# BIMM 121 Laboratory in Microbiology
# Winter 2011

Lakshmi Chilukuri
4070 C York Hall
lchilukuri@ucsd.edu
858-822-2032

**Office hours**: Mondays 11 am -12 noon

**Lecture**: Tuesday/Thursday 8:00 am to 9:20 am, 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: 9:00 am – 1:00 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; and,
- Eye protection (you may wear either safety glasses or goggles, but standard prescription eye glasses are not sufficient).

**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.
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.

## Assignment Deadlines and Submission:

Formatted: Font: 14 pt

Formatted: Font: 14 pt

1. A hard copy of each lab report is due in the first 10 minutes of the lab period or the first 10 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 WebCT. 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.
3. Additional points may be taken for late electronic submissions.

## Regrade Requests:

Formatted: Font: 14 pt

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

## Grading Scheme

| Quiz/Report/Midterm | Points |
|---|---|
| 4 notebook checks at 5 points each | 20 points |
| 2 surveys at 10 points each | 20 points |
| 5 pop quizzes at 5 points each | 25 points |
| Lab report 1 | 100 points |
| Lab report 2 | 100 points |
| Lab report 3 | 25 points |
| Lab and workshop participation | 35 points |
| 3 Midterms | 175 points |
| **Total** | **500 points** |

## Possible assignments.

If an assignment is given, either an exam or a report will have its point value correspondingly reduced.

- Dilution assignment
- Transposon mutagenesis assignment
- Metagenomics assignment

## 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
Start a new page each day
For each 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
  - Draw
  - Questions and connections
- Conclusion or summary
- Answer any questions in the manual or that were raised in class.

## Lab Performance and Participation

In addition to quizzes, midterms, lab reports and 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

Formatted: Font: 14 pt
Formatted: Font: Arial
Formatted: Font: Arial
Formatted: Font: Arial

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 WebCT (https://webctweb.ucsd.edu) and should automatically appear on your WebCT account as soon as you register for the class. We will use WebCT 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.

Formatted: Font: Arial

## Student Responsibility:

Formatted: Font: Arial

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**

**student**s **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.

Formatted: Font: Arial

Formatted: Font: Arial

| Lab | Date | Experiment | Reports, Midterms, Reminders |
|---|---|---|---|
| Lab 1 | Tues/Wed<br>Jan 4-5 | Registration, introductory remarks, safety lecture, etc.<br>**Sterile technique.**<br>Microbes in the environment<br>*E.coli* and toilet paper experiment<br>Aseptic technique, streak and spread plates<br>**Use of pipettors:** Demo | |
| Lab 2 | Thurs/Fri<br>Jan 6-7 | **Workshop:** Intro to microbes, notebook, data analysis, report formats, researching topics | |
| Lab 3 | Tues/Wed<br>Jan 11-12 | **Sterile technique.**<br>Microbes in the environment: Observe results<br>*E.coli* and toilet paper experiment: Observe results<br>Streak and spread plates: Observe results.<br>**Understanding dilutions:**<br>Understanding dilutions- theory<br>**Measuring microbial growth: Yeast**<br>Using a spectrophotometer<br>Counting viable cells using plating<br>**Plant Pathogens:**<br>Set up *Agrobacterium*-kalanchoe infections | |
| Lab 4 | Thurs/Fri<br>Jan 13-14 | **Microscopy:** | |

| | | | |
|---|---|---|---|
| | | Learning to focus the light microscope<br>Calibrating your microscope<br>Observing stained slides<br>**General microbiology:** Introduction to selective and differential media<br>**Soil Enumeration and Enrichment:** Lab Period 1<br>Simple Enumeration: Serial dilution, plating on TSA, SDA, GAA, and MacConkey | |
| Lab 5 | Tues/Wed<br>Jan 18-19 | **Microscopy:**<br>Making a wet mount and Phase Contrast Microscopy: Wet mounts and phase contrast:- view, identify, and measure<br><br>**Microscopy: Staining**<br>Smear preparation and simple staining<br>Gram stain: control organisms only<br>**Characterizing a Standard Organism:**<br>**Introduction**: Receive standard: wet mount and streak plate and slants<br>**Winogradsky column**<br>Understanding the set up | **Report 1 due** |
| Lab 6 | Thurs/Fri<br>Jan 20-21 | **Microscopy: Staining**<br>Complete staining of all controls<br>**Characterization of a Standard Organism**<br>Gram stain<br>MacConkey – inoculate with known G+ and G- organisms | |

