

Genomics Research Initiative Laboratory

BIMM171

Fall 2018

1-5 PM Wednesday

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York Hall 4406

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Tentative Schedule

Date	Laboratory Activities	Laboratory reading - posted on Ted Check frequently for updates		Tentative Lecture Schedule
		Lab manual	Other protocols	
F Sept 28	Laboratory Safety Pipet Exercises Practice sterile technique and streaking samples Collect soil samples – 2 samples needed before next class	Chapters 1, 2, and Chapter 3 – up to pg21 <i>“Eating Away at Bacteria”</i> **Chapter 5, pgs 54- 56 - Read before collecting soil samples. **	Day 1 - Practice exercises protocol	Safety Course overview Importance of pure culture Introduction to bacteriophages
W Oct 3	Cheese tasting Set up Enrichment Cultures – with soil and cheese samples Practice pour plating (time permitting)	Chapter 4, pgs 32-38 <i>“An Introduction to bacteria.”</i> and <i>“An Introduction to the Host Bacteria, Arthrobacter sp.”</i> , Chapter 5 pgs 51 - 53	Enrichment culture protocol – part 1	Introduction to host organism Introduction to cheese bacteria Guest lecture – Dr. Rachel Dutton
F Oct 5	Practice making serial dilution calculations Test enrichment cultures for phage	Chapter 5 pgs 72 – 75 Chapter 6 pgs 84 -87	Enrichment culture protocol – part 2	
W Oct 10	Pick plaques and streak purify Repeat spot titer if necessary Streak cheese rinds for cheese bacteria isolation	Chapter 3 pgs 21 – 27 Chapter 12 pgs 186 - 189	Streak purify phage protocol Cheese bacteria isolation and purification protocol	Breaking and Entering: Details of phage infection – part 1
F Oct 12	Quiz 1 Pick colonies from cheese plates and streak putative cheese bacteria Continue plaque purification – streak purify	Chapter 3 pgs 21 - 27		Breaking and Entering: Details of phage infection – part 2

Oct 15 – 16 M, Tu	Continue plaque purification on own time if necessary			
W Oct 17	Restreak cheese bacterial strains Continue plaque purification – serial dilution and plaque assay	Chapter 3 pgs 27 - 30 Chapter 6 pgs 81-83	Serial dilutions and plaque assay visual outline	Mechanisms of Insensitivity
F Oct 19	Preparation for 1 plate phage lysate – pick single plaque, serial dilutions, plaque assay			
W Oct 24	Flood (~2hrs) and harvest phage lysate from one plate and spot titer phage lysate Restreak cheese bacteria candidates Submit phage name for approval	Chapter 6 pgs 88 – 96	Phage naming rules	
F Oct 26	Calculate titer of 1 plate phage lysate Repeat plaque assay for second round of phage lysate harvest.	Chapter 7 pgs 102 - 109		Bacterial diversity and identification
W Oct 31	Quiz 2 Flood (~2hrs) and harvest the best plates from second round of plaque assay Spot titer phage lysate Save phage lysate at 4 degrees Restreak cheese bacteria candidates			
F Nov 2	Set up 10 plates with correct phage titer to make phage lysate	Chapter 8		Electron microscopy

	for EM/DNA isolation and long-term storage			
W Nov 7	Flood 10 plate lysate in the morning – no later than 11am Harvest 10 plate lysate and spot titer Start liquid culture from two pure colonies of putative cheese bacteria			
F Nov 9	Submit phage for EM (100 µl of best lysate) Bacterial DNA isolation		DNA isolation manual	DNA isolation
W Nov 14	Quiz 3 Begin electron microscopy Start phage DNA isolation	Chapter 9		DNA isolation in bacteria vs phage
F Nov 16	Finish phage DNA isolation Continue electron microscopy			
W Nov 21	Open Lab	Open Lab		No Lecture
F Nov 24	No Class	Thanksgiving		No Class
W Nov 28	Restriction digests and run gel Complete entry on phagesdb	Chapter 10 Chapter 7 pgs 110 - 113		Gel electrophoresis PCR
F Nov 30	Quiz 4 Set up 16s rRNA PCR Estimate genome size Calculate capsid size and tail length		PCR protocol	16s rRNA
W Dec 5	Submit phage lysates for storage Powerpoint presentation of			

	phages			
F Dec 7	Practicum Final Lab Clean up			
Finals Week	No Class	No Class		No Class

Triton Ed: We will be using Triton Ed (<https://triton.ed.ucsd.edu/webapps/login/>) as the course web site. Once you are enrolled in the class you will have access using your ACS username and password. The lab manual, other protocols and any additional reading material will be posted on TEd. Be sure to check TEd frequently for announcements, important deadlines, and schedule updates.

Grade Assignments:

- 100 points – Effort and conduct
- 100 points – Quizzes
- 100 points – Practicum
- 200 points – Notebook
- 100 points – Presentation and Archive report
- 600 points total

Lab effort and conduct (100 points total, 20 collaborative)

Students will be evaluated on overall laboratory performance, mastery of lab methods (including lab safety procedures), professional behavior towards other students, instructors and TA, lab preparation, 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.

Quizzes and Assignments (100 points total)

There will be short four quizzes with 10-15 questions (see schedule above) during the quarter. The quizzes will cover material from the previous weeks – **including protocols, lecture material, and lecture reading assignments**. The goal is to assess understanding of basic lab protocols and phage biology. The best way to study is to read the protocol ahead of time, attend class, take notes, and ask questions. **There are no make-ups for quizzes**. Unexcused absences will result in a zero on that quiz.

Throughout the course there will be assignments that will either be completed during the lab or as homework. These assignments will include problem sets practicing calculations that you will have to do during the course, and group exercises involving primary literature.

Practicum (100 points)

Towards the end of the quarter, we will have a practicum test (rather than a final exam) in which each student will perform a variety of key lab skills (i.e. titrating a phage stock, interpreting results, troubleshooting) at various stations set up throughout the lab. The practicum is cumulative. Attendance is mandatory. There is no way to make-up the practicum. **This year the practicum is on Friday, December 7th.**

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 (!). Just as in any scientific lab, we hope to publish our findings, and your lab notebooks must provide information on exactly where and how each phage was isolated or we cannot include your results or consider it as a candidate for sequencing. Even phage that are not sequenced will be submitted to the Howard Hughes Medical Institute for archiving and possible use in future experiments, but only if your lab notebooks provide sufficient documentation of the phage you isolate.

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 lab manual provides a good overview of lab notebooks.

Since majority of the protocols are online (on Ted), you must read the relevant protocols and write an outline in your lab notebook **before** you come to class, leaving space to write any modifications to this protocol. It is a good habit to write down the overall plan for each lab and note any changes to this plan in the margin as you go. You should use your lab notebook, rather than the lab manual, to guide what you do in the lab. **You need to write down everything you do either when or before you do it, your memory does not suffice!**

Powerpoint presentation of your results and online archive reports (100 points)

At the end of the class, each student will give a 3 to 5 minute presentation of the results of your research. Details regarding the presentation format will be provided in the classroom. In addition, each phage isolated by our class will be archived at the Howard Hughes Medical Institute for potential use in future experiments. Each student will complete an online archive report describing their phage and summarizing the results from various experiments. The

report will be submitted, along with a sample of a phage lysate and phage DNA (if available). 80 points for the presentations, 15 points for submitting the electronic version of the archive report, and 5 points for adding the lysate (and DNA) to the appropriate box in the lab fridge.

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>