BIMM 121 Laboratory in Microbiology
Spring 2013

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***On Ted, in folder labeled "Useful Information"**

Lecture: Tuesday/Thursday 8:00 – 9:20 in PCYNH 122 (Pepper Canyon Hall, by the Gilman Parking Structure)

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

Office hours: Mondays 11:30am-12:30 pm. Location: 4070C York Hall

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 (bound notebook, regular or spiral bound). Carbon notebook not necessary. Loose-leaf binders not allowed.
- 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.

Reading for the lab

Reading ahead of the course: If you wish to read ahead, your best bet is to brush up on any information on microbes, cell structure, and basic microbial physiology (glycolysis, TCA cycle, electron transport chain, central dogma, etc). All information pertinent to topics covered in class is in the textbook and will be discussed in lecture

Reading during the course:

- Read the chapters before you come to lecture. After week 1, I will post guidelines to reading the chapters in the folder labeled “Directed Reading” on TED
- When you are in the classroom, I will go over the basics as required, any fundamental concepts that you do find or might find difficult, that are important, or that are particularly exciting or newsworthy.
- Then you will go to lab and actually see all those tests and concepts in action.
- Then go back and quickly reread the material in light of the lecture and lab work and you will find that it becomes very clear since you are already familiar with most of it.

As often as possible, I will give you questions/problems to think about that should apply the concepts you learned in class. Thinking about and attempting to answer these questions and participating in any classroom/lab discussion is the best practice you can have for midterms, lab reports, and practicing science in general.

Lab Performance and Participation

In addition to quizzes, midterms, lab reports and homework 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
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.**

CLICKERS in Micro Lab

This lab will introduce you to new material and concepts. To increase the depth of your understanding and to give you practice in applying these concepts, we will discuss these concepts from different perspectives in class. Over the last few years, student feedback has shown that class participation has a very positive impact on performance in lab reports and midterms.

We will be using Clickers in class as part of the learning process and to help students stay on top of the concepts and their applications. Participation in the lecture discussion is worth 5% of your grade and requires that you click in at least 75% of the time in each lecture for at least 75% of the lectures. i>Clickers are available for purchase at the UCSD bookstore. Once you have purchased your Clicker, you can register it on Ted. A separate explanation of our Clicker policy is on Ted.

Notebook:

A 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.

Homework and Lab report Deadlines and Submission:

1. A hard copy of each homework is due in the first 5 minutes of the lab period of the day on which your report is due. **All homework assignments submitted more than 10 minutes after start of lab are automatically late and lose 10% of the points. Any homework submitted the next calendar day would lose 50% of the points. No homework will be accepted after the second calendar day.**
2. There is only one lab report and it is due the Mon of finals week. Any lab report turned in on Tues will lose 50% of the points
3. In addition to the hard copy of the assignments/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. Some homework assignments also require Turnitin.com assignments

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

HW#	Description	Due date	Points
1	Practice Excel work	April 2/3	10
2	Toilet paper expt data analysis	April 9/10	10
3	Library tutorial	April 16/17	20
4	Water contamination analysis	April 30/May1	40
5	Dilution problems	May 7/8	10
6	Unknown analysis + research	May23/24	50
7	Growth curve	May 28/29	10
Total			150

Other important dates:

MT1	April 23 (lecture)
MT2	May 14/15 (lab)
MT 3	June 6/7 (lab)
Lab Report	Mon June 10th 1 pm

Grading Scheme

Quiz/Report/Midterm	Points	% of total
Classroom evaluation • Notebook checks (20) • TA eval (10) • Practicum (10)	40	6.2%
Clicker participation	30	~5%
Quizzes 9 quizzes, 1 lowest score dropped	80	12.3%
Homework (7)	150	23
Lab Report (1)	100	15.4
Midterms (3)	250 (70 + 95 + 85?)	38.5
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%

Regrade Requests:

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

Course Website/Ted

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.

Student Responsibility:

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.

