Genomics Research Initiative Laboratory II

BIMM170
Spring 2022
Tata Hall 2301
1-4 PM Tuesday
1-4 PM Thursday

Instructors:
Dr. Joe Pogliano
jpogliano@ucsd.edu
Office: 4111 NSB

Dr. Gary Heussler
gheussler@ucsd.edu
Office: 4600 Tata Hall

Teaching Assistant
Peter Berube
pberube@salk.edu

Preparation Room:
Dr. Ana Gomez
Office: 2105 Tata Hall

Schedule: **Subject to change!

DATE	Class Outline	Presentation/Assignment
1. Tue, March 29th	Lecture: Overview of plan and goals for quarter	**ASSIGNMENT:** Test question on Canvas
2. Thur, Mar 31	Lecture: -How to map Mutations to a sequence -Discussing the different effects of mutations on bacterial behavior depending on what gene is mutated Lab: -Bioinformatic analysis to identify the mutations. Assign students to groups of 4 or 5 to work together to identify the mutations. 7 total mutants. Write down other SNPs, see if they think those are true mutations.	**READING:** Guide on using Geneious for mapping mutants for use in class. **ASSIGNMENTS** **Presentation at the end of class:** each group (only 7) present their results to the class: 1. What genes are mutated? What is the function of this gene? 2. Have these proteins previously been shown to be used as a receptor by phage? **Written assignment (TURN IN ON CANVAS after class):** 1. What genes are mutated? What is the function of this gene? 2. Have these proteins previously been shown to be used as a receptor by phage? Is so describe and example.

3. Tue, Apr 5th	Lecture/Demo: -Brief overview of motility assay including information on bacterial motility Laboratory: -Motility assay using soft agar plates -Include control strains and inoculate for motility assay	**READING:** Instructions on motility assay to test mutants Motility review (make sure to read guide sheet first!) **Written assignment (TURN IN ON CANVAS):** Prior to class answer guided questions for Motility review posted to Canvas.
4. Thur, April 7th	Lecture: -Plasmid Design basics -Expression vector basics -examples of overexpression as a tool in bacteriophage research Laboratory: -Design plasmids to complement the groups specific mutation -Document Motility plates	**READING:** AddGene blog, What is a plasmid? https://blog.addgene.org/plasmids-101-what-is-a-plasmid **Written assignment (TURN IN ON CANVAS):** Prior to class answer guided questions posted to Canvas
5. Tue, April 12th	Lecture: -How mutations in common receptors may protect against other phage -Introduction to biofilm assay, flagella aren't just for motility Laboratory: -Set up biofilm assay	**READING**: Watch JoVE video on Biofilm Assay posted to Canvas **Written assignment (TURN IN ON CANVAS):** Prior to class answer questions related to biofilm assay posted to Canvas

6. Thur April 14th	Lecture: -Biofilms and Phage Laboratory: -Finish biofilm assay by staining wells and quantifying biomass	**READING**: Biofilms and Phage review (make sure to read guide sheet first!) **Written assignment (TURN IN ON CANVAS):** Answer guide questions on Biofilms and Phage review (only read Introduction and sections 2, 2.1, 2.2, and 2.3)
7. Tue, April 19th	Lecture: -Quick overview of transformation Laboratory: -Head up to TATA 4th floor for electroporation -Recover cells for 1 hour -Plate electroporated cells	**READING:** Khan Academy overview of Bacterial Transformation: https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/bacterial-transformation-selection **Written assignment (TURN IN ON CANVAS):** Prior to class answer guided questions posted to Canvas
8. Thur, April 21st	Lecture: **-Discussions on the necessary controls for complementation** -limits of over expressed vector -possible reasons why colonies may appear on selection plate Laboratory: -Purify and confirm that the transformants have the correct plasmid (and not just abx resistant mutants) by restreaking and checking morphology	**READING:** NEB essay, Foundations of Molecular Cloning - Past, Present and Future **Written assignment (TURN IN ON CANVAS):** -Prior to class answer guided questions posted to Canvas

9. Tue, April 26th	Lecture: -Refresher on fundamentals of spot titering Laboratory: -Using mutants, WT, and complemented strains, spot titer 201phi2-1	**READING** -Spot titer protocol
10. Thur, April 28th	Lecture: Interplay between Antibiotic Resistance and Bacteriophage Laboratory: -Repeat Spot titering on same strains as previous lab -Antibiotic resistance testing of complemented mutants	**READING** -Phage-antibiotic combinations review (make sure to read guide questions first!) **Written assignment (TURN IN ON CANVAS):** -Prior to class answer guided questions posted to Canvas
11. Tue, May 3rd	Lecture: Different types of motility and this impacts phage predation Laboratory: -Motility Assay using Complemented Mutant and Mutant with Empty Vector control	
12. Thur, May 5th	Lecture: -Phage phylogeny -the mosaic nature of phage genomes Laboratory: -Generate phylogenetic tree using sequences of other *P. chlororaphis* phage as well as other *Pseudomonas* sp. phage	**READING** -Overview of bacteriophage phylogenic analysis. **Written assignment (TURN IN ON CANVAS):** -Prior to class answer guided questions posted to Canvas
13. Tue, May 10th	Lecture: -Phage communities and the microbiome Laboratory: Spot titer alternative *P. chlororaphis* phage using WT,	**READING**: Phage and Human Microbiome review (make sure to read guide sheet first!) **Written assignment (TURN IN ON CANVAS):**

	mutant, and complemented mutant *P. chlororaphis*	Answer guide questions on Phage and Human Microbiome review
14. Thur, May 12th	Lecture: -Phage communities and bacteriophage therapy Laboratory: -Repeat spot titering assay	
15. Tue, May 17th	Lecture -Basics of Competition Assays Laboratory: - Set up competition assay between complemented mutant *P. chlororaphis* and mutant with empty vector	To Be Updated
16. Thur May 19th	Lecture: - Competition Assay instructions continued Laboratory: -Plate out bacteria from competition assay	To Be Updated
17. Tue May 24th	To Be Updated	To Be Updated
18. Thur May 26th	To Be Updated	To Be Updated

