MCDB 4100: *Experimental Design & CRISPR Mutagenesis in Xenopus*

Semester: Fall 2016
Lecture hour: Monday, 10:00-10:50 AM **Location**: PORTER B436
Lab hours: Tuesday-Thursday, 2-4 PM **Location**: PORTER B425

Instructors: Prof. Michael Klymkowsky and Bilge Birsoy.
Office hour: To be determined depending on student response
Location: To be determined depending on room availability
Contact email: michael.klymkowsky@colorado.edu and bilge.birsoy@colorado.edu

Prerequisites: MCDB3135 & MCDB3145 and by instructor consent
Required reading
Assigned research articles read using the nota bene web system: http://nb.mit.edu/welcome
PUBMED: http://www.ncbi.nlm.nih.gov/pubmed
Google Scholar: https://scholar.google.com
Xenbase: http://www.xenbase.org/

Laboratory Manual: online on course website

Course website: http://virtuallaboratory.colorado.edu/MCDB 4100-MutatingXenopus

Optional background reading: Gilbert, S. Developmental Biology 10th ed.
http://10e.devbio.com/contents.php?sub=1&art=1&full=1

Useful links:
http://www.ncbi.nlm.nih.gov/books/NBK9983/?term=developmental%20biology
https://www.nobelprize.org/nobel_prizes/medicine/laureates/2012/gurdon-lecture.pdf

Required supplies
Lab book (with numbered pages)
Pen
Permanent fine point marker

Overview: MCDB 4100 is a one-semester discovery-based laboratory course where you will (perhaps) discover novel gene function(s) by generating mutations using the latest gene-editing technology, CRISPR-Cas9 and examining their effects on early development of the vertebrate *Xenopus laevis*. You will work in a small group (2-3 students) to learn how to use various scientific databases to select a gene of interest (not previously studied in *Xenopus*), formulate hypotheses about the gene function, and then design and build the reagents needed to i) generate and confirm the presence of mutations in that gene, and ii) characterize the mutant phenotype in terms of gene expression and embryonic phenotype. You will discuss and present your findings to your peers and faculty through a short video presentation.

Course Structure: The course consists of a weekly 1-hour lecture and discussion session and two 2-hour laboratory sessions. Our goal is to provide you with an authentic research experience and get you to experience the scientific process first-hand. The main learning goals are to increase your scientific and experimental literacy and to engage your creativity by enabling you to ask questions and generate new observations by reading scientific publications and finding out what is known and what remains unknown, designing the experiments to study the unknown, analyze the results of these experiments, and to present your results.
The course also aims to provide you with a working introduction and practical experience in

experimental molecular and cell biology and vertebrate embryology through the process of designing and carrying out CRISPR-Cas9-based gene mutation studies and interpretation of the results, using the model organism, *Xenopus laevis* (African clawed frog). You will learn effective communication skills as you present and discuss your experimental results and alternative conclusions and have the opportunity to submit novel findings to online databases, produce resources for the scientific community and contribute to future publications.

The required readings will involve original research articles and review articles and the laboratory manual (available through the course web page). You will learn how to search for articles using PubMed and Google Scholar. Research articles are dense in information. It is necessary that you read the materials carefully and come to class prepared to resolve your confusions and to be ready to discuss the ideas and observations presented. We will be using the web-based *nota bene* system to record your interactions with the text and each other.
Every class meeting will include discussions that you are expected to participate in. As part of the class we will discuss the nature of science, the scientific process, and ethical practices; honesty, rational thinking, and good communication skills (oral and written) will be stressed and you will be encouraged to develop such skills so that you can formulate your ideas and express them clearly and succinctly. Although we will be questioning and critiquing each others' statements, we will do this in a respectful and professional manner as civilized citizens and scientists. Disruptive behaviors and harassment will not be tolerated.
In the laboratory session, you will adhere to lab safety rules at all times and follow good lab practices. You may occasionally be required to come to the lab for short periods of time outside of scheduled lab times in order to complete part of an experiment (e.g. to streak a culture, to put samples in the freezer, etc).

Learning Outcomes

1) Become a more inquisitive individual and a critical thinker
2) Understand the scientific process and become science literate
3) Learn about major concepts and advances in molecular biology (PCR, plasmid construction, DNA isolation, RNA synthesis, experimental analysis of embryonic phenotypes, and CRISPR-Cas9 technology
4) Troubleshoot problems and come up with alternative solutions
5) Learn how to read a research article and evaluate it critically
6) Learn how to design experiments and write a scientific proposal
7) Develop both oral and written science communication skills

Grading: This course requires your active participation throughout the semester.

