BIMM 121 Laboratory in Microbiology
Fall 2011

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Office hours: Mondays 2 pm – 3 pm

Lecture: Mon/Wed/Fri 9:00 – 9:50 PCYH 122 (Pepper Canyon Hall, next to Gilman Parking structure)

Labs: York 2310 and 2332
Tuesday/Thursday: 9:30 am – 1:30 pm
Wednesday/Friday: 10:00 am – 2:00 pm

Course Structure:
This course will introduce you to the fundamentals of microbiology and allow you to explore the many ways in which microbes affect and are used in our lives. We begin the course with a foundation in basic techniques such as sterile techniques, microscopy, methods of quantitating microbes, and preparing and examining stained slides. The remaining duration of the course will comprise four main units: a comprehensive look at bacterial physiology, understanding the complex microbial community of soil, metagenomics as a tool in exploring complex communities, and the use of microbes in various aspects of our lives. Each of these units comprises several multi-day experiments and there will be considerable overlap in the execution, methodology, and analysis of data from each of these units. Throughout the course, you will also receive training in accurate data entry and analysis, scientific reasoning, and in clear and concise scientific writing.

Equipment:
For this lab you will need to purchase:

- A lab notebook (check with instructor to determine if notebook with carbons is required);
- A lab coat
- Eye protection (you may wear either safety glasses or goggles, but standard prescription eye glasses are not sufficient).
- A Sharpie permanent marker pen, preferably fine point (not extra fine or regular)

Attendance and Absences:

1. Your attendance is required at EVERY lab and through the entire lab period, until all the experimental work for the day is completed.
2. Absences will NOT be treated lightly. The labs are set up for groups of two or more and your absence will place an unnecessary burden on your partner. There are no make up labs and you will not be allowed in the lab on non-lab days or in the other Micro lab sections, although you may be asked to make up the work from the day you missed.
3. Documentation will be required for all unavoidable absences.
4. If you are likely to have interviews for graduate school, etc., please schedule them on non-lab days.
5. All absences without prior notification/permission and the appropriate paperwork will be considered unauthorized.
6. **50-point penalty** for the first unauthorized, unexplained absence from the lab. If there is a second such absence, you will be asked to drop the course or will be given an F.
7. If you are ill on a lab day or have an emergency, e-mail or call (instructor or lab partner) before the start of the lab. If you are ill enough to miss lab you must go to the student health center and provide documentation of your illness.

Lab report Deadlines and Submission:

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1. A hard copy of each lab report is due in the first 20 minutes of the lab period or the first 20 minutes of the lecture period of the day on which your report is due. Check with your instructor as to where the report should be turned in. Reports turned in more than 10 minutes after the start of class will be considered late. Penalty for late reports will be 10% for each day late.
2. In addition to the hard copy of the report, you are required to submit an electronic copy to Turnitin.com. A link to the e-submission website will be provided on Ted. Failure to submit on Turnitin.com will results in 0 (zero points) recorded for that report. Check the deadline of the Turnitin.com submission and make sure you adhere to it. Students agree that by taking this course all required papers would be subject to review for textual similarity by Turnitin.com for the detection of plagiarism. All submitted papers will be included as source documents in the Turnitin.com reference database solely for the purpose of detecting plagiarism of such papers. Use of the Turnitin.com service is subject to the terms of use agreement posted on the Turnitin.com site.

Turnitin submission is **not required for Homework** assignments are not required to

3. Additional points may be taken for late electronic submissions.

Regrade Requests:

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All regrade requests should be submitted in writing within one week of receiving the graded material.

Grading Scheme

Quiz/Report/Midterm	Points
Classroom evaluation	70 points
Safety	
Notebook checks	
Techniques	
Pop quizzes	
TA eval	
Homework	100 points
Lab report 1: Data analysis:	60 points
Lab report 2: Unknown	70 points
Lab report 3: Enrichment & Metagenomics	100 points
3 Midterms	250 points
Total	**650 points**

Most Likely Grade Distribution

A = 90% - 100%

B = 80% - 89.9%

C = 70% - 79.9%

D = 60% - 69.9%

F = below 60%

Notebook:

Spiral bound or composition notebook is OK. All notebooks should have a table of contents (handwritten OK) so on the first lab day leave several blank pages at the beginning of your notebook. Number your pages. Entries should be made in chronological order and EVERY day. Each day's entries on each experiment should begin with a brief (1 – 2 sentences) summary of work done on the same experiment the previous day.

