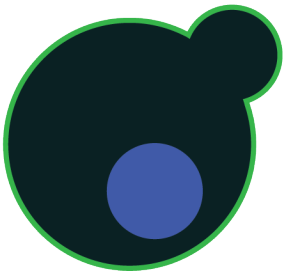
Introduction to Molecular Genetics Laboratory

BioSci 0352
Dr. Allyson O'Donnell

January 7th, 2020 - April 13th, 2020



Molecular Genetics uses precise genome engineering to address increasingly sophisticated biological questions. In this course, students will participate in an authentic research project, defining how protein trafficking adaptors are regulated to dictate selective protein trafficking outcomes. This will be used as a paradigm for students to learn the fundamentals of research design including developing hypotheses, designing experiments to test hypotheses, expanding your molecular and cellular biology toolbox, and analyzing new datasets. Assessment in the course will be aligned to standards for assessing novel findings in the scientific community.

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Spring 2020

General Information

Lab Timing	Mondays 1pm-4:50pm
Location	A146 Langley Hall

Instructor:

Dr. Allyson O'Donnell
A314 Langley Hall
412-648-4270
allyod@pitt.edu
www.odonnelllab.com

Teaching Assistant:

Natalie Hager
A312 Langley Hall
412-648-4289
nah128@pitt.edu

Office Hours

Office hours for Dr. O'Donnell or the teaching assistant, Natalie Hager, can be arranged by appointment between 9:30AM to 5:00PM M-F. Please e-mail to schedule an office visit or schedule a time with one of us during our regular lab meeting schedule.

Pre- and Co-requisites

The prerequisite for the course is BIOSC 0060 or 0067 or 0068 or 0191 or 0080 or BIOL 0102 or 0121 and BIOSC 0350 or 0355 or BIOL 0203 or 0350. Minimum grade for prerequisites is a C. Having taken and passed General Chemistry I will be helpful but is not required.

Course Objectives

We will employ the simple eukaryote *Saccharomyces cerevisiae* to explore molecular genetics and cell biology. The projects planned will lead to the following learning experiences:

1. Nature of Science
   Students should be able to:
   - Predict outcomes of experiments.
   - Interpret results, including an evaluation of positive and negative controls
   - Design an experiment to address a research question of interest
   - Understand the importance of reproducibility
   - Appreciate how data analyses can impact results
   - Develop an appreciation for the scientific literature
   - Maintain a lab notebook and record observations that will be needed for future publication

2. Nature of Lab Research
   Students should be able to:
   - Manipulate yeast strains in a high-throughput manner
   - Assess phenotypes by manual or automated scoring methods
   - Work with databases to assign gene ontologies (bioinformatics)
   - Grow yeast cultures and calculate culture densities
   - Make serial dilutions
   - Set up a polymerase chain reaction
   - Purify a protein of interest from a eukaryotic cell
   - Detect proteins via immunoblotting using IR-dye technology
   - Use antibodies appropriately to bind to target proteins
   - Use a fluorescence microscope to assess protein localization
   - Design a molecular cloning experiment

3. Nature of Yeast Genetics
   Students should be able to:
   - Describe how genes are deleted in yeast
   - Understand the utility of the gene deletion collection
   - Understand what yeast need to grow and how the drugs employed impact yeast growth
   - Describe how plasmids are maintained in yeast (genetic complementation)
   - Appreciate the utility of model organisms in advancing science
   - Understand how proteins are modified post-translationally and the impact that can have on function
   - Describe how mutants of a protein can be used to help define the function for that protein

A tentative class schedule is shown below. More detailed schedules, protocols and background information will be posted on Lab Archives or handed out at the start of each lab session. Note that many lab sessions will have overlapping content and, as with authentic research in a lab, the experiments continue from one lab to the next. This emphasizes the need to build on prior content and ideas so please do come talk to Dr. O'Donnell or the TA if you are unsure of a concept. We are happy to help you!

Tentative Course Schedule

January 6th	Introduction and **Experiment 1**: Manipulation of the yeast deletion collection. ***This is a critical foundational lab class to participate in.*
January 13th	**Experiment 2**. High-throughput yeast transformations with the KinDel library
January 20th	No Class. Martin Luther King Jr. Day
January 27th	**Experiment 3**. Analyses of yeast transformations data and replica pinning to selective medium.
February 3rd	**Experiment 4**. Scoring of phenotypes on selective medium. **Experiment 5**. Data assembly, analyses and deciding on an experimental plan. In consultation with the literature, begin deciding which candidates from the KinDel Screen to pursue and make an outline of experiments to perform. *Strains must be selected for further analyses so that primers can be ordered for use in upcoming experiments.*
February 10th	**Students will continue to explore the literature on their chosen candidates.** **Experiment 6.** Phenotypic assessment of new transformants by serial dilutions and further discussion of tools required for independent projects.
February 17th	**Experiment 7.** Confirming gene deletion strain identification by PCR. **Oral Presentations.** Groups will present a brief review of background literature on candidates pursued in student-driven projects.

