BIOSC 1290: MICROBIOLOGY GENETICS LABORATORY
Spring 2025

Instructor: Dr. Cara Haney, chh333@pitt.edu
Office Hours: last hour of class or by appointment

Course Meeting Times

The lab meets **ONCE** a week.

Section	Time	Classroom
1290	Th 1:30 PM – 5:20 PM	G5 Clapp Hall

Course Description

This authentic research microbiology lab is part of the instructor, Dr. Cara Haney's, Research program in the Department of Biological Sciences. As an authentic research experience, it will involve substantial effort in the lab, and some effort out of the lab. It should provide you with hands on experience that demonstrate an authentic scientific process as well as complement concepts in Microbiology Lecture.

Research objective: We will perform a genetic screen to identify genes involved in regulation of protease activity, antifungal activity, and plant association in the beneficial bacterium *Pseudomonas fluorescens* WCS365.

Research approach: We will use traditional genetic screening methods to perform an *in vitro* screen for *P. fluorescens* WCS365 transposon insertion mutants that have lost ability to make protease as indicated by reduced ability to clear milk agar. Once identified, we will find genetically and phenotypically characterize the mutants in both *in vitro* as well as *in vivo* (plant and fungal) assays.

Learning objectives: Over the course of the semester, you will i) learn basic techniques and methods related to genetic screening, molecular biology, and bioinformatics in bacteria, ii) develop skills in reading, understanding, and implementing experimental protocols, iii) analyze and interpret experimental results including troubleshooting, iv) communicate scientific results in the form of scientific record keeping (lab notebooks), and manuscript writing, v) critically read, analyze, and discuss scientific literature, and vi) learn how to collaboratively communicate and work as a team.

Late Add Policy

Because experimentation and other class activities start on the first day of class, adding the course during the Add/Drop period will result in you missing important course work, including work vital to the course research project. Please be aware that you are responsible for catching up and completing any missed work. **The deadlines for any project-associated work and submissions cannot be extended. Some work may not be possible for you to make up.**

Course Requirements

1. Attendance for Lab
    - All components of this course will occur in person. Attendance is mandatory. See **Absence Policy** for more details.

	2. Technology • A charged device that can access both Canvas and Google Docs during class. • Reliable internet connection outside of class. • PubMed, BLAST or other scientific databases and tools may be used throughout the course. 3. Reading Material • No required textbook or lab manual. • A microbiology textbook may be helpful for background review of topics. • Journal articles and experimental protocols will be provided via Canvas.
Lab Safety	There is **NO** food, drink, cosmetics, etc. allowed in the laboratory classroom. Additionally, your feet and torso must be fully covered, while your thighs must be more than half covered when seated. You will be provided a lab coat for the semester. There are goggles within the classroom to use as needed. If any of the rules above are violated, you will be asked to leave lab and that will count as an **unexcused absence**. **Handling of electronics (phone, tablet, computer) during lab must be done with clean, ungloved hands.** An exception is made for phones if they are within the provided Ziploc phone bag. Additional safety information will be covered during the first lab meeting, and you will be **required to sign a lab safety agreement to participate in this lab**.
Course Tools	**Canvas** will be used extensively in this course to post course assignments, documents, experimental protocols, and up-to-date announcements. There will be a separate Canvas page dedicated to lecture materials. We will use **Google Docs** as an electronic notebook. A single contiguous document with individual sections dedicated to different days and experiments. Entries in Google Docs should be entered in a timely manner (you will forget details if done late!). See **Student Grading** and **Late Assignments** more details. In general, I will check your notebooks within 24 hours of the lab and so they should be completed by the end of each lab class.

Student Grading

Good laboratory work develops as a combination of many different skills. If you are better at the lab bench than at the examination desk, we want your grade to give you credit for that. Consequently, our evaluation of your coursework will proceed at several levels.

Your course grade will be calculated as follows (note that the exact proportion may change during the semester):

Assessment	**Points**	**% of Final Grade**
Lab assignments & Pre-lab reviews	150 pts	30%
Notebooks	150 pts	30%
Final Manuscript/Lab Report	150 pts	30%
Lab Participation & Professionalism	50 pts	10%
Total	**500 points**	**100%**

Your final grade will be rounded to the neared whole number and based off the following scale:

Grade	Grade %
A+	98 - 100
A	92 - < 98
A-	90 - < 92
B+	88 - < 90
B	82 - < 88

Grade	Grade %
C+	78 - < 80
C	72 - < 78
C-	70 - < 72
D+	68 - < 70
D	62 - < 68

Lab assignments and Pre-labs (30%): Periodically there will be homework or reading outside of pre labs assigned. Individual homework assignments will include course surveys, lab skills practice, scientific article review, and updating course-wide data sheets. There will be multiple pre labs during the semester that will help you prepare for the upcoming lab(s). Each lab is worth 10 points and will be an assignment. Pre-labs may include reading protocols, estimating agarose band sizes, performing protocol related math, or updating your lab notebook with information. Each class will start with a group review and discussion of the prelab.

