufl.bluera.com/ufl/. Summaries of course evaluation results are available to students at https://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 umatter@ufl.edu 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.