February 24th	**Experiment 8.** Isolating GST-fused arrestins for immunoblot analyses
March 2nd	**Experiment 9 and Independent Projects 1**. Assessing posttranslational modifications on purified proteins. Students will immunoblot (run gel and transfer; incubate with primary antibody) the pull-downs from last class and begin work on independent research projects.
March 9th	No class. Spring Break
March 16th	**Experiment 10 and Independent Projects 2.** We will assess the localization of GFP-tagged α-arrestins in wild-type cells or those lacking the candidate gene(s) of interest. Students should also work on their Independent Research Projects.
March 23rd	**Independent Projects 3.** Student-driven research projects will be performed. Students can also work on their final poster presentations as well.
March 30th	**Independent Projects 4.** Student-driven research projects will be performed. Students can also work on their final poster presentations as well.
April 6th	**Independent Projects 5.** Student-driven research projects will be performed. Students can also work on their final poster presentations as well.
April 13th	**Poster presentations and Discussion**

Course requirements and grading

Course Attendance:

Attendance at ALL lab sessions is mandatory and your active participation in the lab course is worth 10% of your final grade (*see Lab Conduct and Participation and Grading Plan Summary for more information*). Only extremely extenuating circumstances such as serious illness or a death in the family excuses a student from a lab. All absences must be excused by Dr. O'Donnell, and justification by appropriate documentation

(signed doctors note, etc.; NOT a note from a relative) is required before a makeup lab will be scheduled. If a lab is not easily made-up, missed points associated with the lab will be made-up through an oral discussion of the content for the lab with Dr. O'Donnell (for excused absences only). If permission to be absent from a scheduled lab is not obtained, it will result in loss of all attendance, participation, the laboratory notebook points, and other associated activities will be lost for the days in question. If you miss more than 2 labs it will result in a failing grade.

Pre-course preparation and timeliness:

The lab will commence promptly at 1PM with a discussion of the techniques and approaches for the experiments to be executed that day. You must prepare for the lab in advance so that we can begin executing experiments as soon as the initial lab discussion is completed in order to ensure that you can complete the assigned tasks during the lab period. Each time you are late to class or not prepared for the lab, it will result in loss of attendance/participation points.

Lab conduct, professionalism and participation:

Lab safety is of critical importance. There is no food or drink allowed in the lab. All long hair must be pulled back, and clothing or accessories with dangling parts may not be worn. Close toed shoes must be worn in lab. Backpacks and coats must be stored outside of the lab and not at the bench. You must review the protocols provided before the start of lab and understand the research being executed as well as the equipment and chemical risks before starting.

While in the lab it is important to work both independently to achieve a specific objective, and with your team. Pay attention and ask questions during group discussions to ensure you understand the objectives for the lab and the scientific background. Though you will work in a team, your group members will provide feedback on how evenly the lab work was distributed and the instructor and teaching assistant will also be recording notes for participation and effort throughout the lab. Be sure to label your materials clearly with your lab name, the date, and the content. You must have your lab notebook set up and ready at the start of each class.

You must focus on the science at hand during the duration of the lab. Professionalism in the lab includes, but is not limited to, not using cell phones or computers for anything other than lab-related tasks, keeping conversations directed to lab work, taking careful notes on experimental procedures, and offering to answer questions before being called on. *Your active participation in the lab accounts for 10% or more of your final grade.*

Computers:

You will need access to computers to do many of the analyses and data mining experiments in this lab course. Please, if possible, bring your laptop computers to each lab meeting. In addition, there will be free software downloads we will recommend for your use as the course progresses. All communications through the course will be done via email or Lab Archive and so you will need access to your Pitt login information as well.

Text and Materials for this course:

The text for this course will consist of handouts/protocols prepared by the course instructors and will include material from many sources, all provided by the instructors at the appropriate time. These resources are referenced throughout the handouts. ***Weekly additions or amendments will be given to students in lab or via Lab Archive.*** In addition, students are required to have an updated laboratory notebook before lab work begins. (Even though you will be working in groups, each student should have his/her own Lab Archive notebook.) Instructions for maintaining a lab notebook are in Lab Archive and the expectations for a completed lab notebook prior to the start of a new lab are included for reference below (*please see Keeping a Lab Notebook)*.

Communication and Course Information:

Almost all course information will be provided on Lab Archive or via email to your student University of Pittsburgh accounts. Please be sure that you have access to and regularly check this email account in order to not miss critical information.

Assignments and lab notebook checks:

Unless otherwise indicated, assignments are due at the start of the laboratory class. For course work to be handed in, only PDFs or Microsoft WORD documents will be accepted unless otherwise noted. Follow the naming conventions defined below when submitting work electronically:

LASTNAME_DATE_Title of assignment

Keeping a lab notebook:

(***You must use Lab Archive to maintain an up to date record of what you have accomplished in the lab***.)