Notebooks (30%): Notebooks are an extremely important part of scientific research. Notebooks should be self-contained and organized such that other researchers could understand and reproduce your recorded experiments. Notebook pages will begin with high amounts of scaffolding that will decrease as the semester continues as you gain note keeping skills. While work in this lab will be conducted as a group, **each person will keep their own lab notebook to be completed independently. <u>Notebook entries for are worth 10 points each and will be due at the end of each lab class</u>**. Entries should be entered in a timely manner (you will forget details if done late!).

Notebooks will be retained after completing the course, since the data obtainec may be used by researchers to inform further experiments and/or for publicatio See **Keeping an Electronic Lab Notebook PDF** on Canvas for more information.

Final manuscript/lab report (30%): Each **group** will generate a final manuscript, written in the style of a scientific journal article, describing the research perforn over the term. This will include 6 sections: **Abstract, Introduction, Results, Discussion, Materials and methods, and References**. Different sections (or sub-sections) will be due throughout the term to allow for feedback and revision far advance of the final manuscript.

Lab Participation & Professionalism (10%): It is crucial that you are coming to tl lab prepared by reading the day's protocol(s), completing the appropriate pre-la assignment, as well as keeping up to date on your notebook pages. Additionally, is important that you pay attention to the beginning of the lab where technique protocols and procedures are introduced. Lack of preparation may result in difficulty performing the day's experiments correctly. It is equally important to perform good lab citizenship in a shared lab space. This means prioritizing safet properly handling equipment or reagents, keeping your space clean and organiz being aware of your surroundings, and leaving the lab looking better than you found it. At the end of each lab session, there will be a clean-up sheet to be fille out by the group reviewing the state of your group's bench and where any point deductions for poor clean-up will be noted by the instructor. Professionalism wi also include points for lab meeting discussions and any major group issues that arise.

Assignment Regrading Policy	You may request a re-grade of any portion of an assignment by submitting your request in writing and explaining why you think the grading was in error. You must include a detailed justification for the correctness of your answer, including references to text used in the course (text, page, paragraph) or scientific articles (citation, page, paragraph). This request must be submitted to the instructor within one week (5 business days) after the date the assignment is returned/grade is posted to Canvas. Re-grading requests raised beyond a week after an assignment has been returned/grade is posted will not be addressed.
Late Assignments	Lab and lecture assignments are due at the beginning of the following lab or lecture class as noted on the course schedule. Assignments are considered late if they are turned in after this designated due date time. Late assignment submission penalties (not including point deductions for incomplete/incorrect portions) for lab and lecture assignments are as follows: • Submitted within 24 hours of due date time: -10% • Submitted within 48 hours of due date time: -20% • Submitted after 48 hours of due date time: -50%

	This means that pre-labs or notebook pages edited after the due date may incur late deductions at the discretion of the instructor. Late entries of data within notebook pages that span multiple lab classes (i.e. entering data 1-2 labs after it was initially recorded) are also subject to point deductions. However, extenuating circumstances can occur that may prevent you from completing your work on time (family emergency, medical emergency, etc.). In those circumstances, communication with the instructor as soon as possible (ideally 12+ hours prior to class) with some type of documentation may help facilitate adjusted deadlines if possible. However, **group assignments or assignments that have been graded and returned to the class cannot be submitted late**.
G grading	If you wish to petition for a G grade, you must submit a request for this change in writing and you must document your reason(s). You will be required to make arrangements in person for the specific tasks you must complete in order to remove the G grade. You will be expected to sign documentation describing the work that has to be completed and the due date. All required work must be completed by the specified date otherwise a zero will be assigned for the work and final grade will be determined using this score. Remember that G grades, according to CAS guidelines, are to be given only when students who have been attending a course and have been making regular progress are prevented by circumstances beyond their control from completing the course after it is too late to withdraw.

Absence Policy

All students are permitted one absence, BUT you must contact the instructor and your group members before class. Because the majority of work is done in class and as a group, penalties for additional absences are as follows:

1 absence	No penalty
2 absences	Deduction of 1 letter grade from final grade
>2 absences	Failure for class (if withdrawal or G grade not taken)

Absences will not excuse you from responsibility for assignments, notebooks, or other missed work.

