EEES 4550/5550, **Methods of Microbial Investigation**
3 credit-hours
Spring 2005
Tuesdays from 1:00 – 4:00 in BO 3010

Instructor: Von Sigler, Assistant Professor of Microbial Ecology, Department of Earth, Ecological, and Environmental Sciences
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Office hours: to be determined upon class scheduling and by appointment
Prerequisites: EEES 4540/5540 or consent of instructor

Required text: None. A CD with all protocols, figures, and readings will be provided.

Course objectives In EEES 4540/5540, Microbial Ecology, we discussed the fundamental parameters driving microbial population structure and function in the environment as well as methods to describe microbial populations. This laboratory course complements the lecture material by giving you the opportunity to apply the methods we discussed to an environmental sample of your choice. This sample can be from a research project on which you are currently working, or simply a sample that is interesting to you. You may even find that the information that you generate in this laboratory will fit into your MS or PhD research effort. The objectives of this course are to:

1. become familiar with the promise and pitfalls of the classical and current biochemical and molecular methodology used in microbial community analysis.

2. develop an understanding of experimental design, sample handling, and data analysis.

3. create a portfolio of data describing your particular sample, and if possible, draw comparisons between your sample and those of your lab-mates.

Tentative course set-up/schedule We will meet once per week for three hours. This is not a lot of time to discuss, set-up, execute, and analyze the results of our experiments. However, with proper preparation, we will be able to achieve our objectives. I will expect you to arrive on time and be ready to perform the day's experiments. Prior to each laboratory meeting, I will hand out supplementary reading materials that will describe the methods and provide background information concerning their application. You should read these materials prior to laboratory meetings so that we can progress as efficiently as possible.

Course schedule:

WEEK		TOPIC	EXPERIMENT	OTHER ACTIVITIES
1	Biochemical methods	Introduction and course overview	• Review syllabus, course policies, and protocols	
2		Bacteria quantification	• Dilution Plating (Incubate until WEEK 3)	
3		Microbial activity	• Fluorescein diacetate hydrolysis assay (FDA)	• Count dilution platings from WEEK 2 • Wash plates for culture DNA extraction
4		Community Level Physiological Profiling (CLPP)	• Set up BIOLOG analysis • Perform t=0 reading	• Four other readings will be performed prior to the next meeting
5	**NO LAB**			
6	Molecular methods	DNA isolation I: Soil, sediment, or plant material Water Bacterial culture	• DNA isolation from sample, plate wash, and BIOLOG cultures	• Dispense contents of BIOLOG plates from WEEK 4 for DNA extraction
7		DNA isolation II and Polymerase Chain Reaction (PCR)	• Finish DNA extraction • Set up a PCR to target the 16S rRNA gene of *Bacteria*	• Pour agarose gel to visualize/quantify DNA prior to PCR
8		Community Fingerprinting I: Denaturing Gradient Gel Electrophoresis (DGGE)	• Pour and load a DGGE gel with PCR product from WEEK 7	• Pour agarose gel to visualize PCR products from WEEK 7 prior to DGGE
9		Community Fingerprinting I: Denaturing Gradient Gel Electrophoresis (DGGE)	• Stain and visualize the DGGE gel • Excise bands of interest • Set up RISA PCR	• Introduction to Ribosomal Intergenic Spacer Analysis (RISA)
10		**SPRING BREAK**		
11		Community Fingerprinting II: Ribosomal Intergenic Spacer Analysis (RISA)	• Load, run, and analyze RISA gel	• Pour agarose gel to visualize PCR products from WEEK 9 prior to RISA
12	Case study	Bacterial source marker identification I	• *Escherichia coli* isolation from environmental samples	
13		Bacterial source marker identification II	• Confirm identity of *E. coli* colonies from WEEK 11 • Near full-length 16S rRNA amplification	• Count *E. coli* platings from WEEK 12
14		Bacterial source marker identification III	• Restriction Fragment Length Polymorphism (RFLP) analysis of PCR products from WEEK 13	
15	Data analysis	Bioinformatics software packages	• Analyze RFLP data from case study • BLAST, ENTREZ and multiple alignment	
16		Student Seminars		

Grading Grades will be earned based on your performance in (i) keeping your laboratory notebook, (ii) writing a term paper, and (iii) a final presentation. The point distribution for the notebook, paper, and presentation is as follows:

Lab Notebook (checked periodically and returned)	February 8 March 15 April 19	75 points @ 25 points each
Term paper	April 26	50 points
Final presentation	April 26	50 points
Total		200 points

Laboratory notebook

The notebook will be discussed in detail during the first week of class, but in general, you will be expected to keep detailed notes to aid you in preparing your manuscript and studying for the final exam. Notebooks will be collected three times during the semester and graded. The laboratory notebook should provide you (or more importantly, anybody reading your lab notebook) with enough information to allow the duplication of our experiments. Grades will be assigned based on the following criteria:

1. *Neatness and organization*: Can I quickly locate necessary information? Can I read your entries?

2. *Completeness*: Are all of the experiments accurately documented?

Term paper

A manuscript draft (10-12 pages including references, figures and tables) formatted for submission to a journal of your choice will be due at the end of the term. I do not expect you to include every piece of data that we generate throughout the semester, only the most pertinent. The details of the manuscript will be described as the semester progresses.

Final presentation

You will present the results of your experiments throughout the semester to the class. As with the term paper, you will not be required to present all of your results, but you will certainly generate enough data in this course to put together a substantial presentation. The details of the presentation will be described as the semester progresses.

Attendance Attendance will not be taken. However, as this is a laboratory course, your presence at each class meeting will greatly impact your grade. If you miss a class, it is your responsibility to get the any notes or pertinent from a fellow

student. I will not provide this information with the exception of extreme circumstances.

Missed exams can only be made-up if a written letter explaining the reason for the absence (including a contact phone number of doctor, mechanic, alarm clock manufacturer, etc)) is presented to the instructor *at the time of the student's next attended class*.

Academic dishonesty Academic dishonesty in this course will not be tolerated. Examples of academic dishonesty include:

1. Obtaining or using work other than your own on tests, exams, quizzes or assignments.
2. Unauthorized use of calculators or other programmable equipment during tests, exams, or quizzes.
3. Unauthorized use of study aids, answer or crib sheets.
4. Soliciting or providing answers on exams, tests or quizzes.

Students who violate the above policy can expect disciplinary action. Disciplinary action may consist of receiving a zero on the assignment, failing the course, being reported to the Dean of Students, or other action as deemed appropriate by the course instructors.