

CHM4300L, Laboratory in Biochemistry and Molecular Biology

Spring 2021

Professor: Prof. Rebecca Butcher, butcher@chem.ufl.edu

Laboratory manual: *Characterization of TEM1 β -Lactamase and Discovery of Inhibitors from Streptomyces* (available on Canvas).

Pre-laboratory synchronous lecture: Zoom (see Canvas calendar for link), Thursdays, 9:35am – 10:25am

Laboratory: CCB 110, Fridays, 4:05-7:05pm (TAs: Nasser and Subhradeep “Deep”)

Office hours: Zoom (my personal meeting room: 376-200-3066), Wednesdays and Thursdays 3:3:50pm and by appointment. If you will be joining me for scheduled office hours, please e-mail me at least 5 min before the scheduled Zoom session so that I know you are coming and so that I know to monitor the Zoom session closely.

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 and COVID-19 Safety Precautions:

Students should wear goggles, gloves, and closed toe shoes with hair pulled back at all times while in the laboratory. No shorts, loose clothing, or jewelry are allowed. In response to COVID-19, the following policies and requirements are in place to maintain your learning environment and to enhance the safety of our in-classroom interactions.

- You must maintain your cleared for campus status in order to attend lab.
 - You are required to wear approved face coverings at all times during class and within buildings. Following and enforcing these policies and requirements are all of our responsibility. Failure to do so will lead to a report to the Office of Student Conduct and Conflict Resolution.
 - This course has been assigned laboratory rooms with enough capacity to maintain physical distancing (6 feet between individuals) requirements. Please utilize designated stations and maintain appropriate spacing between students. Do not move from the station you are assigned.
 - Sanitizing supplies are available at each station to wipe down your lab bench at the start and end of the lab. Hand soap will be provided at each sink and should be used prior to lab beginning and before exiting the laboratory.
 - Practice physical distancing to the extent possible when entering and exiting the classroom and when using common-use equipment. Sanitizing solutions will be available to clean common-use equipment before and after each use.
 - If you are experiencing COVID-19 symptoms, please use the UF Health screening system and follow the instructions on whether you are able to attend class. 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.
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| Course grading: | Laboratory notebooks | 40% |
| | Laboratory reports (2) | 40% |
| | 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 bring to each lab. Guidelines for the notebooks will be posted on Canvas. To “turn in” your notebook, you will take a photo of each page, and use these images to create a single .pdf.

- **Labs 1-4** will be turned in **Monday 2/15 @ 5pm** as a single .pdf on Canvas.
- **Labs 5-7** will be turned in **Monday 3/8 @ 5pm** as a single .pdf on Canvas.
- **Labs 8-10** will be turned in **Monday 4/12 @5pm** as a single .pdf on Canvas.

Lab Reports 1 and 2 are due **2/25 & 4/19**, respectively, **@5pm** on Canvas. Guidelines for the reports will be posted on Canvas.

- **Lab Report 1** will cover **labs 1-5 and lab 6-parts 1 & 4**.
- **Lab Report 2** will cover **lab 6-parts 2 & 3 and labs 7-10**.

~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.

Schedule:

| Date | Lab | Title |
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| 1/15 | 1 | PCR Amplification of the <i>tem1</i> Gene |
| 1/22 | 2 | Analysis and Purification of PCR Product; Digestion of the pET28a Vector DNA and <i>tem1</i> DNA for Ligation; Isolation of <i>Streptomyces spp.</i> from Soil |
| 1/29 | 3 | Purification and Quantitation of Restriction Digested DNA; Isolating Pure Cultures of <i>Streptomyces</i> Strain |
| 2/5 | 4 | Ligation of the <i>tem1</i> DNA with the pET28a Vector DNA and Transformation into <i>E. coli</i> TOP10 Cells; Re-streaking <i>Streptomyces spp.</i> |
| 2/12 | 5 | Isolating and Purifying Plasmids from TOP10 Transformants; Preparing an Antibacterial Test for the <i>Streptomyces</i> strain |
| 2/19 | 6 | Analysis of Digested Plasmids to Determine Ligation Results; Pilot Expression of Recombinant BL21(DE3) pET28a-tem1; Performing |

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| | | Antibacterial Tests |
| 2/26 | 7 | SDS-PAGE Analysis of the TEM1 Pilot-Scale Expression Experiment; Starting a <i>Streptomyces</i> Liquid Culture and Creating a <i>Streptomyces</i> Frozen Stock |
| 3/5 | 8 | Expression and Purification of TEM1 from Recombinant BL21(dE3) pET28a-tem1; Isolating Resin from <i>Streptomyces</i> Liquid Cultures |
| 3/12 (Class 28858) OR 3/19 (Class 28859) | 9 | Analysis of TEM1 Purification; Extraction of Metabolites from <i>Streptomyces</i> Culture Resin |
| 3/26 (Class 28858) OR 4/2 (Class 28859) | 10 | Kinetic and Inhibition Assays for TEM1 |

Student Responsibilities & Zoom Etiquette: 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.

- To add a profile picture: Go to ufl.zoom.us/profile
- To ask a question in Zoom: Click on the “Chat” button on the bottom of the screen and a chat window will appear
- To raise your hand in Zoom: Click on the “Participants” button on the bottom of the screen and a window will appear. On the lower right of the window there is an option to raise your hand.

Lecture zoom sessions are to be recorded: Our class sessions may be audio visually recorded for students in the class to refer back and for enrolled students who are unable to attend live. 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.

Laboratory zoom sessions are not recorded, but video presence is required: In order to manage the laboratory as well as maintain safety, it is important that your video is on during laboratory. However, please mute your audio to prevent feedback and communicate via “chat”.

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.

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.
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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.