Lab	Date	Experiment	Reports, Midterms, Reminders
Lab 1	**Tues/Wed April 2/3**	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 **Plant pathogen interaction:** Inoculate *Kalanchoe* plant with *Agrobacterium*	**HW 1 due: Practice Excel (10)**
Lab 2	**Thurs/Fri April 4/5**	**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	**Quiz 1 (10)**
Lab 3	**Tues/Wed April 9/10**	**Sterile technique.** Aseptic technique: streak and spread plates Observe results **Microscopy:** Calibrating your microscope: Demo and complete Making a wet mount and Phase Contrast Microscopy: Wet mounts and phase contrast: - view, identify, and measure (Listed Eukaryotes, Bacteria, and Hay Infusion)	**HW2 due – Toilet paper data analysis (10)**

Lab 4	**Thurs/Fri Apr 11/12**	**Characterizing the Unknown Organisms:** **Introduction**: Receive unknown organisms: make a wet mount, streak plate and set up broth culture with organisms **Understanding dilutions:** Understanding dilutions- theory **Microscopy: Staining** • Smear preparation and simple staining • ~~Gram stain: control organisms only~~ • Gram stain: Complete staining of designated Gram positive and Gram negative controls • Initial staining of Unknown	**Quiz 2 (10)**
Lab 5	**Tues/Wed Apr 16/17**	**Characterization of the Unknown Organisms** Streak stock TSS slant, do wet mounts from both temperatures Gram stain - complete MacConkey – inoculate along with known G+ and G- organisms Sticky test along with known G+ and G- organisms Endospore test – inoculate NSM **Measuring microbial growth: Yeast** • Direct counts using a hemocytometer • Using a spectrophotometer • Counting viable cells using plating	**HW 3 due – online Library tutorial (20)** **Read coliform chapter**
Lab 6	**Thurs/Fri Apr 18/19**	**Data Analysis Workshop:**	**Quiz 3 (10)** **April 18th: 9:30 – 1:30, April 19th: 9:00 – 1:00 York 3010** **April 18th: 3:30 – 6:30 York 3010** **April 19th: 2:00 – 6:00 York 1310**
Lab 7	**Tues/Wed**	**Inoculation of control organisms (to**	**MT 1: in lecture**

	Apr 23/24	**create fresh stocks):** *1. Enterobacter aerogenes* *2. Escherichia coli* 3. *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 8 **Characterization of the Unknown Organisms** NSM – Complete **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	
Lab 8	**Thurs/Fri Apr 25/26**	**Fundamentals of library research: 90 minute hands on workshop** **Characterization of the Unknown 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	**Quiz 4 (10)** **Workshop in York 3010 confirmed** **Lab in usual location before** **April 25th 11:30 – 1:30 and 4:30 – 6:30** **April 26th 11:00 – 1:00 and 4:00 – 6:00**
Lab 9	**Tues/Wed Apr**	**Characterization of the Unknown Organisms:**	**HW4 due – Water contamination data**

	30/May 1	**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 **Special metabolic functions: Unknown 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):** *1. Escherichia coli* 2. *Pseudomonas aeruginosa* Inoculate 1 TSS slant of each per aisle. These slants will be used for the controls for the nitrate test on Lab 10 **Motility** – inoculate plate and deep with unknowns and controls	**analysis (40)**
Lab 10	**Thurs/Fri May 2/3**	**Characterization of the Unknown Organisms:** **Inoculation of control organisms (to create fresh stocks):** *1. Escherichia coli* 2. *Pseudomonas fluorescens* *3. Enterococcus faecalis* 4. *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 11 **How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** • Acid and gas from sugar fermentation - complete • Methyl-Red and Voges Proskauer –	**Quiz 5** **Additional lecture/review time 3010 York** **May 2nd 11:30 – 1:30 and 4:30 – 6:30** **May 3rd 11:00 – 1:00 and 4:00 – 6:00** **Lab in regular lab rooms before**