The purpose of the laboratory notebook in this class is threefold:

1) To provide you with valuable experience in keeping a laboratory notebook.
2) To provide you with a resource to develop your poster and oral presentations for this course.
3) To provide you (and us) with a written record of the successes and failures of the experiments performed in this class and a record of the data generated that could be used in future publications.

The record of every experiment should contain the following:

Title of the experiment

Purpose and Question: One or two sentences that describe the objective/purpose of the experiment/procedure and the question we are trying to address

Flowchart: You will rewrite the posted protocols in the form of a flowchart, with minimal text and diagrams as needed. The flowchart should give you a quick guide to the day's experiment and should be **completed BEFORE you enter the lab**.

These will be graded (see below) and are part of your preparation for the lab each day, ensuring that you have read and understand the protocol we will execute. This can also help you identify places where you have a question or are unsure of a protocol. *Expect instructors to ask you questions about the flowchart at the beginning of each lab.*

Procedure (methods with any modifications): Description of the experiment. This includes the protocols as well as the important materials. Reference the protocol handouts and place them in the notebook at the correct location, if you don't want to rewrite them fully. However, you *must* indicate any change that you made to the protocol. **Be *very* specific.** Indicate any problems you encountered.

Results (include data as an attachment): **Record everything that happens, or doesn't happen—it's all data. Do this AS YOU ARE WORKING and not after the fact**. Add results directly into your notebook as electronic images and make notes in Lab Archives about any changes to the protocol as you are working through the experiment in real time. If your data is in the format of a larger set of computer files (ie. microscopy imaging data set or search of a database) indicate the filename where the data can be found so that you or someone else can find it easily. Typically, you will be able to upload these as well to Lab Archive, but if you have questions about how we can help you figure that out.

Analyses: Some data sets will require computational analyses and, where applicable, these should be included here. Excel files for large data sets should be uploaded in this section as attachments.

Conclusion (your interpretation): A sentence or two summarizing and drawing conclusions from the results. May include comments about why the experiment may or may not have worked. What does the result mean in the bigger context of our research goals for this project?

Remember: A good lab notebook should contain enough detail that any other person could repeat the same experiment and obtain the same results. Reproducibility is a key requisite for any phenomena to become a scientific fact!! Keep your notebook up to date! You should make notes as you do the work, and NOT after the fact. (Your memory is not that good.) For this reason, you MUST bring your notebook to each and every lab, AND use it. EVERY observation is important.

We will be checking your notebooks at the start of every class to make sure that all information from the last experiment is present and up to date.

Grading Summary

Tentative Grading Plan for BioSci 0352 Spring 2020
Maximum of 500 points = 100% of lab grade

Attendance & Participation	50 points
Lab Notebooks (checked daily & final)	80 points
Lab Assignments and Figure Development	250 points
In Lab Oral Presentation	50 points
Final Poster – Results Summary	70 points

An outline of tentative deadlines for each project in the course will be provided separately. Rubrics for each portion of the assessment will be posted with specific assignments closer to the due dates.

Grading Scale:

A+	97-100
A	93-96
A-	90-92
B+	87-89
B	83-86
B -	80-82
C+	77-79
C	70-76
D	60-69
F	<60

Academic Integrity Policy:

You are expected to maintain the high standards of intellectual and ethical honesty that are the hallmark of the scientific community. Further, you must abide by the standards of integrity and honesty set forth in the University of Pittsburgh Policy on Academic Integrity. Please review this at the following link: http://www.cfo.pitt.edu/policies/policy/02/02-03-02.html

To read about what plagiarism is and how to avoid it, go to: https://pitt.libguides.com/academicintegrity/plagiarism

Cheating or plagiarism (or suspected cheating) will result in an automatic zero for the assignment and violations of the University of Pittsburgh's Policy on Academic Integrity will result in mandatory participation in the review process as outlined in this policy. Students will receive a grade of zero on the materials that violate this policy.

Disabilities resource services:

If you are requesting an accommodation for a disability you should contact both Dr. O'Donnell and Disability Resources Services (140 William Pitt Union, 412-648-7890, drsrecep@pitt.edu) as early as possible in the term. Disability Resources Services will determine the accommodation needed for this course. To read more about this process please refer to: https://www.studentaffairs.pitt.edu/drs/

Diversity and Inclusion:

The lab should be a productive, supportive, respectful and welcoming environment in which all can engage in the pursuit of scientific knowledge. Discrimination, harassment, or retaliation based on disability, race, color, religion, national origin, ancestry, genetic information, marital status, familial status, sex, age, sexual orientation, gender identity, veteran status or any other factors as stated in the University's Title IX policy will not be tolerated. Prompt action will be taken to end any hostile environment that interferes with student learning and the University mission. For more information on University policies surrounding diversity and inclusion please refer to: https://www.diversity.pitt.edu/policies-procedures-and-practices

Classroom Recording:

To ensure free and open discussion of ideas, students may not record classroom lectures, discussion and/or activities without the advance written permission of the instructor, and any such recording properly approved of in advance can be used solely for the student's own private use.