19. Tue May 31st	To Be Updated	To Be Updated
20. Thur June 2nd	Lab Clean up	To Be Updated

Campus Safety Requirements and Expectations

Keeping our campus healthy takes all of us. You are expected to follow the campus safety requirements and pursue personal protection practices to protect yourself and the others around you. These include:

- **Participate in the university's daily screening process.**
  Everyone must complete a Daily Symptom Survey to access a university-controlled facility.
- **Participate in the university's testing program.**
  All students are required to participate in the COVID-19 Testing program as required by their vaccination status:
  - Unvaccinated students with approved exceptions must complete a COVID-19 test twice a week.
  - Students who are fully vaccinated must complete a COVID-19 test once a week, for the first four weeks of the quarter.
- **Wear a well-fitted face covering that covers your nose and mouth at all times.**
  Everyone is required to wear face coverings indoors regardless of vaccination status. If you see someone not wearing a face covering or wearing it incorrectly, then kindly ask them to mask up.
- **Monitor the daily potential exposure report.**
  Every day the university will update the potential exposure report with building and some classroom information and the dates of exposure. Download the CA COVID Notify app to your phone to receive an alert if you have been potentially exposed to COVID-19.
- **Assist in the contact tracing process.**
  If you're contacted by a case investigator, it means you have been identified as close contact, please respond promptly. You must assist with identifying other individuals who might have some degree of risk due to close contact with individuals who have been diagnosed with COVID-19.
- **Contact the instructional team if you are impacted by COVID-19**

Please note that due to the ongoing COVID-19 Pandemic, changes may be made in response to new developments and information.

Lab effort and conduct (100 points total, 20 collaborative)
Students will be evaluated on overall laboratory performance, including mastery of lab methods (including lab safety procedures), professional behavior towards other students, instructors and TA, coming to lab prepared, and contributing to collaborative team efforts. Everyone will start off with full credit, with points deducted at the discretion of the instructors and TA for consistently arriving late or leaving early, lapses in safety procedures, failing to clean up properly, breaking or abusing equipment, unexcused absences, and for failing to work well with classmates.

Assigned Reading, Presentations and Homework Questions (100 points total)
Throughout the term there will be reading assignments posted to Canvas. As there is no official textbook for this class, these assignments include various articles and literature reviews which will be accompanied by guide questions posted to the Quizzes tab on the course Canvas. The goal of these assignments is two-fold. One goal is to assess understanding of the assigned reading relating to a laboratory topic, and the other is two help guide what type of information we want you to retain from the readings (specifically relating to literature reviews which can be overwhelmingly detailed). In addition, with certain classes students will give short 3-5 minute presentations on their data from that day. These will be further outlined in class.

Laboratory notebook (200 points)
Students are required to keep a laboratory notebook, in which they record how experiments were performed, their results, data interpretation and future experiments. The general goals of lab notebooks are to (1) record your results, (2) allow anyone to repeat the experiment exactly as you did it, (3) provide a resource for trouble shooting experiments, with sufficient detail to later recognize small differences in experimental protocols (such as slight differences in time or mixing method) that can make the difference between a successful experiment and a failure, and (4) provide a legal record of your discoveries for future patenting activities (!).

Notebooks must be legible and neat enough for others to follow, but they do not have to be beautiful. Write in pen and if you make a mistake cross it out and write the correction. Do not erase or add or remove pages! Tape in photos of gels, plaques, etc. Bring the notebook to class everyday, because it will be periodically checked and graded during class time. The first notebook check will be for feedback so that you can modify your note-taking habits. The section "Before You Start", Part B in the lab manual provides a good overview of lab notebooks.

Notebook grading points distribution:
- Are methods, results and conclusions from all experiments clearly described? (100 points)
-Are key photos included (the location where you found your phage, your plaque morphology, all titer plates) (60 points)
-Does each entry have a title, date and hypothesis? (30 points)
-Do you have a complete table of contents? (10 points)

You need to write down everything you do either when or before you do it, your memory does not suffice! It is a good habit to write down everything you plan to do *before* you do it (noting

any changes to this plan in the margin as you go), and to use this (rather than the lab manual) to guide what you do in the lab. You should read the protocol and write an outline in your lab notebook before you come to class, leaving space to write any modifications to this protocol.

Powerpoint presentation of your overall results (100 points)
Near the end of the term, each student will give a 5-10 minute presentation of the results of your research. Details regarding the presentation format will be provided in the classroom.

Summary:
100 points – Effort and conduct
100 points – Assigned reading (presentations and homework questions)
200 points – Notebook
100 points – Final Presentation
500 points total

Academic Integrity
Cheating is not tolerated. Scientific research is completely dependent on the integrity and transparency of the scientists involved. All work should be your own. This can feel less clear-cut for laboratory classes where you do almost all of your work with a lab partner. You will share data (numbers and outcomes) with your lab partner, but the words, interpretations, and notes should be your own words. The UCSD Office of Academic Integrity defines cheating as follows:

"Cheating occurs when a student attempts to get academic credit in a way that is *dishonest, disrespectful, irresponsible, untrustworthy or unfair."*

All incidents of cheating will be reported to the Office of Academic Integrity. If you have any questions about academic integrity or cheating, please ask any of the instructors or your TA. When in doubt, ask first. We also encourage you to visit the website of the Office of Academic Integrity at UCSD: http://academicintegrity.ucsd.edu