

BB 315/BI 315: Molecular Biology Laboratory, Spring 2019

3 credits

Students will participate in one Lecture/Discussion session and two 3- hour lab sessions each week:

Lecture: Tuesday 4:00-4:50, Location LINC 200

Lab Section:

Monday and Wednesday 1-3:50 pm ALS 0023

Tuesday and Thursday 9-11:50am, ALS 0023

Tuesday and Thursday 1-3:50pm, ALS 0023

Prerequisites: BB 314 [C-], BI 314 [C-], BB 314H [C-], or BI 314H [C-].

Instructors:

Dr. Kari van Zee, Biochemistry-Biophysics Department, ALS 2141

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Dr. Michael Freitag, Department of Biochemistry-Biophysics, ALS 2045

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Allyson Erlendson, PhD candidate, Department of Biochemistry and Biophysics, ALS 2035

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Teaching Assistants:

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Office hours:

Kari and Ally: 11-12 Wednesday in ALS 2040, or by appointment

Course Information:

Molecular Biology Laboratory, BB 315/BI 315 is an intermediate-level laboratory designed for Biochemistry and Molecular Biology and other life science majors. By completing guided-research projects focusing on fundamental molecular biology concepts and essential technologies, participating students will explore the functional relationship between DNA sequence and gene products and the transmission of genetic information from storage through expression to function. Through laboratory projects, lectures, and reading selected primary research papers, the course will introduce students to the design, expression, and use of recombinant proteins and how they are used in advancing the field of molecular biology and biochemistry. Students will attend two three-hour lab sessions each week and one lecture/discussion forum.

This course is designed to expose students to how research is performed, presented, and analyzed in the academic world. Our projects this year are developed in partnership with Dr. Michael Freitag, Professor, and Allyson Erlendson, PhD candidate, in the Department of Biochemistry and Biophysics.

Project 1: Annotation of the *Neurospora crassa* genome

Students will learn how to use sequence analysis tools, data bases, and molecular visualization software by annotating 30-kb regions of the *N. crassa* genome. Students will also learn how to resolve gaps in sequence information using polymerase chain reaction and DNA sequencing methods.

Project 2: Generating a panel of histone H4 mutations to investigate chromatin dynamics and gene expression

Histones, as part of nucleosomes, are responsible for DNA packaging in chromosomes. They also affect DNA expression by a multitude of post-translational modifications that are especially prevalent on the amino terminus of histones. Co-activator protein complexes “write”, “read”, or “erase” histone modifications, and in balance determine which sections of DNA are free of histones and thus can be transcribed or “expressed”. The formation of heterochromatin is crucial for cell differentiation, repression of repetitive DNA elements and protection of chromosome integrity. This term students will investigate the role of histone H4 in transcriptional regulation by systematically mutating amino acids in the amino terminal tail in *Neurospora crassa* histone H4 and replacing the normal gene with the mutated copies. Students will introduce mutations into plasmids containing a histone H4 cassette by a modified “QuickChange” method, validate mutations by sequencing, and transform mutant variants into *N. crassa* by homologous recombination. Transformants will be screened for proper integration, and strains were crossed to reporter strains to yield haploid progeny with mutated *hH4* genes in combination with cytological markers, such as Red Fluorescent Protein (RFP)-tagged centromere proteins or Green Fluorescent Protein (GFP)-tagged proteins involved in gene silencing.

Project 3: Using cytological markers to visualize subcellular localization of hallmarks of heterochromatin in *N. crassa*

The interaction between heterochromatin protein 1 (HP1) and histone H3 methylated at lysine 9 is a well characterized and conserved mechanism for gene silencing in repetitive regions of the genome. Students will cross fluorescently tagged HP1 strains with histone H3 point mutant and visualize any changes to the subcellular localization of HP1 by fluorescent microscopy.

Learning Outcomes

Students will:

1. Design experiments including the proper controls to analyze gene expression, construct, express, and characterize recombinant proteins.
2. Compare different types of gene cloning methods, and identify strengths and limitations of each method.
3. Demonstrate quantitative skills by preparing accurately and reproducibly reagents and solutions for experiments.

4. Operate safely molecular biology laboratory equipment including micropipettes, thermocyclers, centrifuges, gel electrophoresis chambers, power supplies, incubators, and autoclaves.
5. Interpret and evaluate scientific papers related to the research project, analyzing both scientific methods as well as writing style.
6. Use databases, computational tools and other online resources to analyze and interpret genomic sequences.
7. Develop an awareness of the major issues at the forefront of the discipline and discuss ethical issues in the molecular life sciences.
8. Recall and relate foundational molecular biology concepts and laboratory techniques to recent advances in basic research, medicine, and industrial applications.

Resources: Experimental protocols, readings, and resources will be posted on Canvas.

Attendance: This is a time-intensive lab course aimed to expose students to a variety of techniques in the field of molecular biology. The research-based nature of this course requires your presence in lab to move your project forward. Attendance in both lab and lecture are required. Project materials will not be available at a later date to make up experiments.

More than two unexcused absences will result in failure of the course. Students are expected to communicate with instructional team to arrange for excused absences or to notify of illness/emergency preventing lab attendance.