		complete • T-streak plate with unknowns for fresh isolated colonies (for Cyto C and catalase) • Nitrate reduction – inoculate nitrate broth **Motility** – complete	
Lab 11	**Tues/Wed May 7/8**	**How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** • Cytochrome C test – complete • Catalase test – complete • **Nitrate reduction** – complete • H_2S production – inoculate Kligler iron deep • Oxygen requirements – inoculate thioglycolate tube Start all unknown repeats?	**HW5 due- Dilution problems (10 points)**
		Students come in on non lab day to check thioglycolate tube and Kligler iron deep	
Lab 12	**Thurs/Fri May 9/10**	**How energy is produced – aerobic vs. anaerobic breakdown of organic compounds** • Oxygen requirements –complete • H_2S production – complete test Complete all unknown repeats? **Soil Enumeration and Enrichment:** First lab period: • **Enumeration**: Serial dilution, plating on TSA, SDA, GAA, and MacConkey, minimal media + skim milk plates (Extracellular degradation of casein), TSA incubated at high temp (Extreme conditions: thermophiles) Incubation at assigned temperature • **Enrichment of soil organisms**: inoculation of MM = skim milk or TSB as assigned; incubation at assigned	**Quiz 6** **Additional lecture/review time 3010 York** **May 9th 11:30 – 1:30 and 4:30 – 6:30** **May 10th 11:00 – 1:00 and 4:00 – 6:00** **Lab in regular lab rooms before**

		temperature **Nitrogen fixation: Free-living - Anabaena** Inoculate BG11 and BG11-0 with *Anabaena* **Screening for Antibiotic Producers**: Spread plates and grid putative antibiotic producers	
		Non lab day: TAs set up enrichment and subculture of enrichments	
Lab 13	**Tues/Wed May 14/15**	**Soil Enumeration and Enrichment**: Second lab period • **Enumeration**: Colony counts and calculations • **Enrichment**: serial dilution and plating **Screening for Antibiotic Producers**: Check for ZOI **Characterization of an Unknown Organism:** Create elimination flow chart for identification of genera	**Midterm 2 in lab** ***Computer lab – Begin elimination tree for HW 6 – time permitting***
Lab 14	**Thurs/Fri May 16/17**	**Soil Enumeration and Enrichment**: Third lab period • **Enumeration**: Complete colony counts and calculations • **Enrichment**: Colony counts, examination for casein hydrolyzers, calculations **Characterization of a Test Organism:** • Create elimination flow chart for identification of genera	**Quiz 7 (10)** **Computer lab – complete elimination tree for HW 6**
Lab 15	**Tues/Wed May 21/22**	**Soil Enumeration and Enrichment**: Fourth lab period • **Enrichment**: Complete colony counts, examination for casein hydrolyzers, calculations **Evaluation of antibiotics by the Kirby Bauer method** Spread plates with standards and test efficiency of antibiotics	

Lab 16	**Thurs/Fri May 23/24**	**Evaluation of Antibiotics by the Kirby Bauer Method** Measure ZOI, identify any resistant colonies **Growth curve experiment** Growth and graphing of *Vibrio natriegens*	**HW 6 due – identification of unknown (50 points)** **HW 7 – Growth curve – begin work in lab, complete at home**
Lab 17	**Tues/Wed May 28/29**	**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	**Computer lab** **HW 7 due- Growth curve (10 points)** **Complete: Data entry and verification for enrichment study** **Quiz 8 (10 pts)**
Lab 18	**Wed/Fri May 30/31**	**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. **Nitrogen Fixation** 1. Free-living: *Anabaena*: check for heterocysts 2. Symbiotic*: Rhizobium:* Observe nodules **Plant Pathogen** 1. Observe *Agrobacterium*-kalanchoe interaction	**Computer lab** **Complete calculations for LR**
Lab 19	**Tues/Wed June 4/5**	**Transposon mutagenesis:** Lab Period 3 • Count colonies and calculate transposition efficiency Lab clean up and check out	**Quiz 9 (10 pts)**
Lab 20	**Thurs/Fri June 6/7**	Midterm 3 will be held in lab during regular lab hours	**Midterm 3**
	Mon June	**Mon June 10th 1 pm (Mon of finals week)**	

	10	**– Lab Report due**	