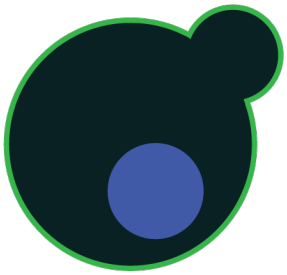
Introduction to Molecular Genetics Laboratory

BioSci 0352

Dr. Allyson O'Donnell and Wes Bowman

January 10th, 2022 - April 18th, 2022



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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Syllabus

Spring 2022

General Information

Lab Timing	Mondays 1:00pm-4:50pm
Locations	Prelab lecture – A221 Langley Hall Lab - A146-148 Langley Hall

Instructors:

Dr. Allyson O'Donnell
A314 Langley Hall
412-648-4270
allyod@pitt.edu
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Ray (Wes) Bowman
A312 Langley Hall
412-648-4289
rwb42@pitt.edu

Teaching Assistants:

Katherine Oppenheimer
KGO5@pitt.edu

Nikolai Makhov
NAM216@pitt.edu

Office Hours

Office hours for Ally O'Donnell or Wes Bowman can be arranged by appointment between 9:30AM to 5:00PM M-F. Please e-mail to schedule a virtual or in person office visit or schedule a time with one of us during our appointed lab meeting time. We are always happy to help you advance your understanding of cell biology!

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
   - Consider how data analyses impacts 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 and protein localization 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
   - Extract proteins from eukaryotic cells
   - Detect proteins using immunoblotting and IR-dye technology
   - Use antibodies appropriately to bind to target proteins
   - Use a fluorescence microscope to assess protein localization
   - Genetically engineer yeast cells to express a protein of interest

3. Nature of Yeast Genetics
   Students should be able to:
   - Describe how genes are deleted or tagged in yeast
   - Understand the utility of library collections of yeast strains
   - Understand what yeast need to grow and how treatments impact growth

- Describe how plasmids are maintained in yeast (genetic complementation)
- Appreciate the utility of model organisms in advancing science
- 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 sent to you via email. 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 Allyson or Wes if you are unsure of a concept. We are happy to help you!***

Tentative Course Schedule

January 10th	**Introduction** ***This session will be held via Zoom only.*** ***This is a critical foundational lab class to participate in and so if you are on the waiting list for this course please plan to attend.*
January 17th	**Martin Luther King Jr Day** ***No class***. Please read the tandem fluorescent timer paper listed in your assignments table for discussion on January 24th.
January 24th	**Experiment 1**: ***Analyses and discussion of TFT library screen*** ***This session will be held via Zoom only.*** Students will be given the tandem fluorescent timer library data sets for WT (control) or *aly1*Δ *aly2*Δ cells (experimental). We will discuss the tandem fluorescent timer technology at length and how this data set was generated. We will discuss the TFT paper that is posted as an assigned reading in a journal club style format. Students will learn to use the *Saccharomyces* Genome Database to find information on a gene's function as this will be needed in subsequent lab classes.
January 31st	**Experiment 2:**

Analyses of yeast high content imaging plates and discussion of results
This session will be in person
We will have completed the high-content imaging of the TFT libraries as described in the last lab and will now examine what membrane proteins showed the most dramatic changes in the absence of *aly1*Δ *aly2*Δ. We will begin to consider the gene candidates together and talk about follow up analyses as well as work on background for a presentation on the membrane proteins of interest. Students should read the Nikko and Pelham paper on α-arrestins and their function for the following week.

February 7th

Experiment 3a:
Cell cytometry
This session will be in person
Students will set up 96-well plates for measurements of fluorescence intensity using the cytometer. We will use multiple conditions here to assess abundance changes. In addition, students will design primers for candidates that seem strong from the fluorescence microscopy datasets, and we will work on background presentations for membrane proteins of interest. We will discuss the Nikko and Pelham paper on α-arrestins in a journal club-style format.

February 14th

Experiment 3b:
Cell cytometry data analyses
This session will be in person
Students will explore the results of their cell cytometry data and make comparisons between this and the fluorescence microscopy imaging data. We will discuss how cell cytometry differs in its abilities compared to microscopy, reviewing pros and cons of each. We will discuss upcoming group presentations.