The majority of the lab work will be performed as a team. **It is your responsibility to inform both your instructor and your group members of your absence** so they can adjust their experimental plan for the day. If you miss a class because of an emergency, please send an email to your instructor or ask someone else to notify your instructor.

Since the introductory and background material for a class is presented at the beginning of the session, **missing the start of a class (20 minutes) counts as a minimum of missing one half of the class.** If you arrive to class late, you will be allowed to participate in the day's experiments *only* at the discretion of the instructor.

Academic Integrity

Students in this course will be expected to comply with the University of Pittsburgh's Policy on Academic Integrity. Any student suspected of violating this obligation for any reason during the semester will be required to participate in the procedural process, initiated at the instructor level, as outlined in the University Guidelines on Academic Integrity. This may include, but is not limited to, the confiscation of the examination of any individual suspected of violating University Policy. Furthermore, no student may bring any unauthorized materials to an exam, including dictionaries and programmable calculators.

To learn more about Academic Integrity, visit the Academic Integrity Guide for an overview of the topic. For hands- on practice, complete the Understanding and Avoiding Plagiarism tutorial.

Please note that academic integrity goes in both directions. Just as we expect you to act in an honest and honorable manner when attending this class, we will treat all students fairly and respectfully. We will not favor any student nor discriminate against any student.

Student Conduct	In keeping with the University of Pittsburgh Student Code of Conduct, all students are expected to behave as respectful and civil members of the university community. All instructors and students will act in a considerate manner in order to create and maintain a classroom atmosphere that is conducive to learning. In addition to being unacceptable on the grounds of common decency, disruptive and disrespectful behavior contributes to unsafe working conditions. Disruptive and disrespectful behavior will not be tolerated! Examples of disruptive behavior include, but are not limited to, repeated tardiness, texting in class, speaking or acting in any sexually, racially, or ethnically harassing manner, cheating, misuse and abuse of laboratory equipment and material, disregarding any safety guidelines. Disruptive students will be referred to the Office of Student Conduct for mediation, discipline, or both.

Email Communication Policy	Each student is issued a University e-mail address (username@pitt.edu) upon admittance. This e-mail address may be used by the University for official communication with students. Students are expected to read e-mail sent to this account on a regular basis. Failure to read and react to University communications in a timely manner does not absolve the student from knowing and complying with the content of the communications. The University provides an e-mail forwarding service that allows students to read their e-mail via other service providers (e.g., Hotmail, AOL, Yahoo). Students that choose to forward their e-mail from their pitt.edu address to another address do so at their own risk. If e-mail is lost as a result of forwarding, it does not absolve the student from responding to official communications sent to their University e- mail address. To forward e-mail sent to your University account, go to http://accounts.pitt.edu , log into your account, click on **Edit Forwarding Addresses**, and follow the instructions on the page. Be sure to log out of your account when you have finished. (For the full E-mail Communication Policy, go to www.bc.pitt.edu/policies/policy/09/09-10-01.html.)
Disability Services	If you have a disability for which you are or may be requesting an accommodation, you are encouraged to contact both your instructor and Disability Resources and Services (DRS), 140 William Pitt Union, (412) 648-7890, drsrecep@pitt.edu, (412) 228-5347 for P3 ASL users, as early as possible in the term. DRS will verify your disability and determine reasonable accommodations for this course.
Preferred Names and pronouns	Course rosters are provided to instructors by the University with students' legal names. We will gladly honor your request to be addressed by an alternate name or gender pronouns. Please let us know of this preference early in the semester so that we can update our records. In addition, there is the NameCoach Roster on Canvas.
Accessibility	Canvas is ADA Compliant and has fully implemented the final accessibility standards for electronic and information technology covered by Section 508 of the Rehabilitation Act Amendments of 1998. Please note that, due to the flexibility provided in this product, it is possible for some material to inadvertently fall outside of these guidelines.