First presentation:	15%
Reading, nota bene contributions, in-class/in-lab discussions:	20%
Quizzes, Lab books and in-lab activities:	30%
Survey / questionniare points	5%
Final project (video+written or oral presentation)	30%

* At professors' discretion, late submissions may be graded up to 80% of total points.
First Presentation: Within the two weeks of classes, working in groups of 2-3, you will learn how to use online resources and databases to identify genes of interest.
Criteria for choosing a gene for experimental analysis:

- The gene may be implicated in an interesting biological process or human disease or may have no known function.
- The gene must have an homolog or ortholog in *Xenopus laevis*.
- The gene function in *Xenopus* should be unknown and not yet published.

You will give a group 8-10 minute oral presentation, justifying your choice.
You should practice what you are going to present. Do not read from notes or slides and do not memorize your sentences, either.

Reading, nota bene contributions, in-class/in-lab discussions: It is essential that you complete the assigned reading and other pre-class assignments before each class and arrive prepared to answer questions and discuss your ideas. Using nota bene, you should highlight any terms you don't know and ask or answer questions about the material.

Quizzes, Lab books and in-lab activities: Before each lab, you will complete pre-lab quizzes. During the lab session, you will be filling out your labbook which will be collected periodically to be graded. You will also be evaluated on good laboratory practices.

Lab-books and other written assignments must be free of typographical and grammatical errors as much as possible. Plagiarism in oral or video presentations will earn you zero points and may cause a failing grade in the course.
Final project 30% (video+written or oral presentation)

Abbreviated Laboratory Safety Rules: You will find detailed information on Lab Safety in your Lab manual and will also have Safety Training with Dr. Birsoy. You will be required to pass the online safety quiz before you can begin any laboratory work. If you fail to follow the safety rules, you will be asked to leave the lab. While in the lab, do not leave your own bench and do not touch reagents or equipment that you have not been trained on. Review the manual and your notes and know what you need to do before start any procedure. If you need help or have a question, ask Dr. Birsoy or Dr. Klymkowsky before proceeding.

Dress code for the lab sessions
You will be wearing personal protective equipment (lab coat, gloves, and nitrile gloves) at all times in the lab. Exposed skin is a risk for contact with hazardous materials. For your protection, you must dress properly for each laboratory session.
No open toed shoes. Wear closed-toed shoes.
No shorts or short skirts. Wear long pants or long skirts covering down to ankles.
Wear long sleeved shirts that fully cover your torso.
Confine long hair, loose clothing and jewelry.

Absolutely, NO food or drinks in the lab. No chewing gum or putting on chapsticks or lipsticks.
These rules aim to prevent accidental ingestion of hazardous material.
No use of cell phones in the lab.
Keep your hands away from your face and mouth. Wash your hands often. Always wash your hands before leaving the lab.
Keep your bench clean and tidy. Always clean up the bench before you start your experiments and after you finish.
Dispose of all waste properly.
Know what to do in case of an emergency.
Report any accident and injury to Dr. Birsoy or Dr. Klymkowsky.

Hazardous materials and other potential hazards in the lab:
Biological: non-pathogenic *E. coli* (BSL-1: no known health risk)
Chemical:
Recombinant or synthetic Nucleic Acids (rsNA)
Ethidium Bromide (EtBr)
Formaldehyde (FA)
Methanol (MeOH)
Ethanol (EtOH)
Phenol
Chloroform
Hydrogen peroxide (H2O2)
Physical: Sharps, Fire (bunsen burner), Electrical (electrophoresis)

Tentative Course Schedule
Blue: bacterial work, Red: live embryo experiments, Green: work with fixed or frozen samples

Week 1: Introduction to the Scientific Process and Model Organisms, Genetics and Molecular Biology review

Monday Scientific Process, Model Organisms (including *Xenopus laevis) and* Embryology
Tuesday Introduction to Genotype and Phenotype, Genes of interest
Thursday Introduction to PubMed, Xenbase, (and Nota Bene)

Week 2: Introduction to Experimental Design and Molecular Biology Techniques, Online databases, CRISPR-Cas9 and Oligo Design

Monday Gene expression and mutagenesis, (promoters, transcription, types of mutations and their effect on mRNA and protein synthesis)
Tuesday Online database use, Experimental Design, MolBio Techniques
Thursday Short presentations on gene of choice

Week 3: Laboratory Safety Training and Design and Cloning of gRNA and in situ probe constructs

Monday NO CLASS (Labor Day)
Tuesday CRISPR sgRNA constructs, oligo design
Thursday Lab Safety Training, Good Lab Practices, Pipetting techniques, Molarity calculations

Week 4: Cloning and purification of gRNAs and in situ probes
Confirm positive colonies and constructs (Diagnostic Digest and Submission for Sequencing)
Setup genomic PCR to test genomic PCR oligos
Week 5: Cloning and Purification continued and followed by Analysis and evaluation results and troubleshooting I (Cloning)
Analyze results, troubleshoot and repeat the necessary steps