How to use your notebook

Table of contents – update everyday – leave at least 4-5 pages for updating
Start a new page each day for each new experiment:

- Purpose of experiment
- Procedure
  - Outline or page from which protocol was taken
  - Note any changes
  - Note who did which part of the procedure – who inoculated controls, etc
  - Note which organisms you used – name and species of the controls, etc
  - Errors
- Observations
  - Write – in detail
  - Draw – enlarged, labeled, and including as much detail as possible
  - Questions and connections
- Conclusion or summary
- Answer any questions in the manual or that were raised in class.
- Number your pages

You may leave space to complete an experiment. When the experiment is complete and all observations have been made, cross off any blank pages or parts of pages following the written portion.

Lab Performance and Participation

In addition to quizzes, midterms, lab reports and howmework assignments, student evaluations will be based on the following criteria:

1. Lab techniques will be evaluated in class
2. Lab workshop participation

Subjective student evaluations will be based on the following criteria:

3. Pre-lab preparation
4. Careful management of lab procedures (e.g., sterile technique, proper waste disposal, experimental procedures, etc.)
5. Ability to adapt to unforeseen procedural changes
6. Caliber of thinking before asking questions
7. Scientific approach (e.g., proper use of notebooks, controls, experimental design)
8. Accuracy
9. Independence

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10. Safety consciousness
11. General neatness in lab

Please note**: You will be expected to get into the habit of methodical, well-planned and organized work by the mid-term. This will help you with the experiments in the second half of the course.**

Course Website

This course is on Ted (https://ted.ucsd.edu) and should automatically appear on your Ted account as soon as you register for the class. We will use Ted to post information on experiments, exams, schedules, readings and practice material, experimental data, report guidelines, etc. We strongly encourage you to use the Discussion board to post questions or answers to questions and to use it as a forum for exploring the material. The TAs and I will routinely check this website and answer any questions but feel free to respond as well. This website will also be used to post any announcements that pertain to the entire class. Please check the site regularly and update yourself on the information provided.

University Policy on Integrity of Scholarship

The principle of honesty must be upheld if the integrity of scholarship is to be maintained by an academic community. The University expects that both faculty and students will honor his principle and in so doing protect the validity of University grading. This means that all academic work will be done by the student to whom it is assigned, without unauthorized aid of any kind. Instructors, for their part, will exercise care in planning and supervising academic work, so that honest effort will be encouraged.

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Student Responsibility:

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Students are expected to complete the course in compliance with the instructor's standards. No student shall engage in any activity that involves attempting to receive a grade by means other than honest effort; for example:

- No student shall knowingly procure, provide, or accept any unauthorized material that contains questions or answers to any examination or assignment to be given at a subsequent time.
- No student shall complete, in part or in total, any examination, or assignment for another person.
- No student shall knowingly allow any examination or assignment to be completed, in part or in total, for himself or herself by another person.
- No student shall plagiarize or copy the work of another person and submit it as his or her own work.

- **If any work is plagiarized from that of another student, both students will be reported to the Office of Academic Integrity, even if one of the students has graduated already. Remember that most graduate schools check the undergraduate records for any indications of dishonesty before awarding a degree.**
- No student shall alter graded class assignments or examinations and then resubmit them for regrading.
- No student shall submit substantially the same material in more than one course without prior authorization.

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Lab	Date	Experiment	Reports, Midterms, Reminders
Lab 1	Thurs/Fri Sept 22/23	Registration, attendance, safety video, responsibility agreements, introductory remarks, Safety lecture **Sterile technique.** Microbes in the environment Why wash your hands? **Use of pipettors:** Demo and exercise	
Lab 2	Tues/Wed Sept 27/28	**Sterile technique.** Microbes in the environment: Observe results *E.coli* and toilet paper experiment: Observe results Aseptic technique: streak and spread plates Demo Lab exercise using a mixed bacterial culture **Microscopy:** Learning to focus the light microscope Demo Lab exercise using prepared (commercial) slides Cleaning your microscope – demo and completion **Plant pathogen interaction:** Inoculate *Kalanchoe* plant with *Agrobacterium*	
Lab 3	Thurs/Fri	**Microscopy:**	