February 21st

Candidate presentations:

This session will be in person
Groups will present a brief review of background literature on membrane protein candidates to be pursued in student-driven projects.

February 28th

Experiment 4:
This session will be in person
PCR to tag membrane proteins with the tandem fluorescent timer in WT and aly1∆ aly2∆ cells
PCR to amplify the tFT cassette with regions of homology to the membrane protein of interest. Yeast transformation to insert cassette into cells.

March 7th

Spring break
No class.

March 14th

Experiment 5:
This session will be in person
PCR to assess genetic engineering of yeast cells
PCR to determine if the tFT cassette has indeed tagged the membrane protein of interest.

March 21st

Experiment 6:
This session will be in person
Immunoblotting of original and newly created membrane proteins tagged with tFT in comparison with strains from original library
Students will extract protein from yeast cells and load SDS-PAGE gels to resolve these extracts. The proteins on the gels will be transferred to a membrane support and immunoblotted to detect the tFT proteins of interest.

March 28th

Experiment 7:

	This session will be in person ***Microscopy of membrane-tFT proteins*** Students will assess the localization of their tFT-tagged membrane proteins in comparison to the original library-borne copy and determine if the original localization data agrees with this new replicate data set.
April 4th	**Data analyses for Expts 6 & 7**: ***This session will be in person*** ***Image and immunoblot analyses*** Students will assess the results of Experiments 6 and 7. Data will be quantified, and interpretations considered.
April 11th	**Preparing presentations and discussion of data sets** ***This session will be in person*** Students will have the opportunity to work on their final PowerPoint presentations and show elements to the instructors for review and feedback. We will also discuss the final set of results from all student-driven experiments.
April 18th	**Student presentations and Discussion** ***This session will be in person*** Students will present short talks on their research this semester followed by a Q & A period.

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 attendance at the assigned lab time. ***With the exceptions noted above, this course is ONLY offered in person.*** All absences must be excused by Dr. O'Donnell, and justification by appropriate documentation (signed doctor's note, etc.; NOT a note from a relative) is required before a makeup. The makeup will take the form of discussion of the content for the lab with Dr. O'Donnell. If permission to be absent from a scheduled lab is not obtained, it will result in loss of all participation, laboratory

notebook points, and other associated activities 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 1:00PM 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 lab periods.*** Each time you are late to class, or are not prepared for the lab, it will result in loss of 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 occasionally work as a socially distanced team on Zoom, ALL group members should be providing feedback on how evenly the lab work was distributed and the instructor and teaching and participating. Be sure to label your materials clearly with your lab name, the date, and the content. You must have your virtual lab notebook set up and ready at the start of each class as **we will be grading a prelab assessment for each experiment to help gauge your preparedness for the day's experiment.**

You must focus on the science at hand for 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.***

COVID-19 SAFETY

There is no doubt that this semester has the potential to continue to be a shifting situation given the ongoing COVID-19 pandemic. For the most up-to-date information and guidance, please visit **coronavirus.pitt.edu** and check your Pitt email for updates before each class. To ensure the safety of students and instructors during the lab course, and providing you with a high-quality learning experience, the following policies will be implemented and followed without exception:

- **When in the lab you must wear a face mask and gloves AT ALL TIMES.** You will not be permitted in the building or the lab without a mask. You cannot remove your mask or gloves (other than to change them) while you are in the lab space. The CDC has guidance on masks and how they are to be worn in indoor situations, such as our lab environment, to help prevent the spread of COVID19 (https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/about-face-coverings.html).
- **All benches will be disinfected prior to your start in the lab, and you must disinfect your work area at the end of each lab session with the provided cleaners.**
- **The safety of all lab members is the highest priority. If you are unwell, please do not come to class and alert Dr. O'Donnell and Mr. Bowman of the situation.**

Be flexible and resilient. We will work with you to accommodate changes as they may arise in this every changing environment. Know that we are trying our best to provide you with a cutting-edge research experience in challenging circumstances and we are aware that you are navigating some tough situations surrounding the pandemic as well. We will always try to be supportive but clear and open communication is needed.