Week 6: Injection of *Xenopus* embryos with gRNA constructs
Embryo injections, Phenotype analysis and sample preparation (freeze and fix samples regardless of phenotype)
Week 7: Phenotype analysis and Sample preparation for genotyping (continued)
Week 8: Genomic PCR and Genotyping
PCR uninjected controls along with gRNA injected samples
Both for tyrosinase control as well as genes of interest
Week 9: Analysis and evaluation of results and troubleshooting II (Phenotypes and Genotypes)

Week 10: In situ hybridization
Probe synthesis, dot blots, start in situs if probes are good
Week 11: In situ hybridization continued
Week 12: Analysis and evaluation of results and troubleshooting III (In situ hybridization)

Week 13: How to generate figures and present data
Week 14: No classes
Week 15: Group Presentations
Week 16: Group Presentations

Attendance: Attendance is mandatory. Since continuous participation is required, failing to attend lectures or laboratories or lack of active participation will reduce your grade. You will be allowed two unexcused absences as long as they are not on your presentation days or final projects. If possible, notify us that you will not be attending in advance of the class or lab.
Excused absences include illness with a note from your physician, death or grave illness in your immediate family with appropriate documentation (e.g. death certificate and relationship) and significant scheduling conflicts such as school/job interviews, athletic participation in NCAA events.
There are no make-up labs, exams or projects due to the nature of this lab course. You may come to the office hours if you need extra help.

Classroom Behavior: Students and faculty each have responsibility for maintaining an appropriate learning environment. Those who fail to adhere to such behavioral standards may be subject to discipline. Professional courtesy and sensitivity are especially important with respect to individuals and topics dealing with differences of race, color, culture, religion, creed, politics, veteran's status, sexual orientation, gender, gender identity and gender expression, age, disability, and nationalities. Class rosters are provided to the instructor with the student's legal name. I will gladly honor your request to address you by an alternate name or gender pronoun. Please advise me of this preference early in the semester so that I may make appropriate changes to my records. For more information, see the policies on classroom behavior and the student code.

Discrimination and Harassment: The University of Colorado Boulder (CU-Boulder) is committed to maintaining a positive learning, working, and living environment. CU-Boulder will not tolerate acts of discrimination or harassment based upon Protected Classes or related retaliation against or by any employee or student. For purposes of this CU-Boulder policy, "Protected Classes" refers to race, color, national origin, sex, pregnancy, age, disability, creed, religion, sexual orientation, gender identity, gender expression, veteran status, political affiliation or political philosophy. Individuals who believe they have been discriminated against should contact the Office of Institutional Equity and Compliance (OIEC) at 303-492-2127 or the Office of Student Conduct and Conflict Resolution (OSC) at 303-492-5550. Information about the OIEC, the above referenced policies, and the campus resources available to assist individuals regarding discrimination or harassment can be found at the OIEC website. The full policy on discrimination and harassment contains additional information.

Honor Code: All students of the University of Colorado at Boulder are responsible for knowing and adhering to the academic integrity policy of this institution. Violations of this policy may include: cheating, plagiarism, aid of academic dishonesty, fabrication, lying, bribery, and threatening behavior. All incidents of academic misconduct shall be reported to the Honor Code Council (honor@colorado.edu;303-735-2273). Students who are found to be in violation of the academic integrity policy will be subject to both academic sanctions from the faculty member and non-academic sanctions (including but not limited to university probation, suspension, or expulsion). Additional information regarding the Honor Code policy can be found online and at the Honor Code Office.

Accommodation of disabilities and special needs: If you qualify for accommodations because of a disability, please submit to me a letter from Disability Services in a timely manner (for exam accommodations provide your letter at least one week prior to the exam) so that your needs can be addressed. Disability Services determines accommodations based on documented disabilities. Contact Disability Services at 303-492-8671 or by e-mail at dsinfo@colorado.edu. If you have a temporary medical condition or injury, seeTemporary Injuries guidelines under the Quick Links at the Disability Services website and discuss your needs with me as soon as possible.

Counseling and Psychological Services (CAPS) offers free and confidential counseling services for all CU-Boulder students. CAPS provides outreach and consultation services to students, staff and faculty, as well as couples and group counseling and workshops on stress management, sleep, relationships and coping skills. CAPS is open Monday–Friday, 9 a.m.–5:00 p.m. Walk-in services are available Monday–Friday, 10 a.m.–4 p.m., and no appointment is needed for a first visit. CAPS is

located in the Center for Community, room S440.

Religious observances: Campus policy regarding religious observances requires that faculty make every effort to deal reasonably and fairly with all students who, because of religious obligations, have conflicts with scheduled exams, assignments or required attendance. If you have a conflict with a religious holy day and would like accommodation, you must bring it to my attention at least two weeks in advance of the date(s) you'll need accommodations for.