	Sept 29/30	Calibrating your microscope: Demo and complete Making a wet mount and Phase Contrast Microscopy: Wet mounts and phase contrast:- view, identify, and measure (all with Hay Infusion) **Understanding dilutions:** Understanding dilutions- theory **Measuring microbial growth: Yeast** Direct counts using a hemocytometer Using a spectrophotometer Counting viable cells using plating	
Lab 4	Tues/Wed Oct 4/5	**Microscopy:** Continue/complete all wet mounts (all other bacterial and yeast) **Microsopy: Staining** Smear preparation and simple staining Gram stain: control organisms only **Characterizing the Test Organisms:** **Introduction**: Receive 2 test organisms per group: make a wet mount, streak plate with organisms **Winogradsky column** Understanding the set up, a first look	
Lab 5	Thurs/Fri Oct 6/7	**Characterization of the Test Organisms** Streak stock TSS slant **Data Analysis Workshop:**	**Need room for 50** **York 3010 confirmed**
Lab 6	Tues/Wed	**Microscopy: Staining**	

	Oct 11/12	Complete staining of designated Gram positive and Gram negative controls **Characterization of the Test Organisms** Gram stain MacConkey – inoculate along with known G+ and G- organisms Sticky test along with known G+ and G- organisms Endospore test – inoculate NSM **Macronutrient use – how organisms get energy to survive:** Introduction: Hydrolysis and use of large extracellular materials Polysaccharides: Starch plates - inoculate Proteins: Skim milk plates and gelatin deeps - inoculate Lipids: Rhodamine plates - inoculate **Inoculation of control organisms (to create fresh stocks):** *Enterobacter aerogenes* *Escherichia coli* *Proteus vulgaris* Inoculate 1 TSS or TSA of each per aisle. These slants will be used for the controls for the urease test on Lab 7	
Lab 7	Thurs/Fri Oct 13/14	**Characterization of the Test Organisms** **Macronutrient use – how organisms get energy to survive** Polysaccharides: Starch plates - complete Proteins: Skim milk plates and gelatin deeps - complete Lipids: Rhodamine plates – complete **Special metabolic functions: Test organisms only**	

		• Indole production from tryptophan, catabolite repression – inoculate • Urease test – inoculate • Differential utilization of citrate by enterics - inoculate **How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** Acid and gas production from sugar fermentation – inoculate Methyl-Red and Voges-Proskauer – inoculate	
Lab 8	Tues/Wed Oct 18/19	**Characterization of the Test Organisms:** **How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** Acid and gas from sugar fermentation - complete Methyl-Red and Voges Proskauer – complete Oxygen requirements – inoculate thioglycolate tube T-streak plate for fresh isolated colonies (for Cyto C and catalase) H_2S production – inoculate NSM – Complete **Special metabolic functions: Test organisms only** • Indole production from tryptophan, catabolite repression – • complete • Urease test – complete • Differential utilization of citrate by enterics – complete **Inoculation of control organisms (to create fresh stocks):**	

		Escherichia coli *Pseudomonas fluorescens* *Enterococcus faecalis* *Staphylococcus epidermidis* Inoculate 1 TSS or TSA of each per aisle. These slants will be used for the controls for the Cyto C and catalase tests on Lab 9 *Escherichia coli* *Pseudomonas aeruginosa* Inoculate 1 TSS slant of each per aisle. These slants will be used for the controls for the nitrate test on Lab 9	
		Students come in on non lab day to check thioglycolate tube and Kligler iron deep	
Lab 9	Thurs/Fri Oct 20/21	**Fundamentals of library research: a practice run- workshop- location to be determined** **Characterization of the Test Organisms:** **How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** • Oxygen requirements –complete • H_2S production – complete test • Cytochrome C test – complete • Catalase test – complete • Nitrate reduction - inoculate **Winogradsky column** Examine for evidence of anaerobiosis and H_2S production	**York 3010 confirmed**

Lab 10	Tues/Wed Oct 25/26	**Characterization of the Test Organisms:** **Motility** – inoculate plate and deep with test organism **Nitrate reduction** – complete **Survival in extreme conditions:** • Low pH • High pH • Low temp • High temp • High salt • **Control** inoculate appropriate broth with test organism **Soil Enumeration and Enrichment:** First lab period: • **Simple Enumeration**: Serial dilution, plating on TSA, SDA, GAA, and MacConkey • **Extracellular degradation**: **Enumeration**: Serial dilution and plating of soil sample on minimal media + starch, minimal media + skim milk plates **Enrichment of soil organisms**: inoculation of minimal media containing starch/skim milk with soil • **Extreme Conditions:** **Enumeration**: Serial dilution and plating of soil sample on TSA or TSA + low pH plates as assigned; incubation at assigned temperature. **Enrichment of soil organisms**: inoculation of TSB or TSB +	

