
Professor	Jon D. Stewart Office: 102 Leigh Hall Phone: 352.846.0743 E-mail: jds2@chem.ufl.edu
Office hours	Monday, 2 nd period, 8:30 – 9:20 a.m. Monday, 6 th period, 12:50 – 1:40 p.m. Thursday, 2 nd period, 8:30 – 9:20 a.m.
Teaching Assistants	Francis Agama, agamfran@ufl.edu Ian De Vera, idevera@ufl.edu Timothy Gould, tgould@ufl.edu Huiyuan Hu, huhuiyuan@ufl.edu Trey Powell, rwpowell@chem.ufl.edu
Course Objectives	CHM 4300L provides a hands-on opportunity to learn basic techniques in molecular biology and biochemistry including the polymerase chain reaction, DNA cloning, plasmid isolation and characterization, protein purification and steady-state kinetics.
Prerequisites	CHM 2211, CHM 2211L and either CHM 3218 or BCH 4024.
Grading	Three lab reports (100 points each) will be due over the course of the semester (due on October 4/5 , November 1/2 [depending on your section] and December 10 [all sections]). Guidelines for each lab report will be distributed at least two weeks before each due date. Lab notebooks will also be graded at these times for accuracy and completeness (100 points total). Four short (20 minute) quizzes will be given at the beginning of pre-lab lectures at unannounced times during the semester (total 100 points). These quizzes will cover material for that week's lab only. 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. You will be expected to analyze critically where the problem(s) lay in your lab report, however, and this will be graded (see above). Current UF grading policies can be found at https://catalog.ufl.edu/ugrad/current/regulations/info/grades.aspx .
Class Attendance	Attendance is <u>required</u> at all laboratory sessions; please be on time! Attendance at pre-lab lectures is strongly suggested. Please do not schedule school visits, interviews, etc. during lab periods since these are not official U.F. excused absences and no make-up work will be available.
Make-Up Work	Because they are unannounced, there are no make-up quizzes. Students with an allowable excuse should see the Instructor to arrange accommodations if they miss a quiz.

Required Textbook *Cloning, Expression & Characterization of Human Carbonic Anhydrase II. Experimental Manual.* Fall 2012 Edition (available at Target Copy Center).

Laboratory Schedule A calendar of scheduled experiments is available at the course e-Learning site (<http://lss.at.ufl.edu>).

Laboratory Attire Department of Chemistry approved SAFETY GLASSES or GOGGLES (only Astro OTG 3001 or American Optical 91214 Goggles) must be worn any time you are in the laboratory. Protective clothing (long pants, shirt/blouse and proper shoes) is required any time you are in the laboratory and it must be worn at all times while in the laboratory. A more detailed discussion of laboratory safety is available on the course e-Learning site (<http://lss.at.ufl.edu>).

Academic Honesty We, the members of the University of Florida community, pledge to hold ourselves and our peers to the highest standards of honesty and integrity. On all work submitted for credit by students at the University of Florida, the following pledge is either required or implied: "On my honor, I have neither given nor received unauthorized aid in doing this assignment." *All portions of the quizzes and lab reports are to be completed individually.*

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.

List of Experiments

- Laboratory 1 Introduction to CHM 4302L Laboratory Techniques
- Laboratory 2 Cloning Strategy, Introduction to Restriction Enzymes, Agarose Gel Electrophoresis and the Polymerase Chain Reaction
- Laboratory 3 Estimating the Concentration of the PCR Amplification Product by Gel Electrophoresis, PCR Clean-up, Digestion of the Expression Vector and PCR Product
- Laboratory 4 Purification of DNA by Preparative Agarose Gel Electrophoresis and Purification of DNA from Agarose Gels
- Laboratory 5 DNA Quantification of Purified hCA2 DNA and pETBlue-2 Vector DNA, Ligation of hCA2 DNA to pETBlue-2 Vector DNA and Transformation into *E. coli* NovaBlue
- Laboratory 6 Screening pETBlue-2 Recombinants for hCA2 Insertion
- Laboratory 7 Transformation of pETBlue-2 / hCA2 into *E. coli* TunerTM(DE3) pLacI
- Laboratory 8 Pilot Expression of Recombinant TunerTM(DE3) pLacI pETBlue-2 / hCA2
- Laboratory 9 SDS-PAGE Analysis of the hCA2 Pilot-Scale Induction Experiment
- Experiment 10 Expression and Partial Purification of Carbonic Anhydrase from Recombinant *E. coli*
- Experiment 11 Gel Filtration Chromatography of Partially-Purified Carbonic Anhydrase
- Experiment 12 Constructing a Purification Table for Carbonic Anhydrase Isolation and Kinetic Assays