

Laboratory in Biochemistry and Molecular Biology

CHM 4300L

Spring 2015

Instructor: Steve Bruner bruner@ufl.edu Office: Leigh Hall 404

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

Weekly pre-laboratory discussion: Leigh Hall 142, Wednesdays 12:50-1:40pm

Office hours: Prof. Bruner: Mondays 10-11am, Wednesday 3:00-4:00pm 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: 2/25 & 4/15)
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 weeks of 2/9, 3/16 & 4/20) Lab notebooks do not leave the lab. Lab reports will cover labs 1-6 and 7-12 and due 2/25 & 4/15. 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 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 can't 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.

Tentative lab schedule:

Week of:

1	January 12th	Introduction to CHM 4300L Laboratory Techniques
2	January 19th	Cloning Strategy, Introduction to Restriction Enzymes, Agarose Gel Electrophoresis and PCR
3	January 26th	Estimating the Concentration of the PCR Amplification Product by Gel Electrophoresis
4	February 2nd	Purification of DNA by Preparative Agarose Gel Electrophoresis and Purification of DNA
5	February 9nd	Quantification of Purified hCA2 DNA and Vector DNA, Ligation of hCA2 DNA and Transformation
6	February 16th	Screening pETBlue-2 Recombinants for hCA2 Insertion
7	February 23rd	Transformation of pETBlue-2 / hCA2 into E. coli Tuner TM (DE3) pLacI
	March 2nd	<i>spring break</i>
8	March 9th	Pilot Expression of Recombinant Tuner TM (DE3) pLacI pETBlue-2 / hCA2
9	March 16th	SDS-PAGE Analysis of the hCA2 Pilot-Scale Induction Experiment
10	March 23rd	Expression and Partial Purification of Carbonic Anhydrase from Recombinant E. coli
11	March 30th	Gel Filtration Chromatography of Partially-Purified Carbonic Anhydrase
12	April 6th	Constructing a Purification Table for Carbonic Anhydrase Isolation and Kinetic Assays