Your Well-being Matters	College/Graduate school can be an exciting and challenging time for students. Taking time to maintain your well-being and seek appropriate support can help you achieve your goals and lead a fulfilling life. It can be helpful to remember that we all benefit from assistance and guidance at times, and there are many resources available to support your well-being while you are at Pitt. You are encouraged to visit Thrive@Pitt to learn more about well-being and the many campus resources available to help you thrive. If you or anyone you know experiences overwhelming academic stress, persistent difficult feelings and/or challenging life events, you are strongly encouraged to seek support. In addition to reaching out to friends and loved ones, consider connecting with a faculty member you trust for assistance connecting to helpful resources. The University Counseling Center is also here for you. You can call 412-648-7930 at any time to connect with a clinician. If you or someone you know is feeling suicidal, please call the University Counseling Center at any time at 412-648-7930. You can also contact Resolve Crisis Network at 888-796-8226. If the situation is life threatening, call Pitt Police at 412-624-2121 or dial 911.
Equity, Diversity, and Inclusion	The University of Pittsburgh does not tolerate any form of discrimination, harassment, or retaliation based on disability, race, color, religion, national origin, ancestry, genetic information, marital status, familial status, sex, age, sexual orientation, veteran status or gender identity or other factors as stated in the University's Title IX policy. The University is committed to taking prompt action to end a hostile environment that interferes with the University's mission. For more information about policies, procedures, and practices, visit the Civil Rights & Title IX Compliance web page. I ask that everyone in the class strive to help ensure that other members of this class can learn in a supportive and respectful environment. If there are instances of the aforementioned issues, please contact the Title IX Coordinator, by calling 412648-7860, or e-mailing titleixcoordinator@pitt.edu. Reports can also be filed online. You may also choose to report this to a faculty/staff member; they are required to communicate this to the University's Office of Diversity and Inclusion. If you wish to maintain complete confidentiality, you may also contact the University Counseling Center (412-648-7930).
Statement on Classroom Recording	To ensure the 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 in advance can be used solely for the student's own private use.
Copyright Notice	These materials may be protected by copyright. United States copyright law, 17 USC section 101, et seq., in addition to University policy and procedures, prohibit unauthorized duplication or retransmission of course materials. See Library of Congress Copyright Office and the University Copyright Policy.

BIOSC 1290: MICROBIOLOGY GENETICS LABORATORY
Spring 2025; Lecture Schedule as of January 7th

Note: As this is an authentic research experience, the schedule is subject to change as issues arise and we learn new information.

Date (Thurs.)	**Lab #**	**Class Focus**	**Assignments (due by the start of class unless otherwise noted)**
1/9	Lab 1	**Lecture**: Introduction to course and course project **Lab:** Unit conversions, streaking, sterile technique, start lab notebook	**Pre-Lab Survey (by end of class)**
1/16	Lab 2	**Lecture:** Introduction to course project **Lab:** Assign to groups, peer contracts, assess streaking from last week, serial dilutions to quantify CFU to OD conversations.	**Pre-Lab 2**
1/23	Lab 3	**Lecture:** More on genetic screening. **Lab:** Start genetic screen.	**Pre-Lab 3**
1/30	Lab 4	**Lecture:** Overview of mapping mutations **Lab:** Groups streak candidate mutants and document phenotypes	**Pre-Lab 4**
2/6	Lab 5	**Lecture:** DNA preps and freezer stocks **Lab:** Make glycerol stocks from overnight cultures (started by instructor), verify phenotype from streak plates last week, DNA extractions, quantify DNA.	**Pre-Lab 5**
2/13	Lab 6	**Lecture:** Mapping transposons: arbitrary PCR background and overview **Lab:** Set up arbitrary PCR first round; PCR cleanup	**Pre-Lab 6** **Manuscript:** Methods section and results to date
2/20	Lab 7	**Lecture:** Review arbitrary PCR **Lab:** Set up second round arbitrary PCR, check on gel, send for sequencing	**Pre-Lab 7**
2/27	Lab 8	**Lab:** Catchup; Run Arbitrary PCR on gels, PCR cleanup, troubleshoot those that didn't work	**Pre-Lab 8**
3/6		No Class, Pitt Spring Break	
3/13	Lab 9	**Lecture:** BLAST overview **Lab:** Annotation of Sequence insertion, catchup time	**Pre-Lab 9**
3/20	Lab 10	**Lecture:** Functional annotations **Lab:** Continue annotation of BLAST sequences; Present and discuss candidate insertion sites in the class	**Pre-Lab 10** **Manuscript:** Draft of introduction; Methods section and results to date
3/27	Lab 11	**Lecture:** Functional assays discussion **Lab:** Functional assays, transfer seedlings, inoculate plants with mutants	**Pre-Lab 11**

		Spot dilutions onto milk agar to quantify zone of clearing	
4/3	Lab 12	**Lab:** Image plants, measure root length; set up Fungal inhibition assays and growth curves	**Pre-Lab 12**
4/10	Lab 13	**Lab:** Quantify fungal inhibition, analyze growth curve data	**Pre-Lab 13**
4/17	Lab 14	Data analysis help, writing help, catchup.	**Pre-Lab 14** **Manuscript:** Full draft minus last few pieces of data (final manuscript due 1 week after class).