| | | | |
|---|---|---|---|
| | | Sticky test with known G+ and G- organisms<br>Endospore test – incoulate NSM<br>**Macronutrient use – how organisms get energy to survive:**<br>Introduction: Hydrolysis and use of large extracellular materials<br>Polysaccharides: Starch plates - inoculate<br>Proteins: Skim milk plates and gelatin deeps - inoculate<br>Lipids: Rhodamine plates - inoculate<br>Inoculate with standard organism<br>**Soil Enumeration and Enrichment**: Lab Period 2<br>• **Simple enumeration**: colony counts<br>• **Extracellular degradation**:<br>**Enumeration**: Serial dilution and plating of soil sample on starch/rhodamine/casein plates<br>**Enrichment of soil organisms**: inoculate minimal media containing starch/olive oil/skim milk with soil | |
| Lab 7 | Tues/Wed<br>Jan 25-26 | **Characterization of a Standard Organism**<br>**Macronutrient use – how organisms get energy to survive**<br>Polysaccharides: Starch plates - complete<br>Proteins: Skim milk plates and gelatin deeps - complete<br>Lipids: Rhodamine plates – complete<br>**How energy is produced – aerobic vs. anaerobic breakdown of organic compounds**<br>Oxygen requirements – inoculate thioglycolate tube<br>Acid and gas production from sugar fermentation – inoculate | **Midterm 1** |

| | | | |
|---|---|---|---|
| | | Methyl-Red and Voges-Proskauer – inoculate<br>T-streak plate for fresh isolated colonies<br>**Soil Enumeration and Enrichment**: Lab Period 3<br>• **Extracellular degradation**:<br>**Enumeration**: test differential media and count<br>**Enrichment**: subculture<br>**Winogradsky column**<br>Examine for evidence of anaerobiosis | |
| | | **Non-lab day:** You may check the growth in the thioglycolate tube to determine the oxygen requirement | |
| Lab 8 | Thurs/Fri<br>Jan 27-28 | **Characterization of a Standard Organism:**<br>**How energy is produced – aerobic vs. anaerobic breakdown of organic compounds**<br>Oxygen requirements –complete<br>Acid and gas from sugar fermentation - complete<br>Methyl-Red and Voges Proskauer – complete<br>Cytochrome C test – complete<br>Catalase test – complete<br>Nitrate reduction - inoculate<br>$H_2S$ production – inoculate<br>**Soil Enumeration and Enrichment**: Lab Period 4<br>• **Extracellular degradation**:<br>**Enrichment**: | |

| | | | |
|---|---|---|---|
| | | • Serial dilutions and plating to enumerate enrichment<br>• Centrifuge aliquot of enrichment and freeze pellet | |
| Lab 9 | Tues/Wed<br>Feb 1-2 | **Characterization of a Standard Organism:**<br>**How energy is produced – aerobic vs. anaerobic breakdown of organic compounds**<br>• Nitrate reduction – complete test<br>• $H_2S$ production – complete test<br>**Special metabolic functions: Standards only**<br>• Indole production from tryptophan, catabolite repression – inoculate<br>• Urease test – inoculate<br>• Differential utilization of citrate by enterics - inoculate<br>**Motility** – inoculate plate and deep with standard organism<br>**Survival in extreme conditions:**<br>• Low pH<br>• High pH<br>• Low temp<br>• High temp<br>• High salt<br>• **Control**<br>• inoculate appropriate broth with standard organism<br>**Soil Enumeration and Enrichment**: Lab Period 5<br>• **Extracellular degradation**: | |

| | | | |
|---|---|---|---|
| | | **Enrichment**: Complete colony counts and calculations<br>• **Extreme conditions**<br>**Enrichment**: Inoculate medium as assigned<br>**Winogradsky column**<br>Examine for evidence of anaerobiosis and $H_2S$ production | |
| Lab 10 | Thurs/Fri<br>Feb 3-4 | **Characterization of a Standard Organism:**<br>**Special metabolic functions: Standards only**<br>• Indole production from tryptophan, catabolite repression –<br>• complete<br>• Urease test - complete<br>• Differential utilization of citrate by enterics – complete<br>**Motility** – complete<br>**Survival in extreme conditions:**<br>• Score growth/no growth in each tube<br>**Soil Enumeration and Enrichment**: Lab Period 6<br>• **Extreme conditions**<br>**Enrichment**:<br>Serial dilution and plating of enriched sample<br>Centrifuge aliquot and freeze pellet | |
| | | **TAs set up serial dilution and plating of soil sample for "Screening for Antibiotic Producers"** | |
| Lab 11 | Tues/Wed<br>Feb 8-9 | **Soil Enumeration and Enrichment**: Lab Period 6<br>• **Extreme conditions**<br>**Enrichment**: | **Report 2 due** |