Evaluation of Student Performance:

Lab Safety, Technique, and Organization (15%): Students are expected to attend every lab session, complete safety training, observe safety policies while working in the lab, demonstrate proper care of equipment and reagents, and be responsible for moving their research project forward. Students are expected to organize and label their research materials so that their research collaborators can access and use them. Assignments will include completion of OSU on-line safety training modules. Points will be deducted for students who fail to follow safety guidelines, leave messes behind for TAs or another group to clean-up, or who do not display proper lab etiquette.

Primary Literature assignments (15%): An important component of the “practice of everyday science” is reading scientific articles published in peer-reviewed journals. The goals of these assignments are to help you 1) develop skills in critical thinking, 2) practice scientific journal article- reading and learn how to extract information from a scientific paper, 3) make connections between the research presented in an article and its significance in the broader world, and 4) have fun learning about research advances in a variety of fields.

Literature assignments will be submitted via Canvas **Friday April 19th, April 26th, May 17th, and May 31st.**

Pre-lab assignments (15%): Students are required to complete pre-lab assignments and submit via Canvas weekly. These assignments will test your knowledge on the protocol that will be performed, as well as concepts that will be utilized in the lab. Pre-labs are due every Monday by 9 am (**except Pre-lab 1, Due Wednesday April 3rd at 9am**), regardless of when

your lab section is. Other deliverables will be assigned during lecture.

Notebook (20%): Each student will maintain a scientific laboratory notebook recording experimental design notes, procedures, calculations, data, and conclusions. You are expected to supply your own notebook and bring it to every class. Guidelines for keeping a scientific notebook will be provided in class. Notebooks will be submitted twice throughout the term for grading. **Mid-term at the end of Week 4 Day 2 lab session and final notebook Friday 6/7 by 4pm to Kari's office ALS 2141.**

Genome Annotation Project Milestones/Deliverables (20%)

Assignment and submission instructions will be posted on Canvas.

Deliverable 1: BLAST N, BLAST X and SnapGene map (5%) **Due Friday 4/12 at 4pm**

Deliverable 2: Fill in genome gaps (5%) **Due Friday 5/03 at 4pm**

Deliverable 3: Annotations to genes in 30 kb segment (5%) **Due Friday 5/10 at 4pm**

Deliverable 4: Final annotation report and BLAST P analysis for 5 hypothetical proteins (5%) **Due Friday 5/24 at 4pm**

**Final Report on favorite hypothetical gene from annotated region (15%)
Due Friday 6/7 at 4pm**

Personal Protective Equipment (PPE) Safety Requirements:

Close-toed shoes and full-length pants, or their equivalent, are required in the lab. Additionally, students will wear lab coats, gloves, and protective eyewear while they work. Lab coats and safety glasses (splash resistant and UV protective) are available in the BB Teaching Lab or students may provide their own.

University Policies:

Diversity Statement: The College of Science strives to create an affirming climate for all students including underrepresented and marginalized individuals and groups. Diversity encompasses differences in age, color, ethnicity, national origin, gender, physical or mental ability, religion, socioeconomic background, veteran status, sexual orientation, and marginalized groups. We believe diversity is the synergy, connection, acceptance, and mutual learning fostered by the interaction of different human characteristics.

Religious Holiday Statement: Oregon State University strives to respect all religious practices. If you have religious holidays that are in conflict with any of the requirements of this class, please see me immediately so that we can make alternative arrangements.

Statement Regarding Students with Disabilities: Accommodations for students with disabilities are determined and approved by Disability Access Services (DAS). If you, as a student, believe you are eligible for accommodations but have not obtained approval please contact DAS immediately at 541-737-4098 or at <http://ds.oregonstate.edu>. DAS notifies students and faculty members of approved academic accommodations and coordinates implementation of those accommodations. While not required, students and faculty members are encouraged to discuss details of the implementation of individual accommodations.

The University student conduct code can be found at:

<http://oregonstate.edu/studentconduct/offenses-0>

Cheating or plagiarism by students is subject to the disciplinary process outlined in the Student Conduct Regulations.

Students are expected to be honest and ethical in their academic work. Academic dishonesty is defined as an intentional act of deception in one of the following areas:

- ◆ Cheating-use or attempted use of unauthorized materials, information or study aids
- ◆ Fabrication-falsification or invention of any information
- ◆ Assisting-helping another commit an act of academic dishonesty
- ◆ Tampering-altering or interfering with evaluation instruments and documents
- ◆ Plagiarism-representing the words or ideas of another person as one's own

Behaviors disruptive to the learning environment will not be tolerated and will be referred to the Office of Student Conduct for disciplinary action.

“The goal of Oregon State University is to provide students with the knowledge, skill and wisdom they need to contribute to society. Our rules are formulated to guarantee each student's freedom to learn and to protect the fundamental rights of others. People must treat each other with dignity and respect in order for scholarship to thrive.

Behaviors that are disruptive to teaching and learning will not be tolerated, and will be referred to the Student Conduct Program for disciplinary action. Behaviors that create a hostile, offensive or intimidating environment based on gender, race, ethnicity, color, religion, age, disability, marital status or sexual orientation will be referred to the Affirmative Action Office”