CHM4300L, Laboratory in Biochemistry and Molecular Biology
Spring 2018

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

Laboratory manual: *Cloning, Expression, & Characterization of Human Carbonic Anhydrase II, Experiment Manual.* (available at Target Copy Center). Safety glasses, and proper lab attire required.

Pre-laboratory lecture: Leigh Hall 207, Tuesdays, 8:30am – 9:20am
Laboratory: Leigh Hall 200, Wednesdays, 9:35am-12:35pm (Section 2827) and 12:50-3:50pm (Section 1C41)
Office hours: Prof. Butcher: Tuesdays 9:30-10:30am, Fridays 3-4pm and by appointment (email).

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.

Course grading:	Laboratory notebooks	40%	
	Laboratory reports (2)	40%	(due: 02/27 & 04/17)
	Lab performance and quizzes	20%	

Course grades will be assigned on a curve with the following percentages used for guidance: 100-85% A, 84-72% B, 71-60% C, 59-50% D, 50-00% F. For information on UF's Grading Policy, see:
http://www.registrar.ufl.edu/catalog/policies/regulationgrades.html;

Laboratory notebooks will be graded at three times during the semester for accuracy and completeness (graded the weeks of 2/05, 03/12 & 04/09). Lab notebooks do not leave the lab. Lab reports will cover labs 1-6 and 7-12 and are due 02/27 & 04/17. 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 that week's lab.

Attendance: Attendance is required for all lab sessions. Due to the continuity of the labs in the course, missed labs cannot be made up. Attendance at the pre-lab lecture is strongly encouraged.

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:

Week of	Lab	Title
01-08	**1**	Introduction to CHM 4300L Laboratory Techniques
01-15	**2**	Cloning Strategy, Introduction to Restriction Enzymes, Agarose Gel Electrophoresis and PCR
01-22	**3**	Estimating the Concentration of the PCR Amplification Product by Gel Electrophoresis
01-29	**4**	Purification of DNA by Preparative Agarose Gel Electrophoresis and Purification of DNA
02-05	**5**	Quantification of Purified hCA2 DNA and Vector DNA, Ligation of hCA2 DNA and Transformation
02-12	**6**	Screening pETBlue-2 Recombinants for hCA2 Insertion
02-19	**7**	Transformation of pETBlue-2 / hCA2 into E. coli TunerTM(DE3) pLacI
02-26	**8**	Pilot Expression of Recombinant TunerTM(DE3) pLacI pETBlue-2 / hCA2
03-12	**9**	SDS-PAGE Analysis of the hCA2 Pilot-Scale Induction Experiment
03-19	**10**	Expression and Partial Purification of Carbonic Anhydrase from Recombinant E. coli
03-26	**11**	Gel Filtration Chromatography of Partially-Purified Carbonic Anhydrase
04-02	**12**	Constructing a Purification Table for Carbonic Anhydrase Isolation and Kinetic Assays

Tentative lab schedule:

1	01-08-2017	Lab 1 Introduction to CHM 4300L Laboratory Techniques
2	September 4th	Cloning Strategy, Introduction to Restriction Enzymes, Agarose Gel Electrophoresis and PCR
3	September 11th	Estimating the Concentration of the PCR Amplification Product by Gel Electrophoresis
4	September 18th	Purification of DNA by Preparative Agarose Gel Electrophoresis and Purification of DNA
5	September 25th	Quantification of Purified hCA2 DNA and Vector DNA, Ligation of hCA2 DNA and Transformati
6	October 2nd	Screening pETBlue-2 Recombinants for hCA2 Insertion
7	October 9th	Transformation of pETBlue-2 / hCA2 into E. coli TunerTM(DE3) pLacI
8	October 16th	Pilot Expression of Recombinant TunerTM(DE3) pLacI pETBlue-2 / hCA2
9	October 23rd	SDS-PAGE Analysis of the hCA2 Pilot-Scale Induction Experiment
10	October 30th	Expression and Partial Purification of Carbonic Anhydrase from Recombinant E. coli
11	November 6th	Gel Filtration Chromatography of Partially-Purified Carbonic Anhydrase
12	November 13rd	Constructing a Purification Table for Carbonic Anhydrase Isolation and Kinetic Assays
	November 20rd	