Computers:

You will need access to computers and the internet to participate in data mining experiments and other data manipulations 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, Canvas and 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 via Canvas, Lab Archive, and/or email.*** 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 their own Lab Archives notebook and make independent updates.) Instructions for maintaining a lab notebook are in Lab Archives 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 Canvas and/or Lab Archives 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. Any video recordings will be linked from Lab Archives/Canvas in Panopto and you can also access videos via direct access to Panopto.

Assignments, prelab questions and lab notebook checks:

Unless otherwise indicated, assignments are due at the start of the laboratory class. Most assignments (probably ALL!) will be submitted via Canvas or Lab Archives to make this process streamlined. ***There is a summary of prelab questions and assignment dates in the Course Schedule document that is also posted on Canvas/Lab Archives. These dates may change but an announcement will be provided if that is the case.***

For course work to be handed in, only PDFs, Microsoft WORD, Excel or PowerPoint documents will be accepted unless otherwise indicated. Follow the naming conventions defined below when submitting work electronically:

LASTNAME_DATE_Title of assignment

We will be checking your Lab Archive notebook throughout the semester to make sure it is complete and up to date. **Maintaining your lab notebook is worth >10% of your grade and so please work to keep this management system up to par.** Have you updated the data in your lab notebook with the results from the last experiment? Have you presented a summary and interpretation of the results? These will be graded by the end of each Monday and so please ensure they are done prior to the start of the lab.

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 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 as part of your lab notebooks 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. *Be ready for 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 (i.e. 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. There will be several examples of Excel files as raw data for experiments that will need to be managed. Where needed we will help you learn to maintain these files in a way that makes sense.

Analyses: Some data sets will require computational and/or quantitative analyses and, where applicable, these should be included here. In many forms, these will be Excel files for large data sets that have been manipulated or sorted in specific ways and they should be uploaded in this section as attachments. Alternatively, you may have statistical analyses that you have performed in Prism software to upload. Anything you have done to assess the data generated goes into analyses.

Conclusion (your interpretation): Please summarize the data and draw conclusions about your findings based on the results. This can include comments about why the experiment may or may not have worked as anticipated. 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 lab AND use it. EVERY observation is important.

We will be checking your notebooks throughout the semester to make sure that all information from the last experiment is present and up to date.

Grading Summary

Tentative Grading Plan for BIOSC 0352 Spring 2022
Maximum of 500 points = 100% of lab grade

Attendance & Participation	50 points
Prelab questions (5 points each)	60 points
Lab Notebooks (checked 3x in semester)	60 points
Lab Assignments and Figure Development (Typically, 18 points each with noted exceptions)	180 points
1st Oral Presentation	75 points
2nd Oral Presentation	75 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

Zoom Policy

Students and instructors are expected to work together to ensure a respectful and productive online learning environment. The University of Pittsburgh Student Code of Conduct applies to all behaviors, including online and in-person communications and interactions with classmates and instructors. We expect you to be professional and respectful to others when attending classes on Zoom. The following policies will be in effect for the duration of our online portion of the course using Zoom. Please carefully review these policies and direct questions to your section instructor. All students are

expected to adhere to these policies in order to facilitate a professional and safe learning environment. Please note that these policies were drafted with your best interest in mind. *All of you want to get as much as possible out of this class. The policies outlined below will help with that and make the online class experience as ideal as possible. We understand it is not the same as meeting in the lab in person to perform all the experiments. But this is how we have to do it for the first two labs this year and we are all in this together. We need each other's support in order to make this a good experience for everyone.*

NOTE: Class meetings on Zoom (including video, audio, and chat text) will be recorded and made accessible to all students enrolled in this course. Please do not share them with individuals who are not enrolled in the class. Violations are subject to the University of Pittsburgh Student Code of Conduct.

