

<b>Professor</b>	<b>Jon D. Stewart</b> Office: 102 Leigh Hall Phone: 352.846.0743 E-mail: jds2@chem.ufl.edu
<b>Office hours</b>	Monday, 2 <sup>nd</sup> period (8:30 – 9:20 a.m.) Monday, 7 <sup>th</sup> period (1:55 – 2:45 p.m.) Tuesday, 3 <sup>rd</sup> period (9:35 – 10:25 a.m.)
<b>Teaching Assistants</b>	<b>Erica Amato</b> (amatoe1@chem.ufl.edu) <b>Jeff Arciola</b> (jarciola@chem.ufl.edu) <b>HyunJun Choe</b> (hchoe1@ufl.edu) <b>Kevin Fisher</b> (kevinjfisher@chem.ufl.edu) <b>Louis Mouterde</b> (louis.mouterde@gmail.com) <b>Yuting Wang</b> (yutingwang@chem.ufl.edu) <b>Richard Watkins</b> (rrwatkinrye@gmail.com)
<b>Course Objectives</b>	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.
<b>Prerequisites</b>	CHM 2211, CHM 2211L and either CHM 3218 or BCH 4024.
<b>Grading</b>	Three lab reports (100 points each) will be due over the course of the semester [due on <b>February 15 or 16</b> (depending on section), <b>March 22 or 23</b> (depending on section) and <b>April 24</b> (all sections)]. Guidelines for each lab report will be distributed at least one week 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). <i>These quizzes will cover material for that week's lab only.</i> 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 <a href="https://catalog.ufl.edu/ugrad/current/regulations/info/grades.aspx">https://catalog.ufl.edu/ugrad/current/regulations/info/grades.aspx</a> .
<b>Class Attendance</b>	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.
<b>Make-Up Work</b>	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. Late reports and lab notebooks will not be accepted for grading.

**Required Textbook**

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

**Laboratory Schedule**

A calendar of scheduled experiments is available at the Canvas e-Learning site (<http://elearning.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 with disabilities requesting accommodations should first register with the Disability Resource Center (352-392-8565, [www.dso.ufl.edu/drc/](http://www.dso.ufl.edu/drc/)) by providing appropriate documentation. Once registered, students will receive an accommodation letter which must be presented to the instructor when requesting accommodations. Students with disabilities should follow this procedure as early as possible in the semester.

**Additional Information**

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 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](mailto: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 9-1-1.

## List of Experiments

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| 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 <i>E. coli</i> NovaBlue |
| Laboratory 6  | Screening pETBlue-2 Recombinants for hCA2 Insertion  |
| Laboratory 7  | Transformation of pETBlue-2 / hCA2 into <i>E. coli</i> Tuner <sup>TM</sup> (DE3) pLacI   |
| Laboratory 8  | Pilot Expression of Recombinant Tuner <sup>TM</sup> (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 <i>E. coli</i>  |
| Experiment 11 | Gel Filtration Chromatography of Partially-Purified Carbonic Anhydrase   |
| Experiment 12 | Constructing a Purification Table for Carbonic Anhydrase Isolation and Kinetic Assays  |