CHM4300L, Laboratory in Biochemistry and Molecular Biology
Fall 2018

Instructor: Rebecca Butcher, butcher@chem.ufl.edu, office: Hernandez Hall 302B

Laboratory manual: *Characterization of TEM1 β-Lactamase and Discovery of Inhibitors from* Streptomyces (available at Target Copy Center).

Pre-laboratory lecture: Leigh Hall 142, Tuesdays, 10:40am – 11:30am
Laboratory: Leigh Hall 200, Wednesdays, 9:35am-12:35pm (Section 0816) and 12:50-3:50pm (Section 1465)
Office hours: Prof. Butcher: Tuesdays 3-3:50pm, Fridays 3-3:50pm and by appointment (please schedule by email in advance!).

Lab attire: Safety glasses, close toed shoes, hair pulled back. No loose clothing or jewelry.

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.

Course grading:	Laboratory notebooks	40%	
	Laboratory reports (2)	40%	(due: 10/16 & 12/04)
	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 (graded the weeks of 09/24, 10/22 & 11/19). Lab notebooks do not leave the lab. Lab Report 1 will cover labs 1-5, lab 6 (parts 1-3), and lab 7 (part 1), and Lab Report 2 will cover lab 6 (parts 4 and 5), lab 7 (parts 2 and 3), and labs 8-12. Lab Reports 1 and 2 are due 10/16 & 12/04, respectively. The guidelines for the reports will be given at least 2 weeks before the due date. At various times, announced quizzes (~4) will be given during pre-lab lecture to cover basic principles and concepts related to the labs. 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 safety glasses, with proper attire, and having read the experiment thoroughly in advance. 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.

Academic honesty: Any act of academic dishonesty will be reported to the Dean of Students, and may result in failure of the assignment in question and/or the course. For University of Florida's honor code, see http://www.dso.ufl.edu/sccr/honorcodes/honorcode.php.

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.

Schedule:

Date	Lab	Title
08-29	**1**	Isolation of *Streptomyces* Strains from Soil
09-05	**2**	PCR Amplification of *tem1*; Picking Candidate *Streptomyces* Colonies
09-12	**3**	Estimating the Concentration of the PCR Product by Gel Electrophoresis, PCR Clean-up, Digestion of the Expression Vector and PCR Product; Restreaking of *Streptomyces* strain
09-19	**4**	Purification of DNA by Preparative Gel Electrophoresis and Purification of DNA from Agarose Gels
09-26	**5**	DNA Quantification of Purified *tem1* DNA and pET28a Vector DNA, Ligation of *tem1* DNA to pET28a Vector DNA and Transformation into TOP10 Cells
10-03	**6**	Screening pET28a Recombinants for *tem1* Insertion; Streaking of *Streptomyces* Strains for Antibiotic Tests
10-10	**7**	Analysis of Digested Ligation Products; Performing Antibiotic Tests; Extraction of *Streptomyces* Cultures
10-17	**8**	Pilot Expression of Recombinant BL21(DE3) pET28a-*tem1*
10-24	**9**	SDS-PAGE Analysis of the TEM1 Pilot-Scale Induction Experiment
10-31	**10**	Expression and Purification of TEM1 from Recombinant BL21(DE3) pET28a-*tem1*

11-07	**11**	Analysis of TEM1 Purification Fractions by SDS-PAGE and Constructing a Purification Table for TEM1 Purification
11-14	**12**	Kinetic and Inhibition Assays for TEM1