- **Please sign in to Zoom using your Pitt Zoom account. Please also use your preferred first name and last name as listed on the class roster. You will be entered into the course waiting room and will be individually admitted into the meeting by your section instructor to ensure the security of the course meeting.** Your instructor relies on recognizing student names to take attendance and to form student groups in Breakout rooms***. If you prefer to use a nickname or other preferred name, please inform your instructor on the first day of class***. If you have changed your name to better reflect your gender identity, please email the instructors so that they can update the course roster with your preferred name and pronouns. You can change your preferred name within the Pitt system by following these instructions. **If your instructor is unable to match your Zoom display name with the official course roster, you will not be admitted into the Zoom meeting.**

- If you do not have access to a computer or smartphone with internet access, you can participate in the Zoom meeting by calling with a landline telephone. This is not optimal, but it will allow you to participate in the meeting via audio. Please contact your instructor if you are having difficulty in obtaining an internet-enabled device with access to the internet to use for class.

- **Stay focused**. It is very tempting to multitask during an online class, but multitasking has been shown to increase the time students need to study for a course as well as lowering students' grades. Additionally, use of other apps on your device that are not relevant to class will reduce your available bandwidth, resulting in poor quality Zoom connections and frequent disconnections to Zoom. We recommend only running apps that are currently being used in the course while you are participating on Zoom.

- **Find a comfortable environment for class.** Review some good practices for staying organized in online classes and setting up an ideal home workspace. Feel free to keep snacks/drinks readily available in your work area. Take a quick stretch break when needed.

- **Do not share Zoom links with any person NOT registered for this course.** We respect students' rights to privacy and to a safe classroom environment free of outside disruption. Unauthorized participants will be immediately removed from the meeting and violations will be reported to Student Affairs.

- If you need technical help, you can contact the Pitt IT Help Desk by calling 412-624-4357, emailing helpdesk@pitt.edu, submit a help request online, or chat live with a Help Desk technician. Help Desk technicians are available 24 hours per day, 7 days per week.

- **Turn on your video when possible.** It is helpful to be able to see each other, just as in an in-person class. However, in cases of limited internet bandwidth or no availability to a webcam, we recommend an audio-only connection.

- Using a virtual Zoom background can be very helpful if you cannot find an environment without a lot of visual distractions or if you aren't comfortable sharing your background. Get creative, but please keep your background choice professional and respectful to others.

- **Keep it clean!** Don't share anything on the video or during screensharing that you wouldn't want put up on the projector in class.

- **Mute your microphone when you are not talking.** This helps to eliminate background noise.

- **Use a headset if possible.** If you have access to headphones with a microphone, please use them. This will improve audio quality and is particularly important when you Zoom with your remote lab group partners during lab time.

- **Work in a quiet place if possible.** Turn off any music, videos, etc. in the background.

- **In the Chat - Stay on topic.** Please use the chat window for questions and comments that are relevant to the class; the chat window is not an appropriate place for socializing or posting distracting comments. The chat window should be kept free of off-topic information to allow others to quickly sort through information needed to address questions/comments about course material.

- **Disrespectful comments and hate speech will not be tolerated in the chat.** Just as in the physical classroom, respectful behavior is expected from all class participants. In order to protect the safety of all students in the course, any student making disrespectful comments or hate speech in the Zoom meeting or in the Chat will be removed from the Zoom meeting for the day and will not be permitted to rejoin the course until meeting with the course instructors to review appropriate behavior standards. Repeated violations will be reported to Student Affairs.

- **Chat transcripts will be archived and made available for review by all students in the course.** Private chats are disabled for this course (Zoom does record all private chat transcripts and makes them available to the meeting host – keep that in mind in general), so please use the main chat room or Breakout chat room to post all of your questions and comments.

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 that all individuals have full access to the introductory sessions and any virtual discussions that occur in the main Zoom room for the course, we will be recording the lecture and initial meeting portion of the course. These will be posted to Panopto and linked to on Lab Archives. Having access to the recording in no way replaces being present--either in person or via Zoom--for the course at its assigned time so that you can participate in experiments. These recordings will only be made accessible to individuals enrolled in the course.