| | | | |
|---|---|---|---|
| | | Colony counts and calculations<br>**Nitrogen fixation: Free-living - Anabaena**<br>• Inoculate BG11 and BG11-0 with *Anabaena*<br>**Metagenomics**: Lab Period 1<br>• Step 1: Chromosomal DNA preps from frozen cell pellets from various soil enrichments<br>• Step 2: Set up 16S rRNA PCR | |
| Non lab day | | **TAs run gel of PCR reactions** | |
| Lab 12 | Thurs/Fri<br>Feb 10-11 | **Metagenomics**: Lab Period 2<br>• Step 3: Evaluate PCR results<br>• Step 4: Purify PCR product<br>• Step 5: Set up ligations in pGEM-T<br>**Screening for Antibiotic Producers**: grid plates | |
| Lab 13 | Tues/Wed<br>Feb 15-16 | **Metagenomics**: Lab Period 3<br>• Step 6: Transform ligations and plate on selective media<br>**Screening for Antibiotic Producers**: Identify antibiotic producers, measure ZOI<br>**Evaluation of antibiotics by the Kirby Bauer method**<br>Spread plates with standards and test efficiency of antibiotics<br>**Nitrogen Fixation – Free-living - Anabaena**<br>Subculture in BG11 and BG11-0 **– check materials list** | |
| Lab 14 | Thurs/Fri | **Metagenomics:** Lab Period 3 | **Midterm 2** |

| | | | |
|---|---|---|---|
| | Feb 17-18 | • Step 7: Select white colonies and streak out for sequencing<br>**Evaluation of Antibiotics by the Kirby Bauer Method**<br>Measure ZOI, identify any resistant colonies<br>**Growth curve experiment**<br>Growth and graphing of *Vibrio natriegens*<br>**Identification of an Unknown Organism:** Lab Period 1<br>• Receive unknown<br>• Streak on plates and slants<br>• Check morphology by microscopy – wet mount<br>• Gram stain | |
| Lab 15 | Tues/Wed<br>Feb 22-23 | **Metagenomics:** Lab Period 4<br>• Step 8: Analyze sequence data – computer lab<br>• Complete after lab as necessary<br>**Characterization of a Standard Organism:**<br>• Brief workshop where each group or set of groups outlines and explains characteristics of their assigned standard organism<br>• Create elimination flow chart for identification of genus<br>**Transposon mutagenesis:** Lab Period 1<br>• Step 1: Set up conjugation of *E.coli* and *Salmonella* | **Computer lab day** |
| Lab 16 | Thurs/Fri<br>Feb 24-25 | **Transposon mutagenesis:** Lab Period 2<br>• Step 2: Plate exconjugants for selection and counterselection<br>**Identification of an Unknown Organism:** Lab Period 2<br>• Inoculate all media provided with your unknown organism | |

| | | | |
|---|---|---|---|
| Lab 17 | Tues/Wed<br>Mar 1-2 | **Identification of an Unknown Organism:** Lab Period 3<br>• Briefly examine all tests with easily identified results<br>• Save all tests for reexamination<br>**Soil Workshop**: Collaborative understanding of all soil and metagenomics experiments | |
| Lab 18 | Thurs/Fri<br>Mar 3-4 | **Identification of an Unknown Organism:** Lab Period 4<br>• Complete all tests<br>**Transposon mutagenesis:** Lab Period 3<br>• Step 3: Screen transposants (mutants) for loss of function mutations<br>**Nitrogen Fixation**<br>Free-living: *Anabaena*: check for heterocysts<br>Symbiotic*: Rhizobium:* Observe nodules<br>**Plant Pathogen**<br>Observe *Agrobacterium*-kalanchoe interaction | |
| Lab 19 | Tues/Wed<br>Mar 8-9 | **Identification of an Unknown Organism:**<br>• Report due<br>**Transposon mutagenesis:** Lab Period 4<br>• Check screens<br>**Winogradsky column**<br>Observation and sampling<br>Review<br>Potluck | **Unknown Report due** |
| Lab 20 | Thurs/Fri | Midterm 3 will be held during normal lab hours. No lecture today | **Midterm 3** |

| | | | |
|---|---|---|---|
| | Mar 10-11 | | |