		low pH as assigned; incubation at assigned temperature	
		Non lab day: TAs set up subculture of enrichments	
Lab 11	Thurs/Fri Oct 27/28	**Motility** – complete **Survival in extreme conditions:** Score growth/no growth in each tube **Soil Enumeration and Enrichment**: Second lab period • **Simple Enumeration**: Colony counts and calculations • **Extracellular degradation**: **Enumeration**: test differential media, count, and calculate **Enrichment**: serial dilution and plating; centrifuge cell suspension and freeze pellet • **Extreme conditions** **Enumeration**: Colony counts and calculations **Enrichment**: serial dilution and plating; centrifuge cell suspension and freeze pellet **Nitrogen fixation: Free-living - Anabaena** • Inoculate BG11 and BG11-0 with *Anabaena*	
Lab 12	Tues/Wed Nov 1/2	**Soil Enumeration and Enrichment**: Third lab period • **Extracellular degradation**: **Enrichment**: test differential media, count, and calculate • **Extreme conditions** **Enrichment**: Colony counts and calculations **Metagenomics**: First lab period	

		• Step 1: Chromosomal DNA preps from frozen cell pellets from various soil enrichments • Step 2: Set up 16S rRNA PCR **Screening for Antibiotic Producers**: grid plates	
Lab 13	Thurs/Fri Nov 3/4	**Metagenomics**: Second lab period • Step 3: Run gel and evaluate PCR results • Step 4: Purify PCR product **Screening for Antibiotic Producers**: Identify antibiotic producers, measure ZOI **Evaluation of antibiotics by the Kirby Bauer method** Spread plates with standards and test efficiency of antibiotics **Nitrogen Fixation – Free-living - Anabaena** Subculture in BG11 and BG11-0 **– check materials list**	
		Non lab period: TAs run gel of purified PCR product and set up ligations (Step 5 of Metagenomics)	
Lab 14	Tues/Wed Nov 8/9	**Metagenomics:** Third lab period • Step 6: Transform ligations and plate on selective media **Evaluation of Antibiotics by the Kirby Bauer Method** Measure ZOI, identify any resistant colonies **Characterization of a Test Organism:** • Each group or set of groups outlines and explains characteristics of their assigned test organism • Create elimination flow chart for identification of genera	**Computer lab**
Lab 15	Thurs/Fri	**Metagenomics:** Fourth lab period	

	Nov 10/11	• Step 7: Select white colonies and streak out for sequencing **Growth curve experiment** Growth and graphing of *Vibrio natriegens*	
		Fri: send all Thurs plates for sequencing, set up sequencing order of Fri plates for pick up on Mon? **Sat: remove and refrigerate Fri plates**	
Lab 16	Tues/Wed Nov 15/16	**Transposon mutagenesis:** Lab Period 1 Step 1: Set up conjugation of *E.coli* and *Salmonella* **Yogurt:** Inoculate milk with starter yogurt **Metagenomics:** Analysis of sample sequence data and sample construction of phylogenetic tree– computer lab	**Room and computer lab?**
Lab 17	Thurs/Fri Nov 17/18	**Transposon mutagenesis:** Lab Period 2 • Step 2: Plate exconjugants for selection and counterselection • Save LB recipient control plates for later use **Yogurt:** measure pH, gram stain **Metagenomics**: Step 8: Begin/complete analysis of all sequences, construction of phylogenetic trees.	**Computer lab**
Lab 18	Tues/Wed Nov 22/23	**Transposon mutagenesis:** Lab Period 3 • Count colonies and calculate transposition efficiency • Step 3: Screen transposants (mutants) for loss of function mutations **Nitrogen Fixation** Free-living: *Anabaena*: check for heterocysts Symbiotic*: Rhizobium:* Observe nodules	**Computer lab**

		Plant Pathogen Observe *Agrobacterium*-kalanchoe interaction **Metagenomics**: Complete analysis/discussion of all sequences	
Lab 19	Tues/Wed Nov 29/30	**Transposon mutagenesis:** Lab Period 4 • Check screens • Streak out mutants on LB/TSA plate for future use **Winogradsky column** Observation and sampling Check out Review	
Lab 20	Thurs/Fri Dec 1/2	Midterm 3 will be held during normal lecture or lab hours.	**Midterm 3**