COURSE SYLLABUS AND POLICIES

Spring, 2005 CENG 449/649 Environmental Molecular Microbiology

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Prerequisites: Instructor’s permission

Office Hrs: Wednesday 10:00-12:00 and Wednesday 2-4
Also During Individual Lab Sections and by Appointment.

Humana Press Inc. Totowa, NJ.

Recommended readings
Norfolk, England.

Course Objectives and Statement:
It was estimated that potentially less than 1% of microorganisms are recovered in the environmental samples through traditional culturing techniques. Molecular techniques provide fast, accurate, and unbiased analysis of environmental microorganisms and have been widely adopted by environmental engineers and scientists in recent years. The goal of this course is to provide environmental engineers and scientists, whom have no prior experience in molecular biotechnology, the necessary training to understand and apply current molecular techniques used in environmental profession. The course will start out with introductory level microbiology and molecular microbiology, continue with background theories of individual molecular techniques, and conclude with case discussions on current environmental research using molecular techniques such as PCR-DGGE, t-RFLP, PLFA, FISH, and qPCR etc. Special focuses will target on environmental samples which contain complex microbial communities.

The laboratory is designed to provide students with hands-on molecular techniques experience, especially for those who are interested in incorporating molecular analysis into their research. The laboratory allows students to bring in a set of environmental samples of interest at the beginning of the class, analyze them with a series of molecular techniques throughout the semester, and conclude with data interpretations and preparation of a scientific report.
After taking this course students will be able to:

1. Have a general idea of the science of molecular microbiology.
2. Understand the background theories of individual environmental molecular techniques.
3. Describe and communicate environmental molecular techniques with other fellow engineers and scientists.
4. Design experiment and select suitable molecular techniques for research projects.
5. Obtain information needed for research either through books, journals, internet, or technical support.
6. Handle and manipulate DNA.
7. Understand how to prevent microbial contamination and DNA contamination.
8. Operate instruments for molecular analysis, such as electrophoresis, thermalcycler, and imaging system.
9. Interpret results using available software and database.
10. Prepare a good scientific laboratory notebook.
11. Prepare a scientific report for peer-reviewed journals.
12. Demonstrate confidence in incorporating molecular techniques in your future work.

**Attendance.** Although attendance will not be taken during lecture periods, you are expected to attend class and you are responsible for obtaining any missed material from your classmates. Students are also required to reschedule a time to complete laboratories missed.

**Weekly lab reports** There will be weekly laboratory reports. Laboratory reports are due one week after the completion of the lab. Any reports turned in after this time will be considered late, and will receive a grade of zero, no excuses. The lab reports should include the following sections: goal (1 or 2 sentences), introduction which includes background and theory of the method (up to 5 pages), material and method, results, and discussion. The reports should not exceed a total of 20 pages, and students should try to prepare a well organized and concise report.

**Final lab report.** A final lab report is due on the day of final exam. The report should be a compilation of the data obtained throughout the semester and be written in a scientific journal publication format. The preferred format should follow the general publication requirements of the journal “Applied and Environmental Microbiology”, which can be obtained through [www.asm.org](http://www.asm.org) at the “instruction to authors” section under the specified journal.

**Laboratory performance.** Students are required to prepare a scientific laboratory notebook for data keeping. Instructions on how to keep a good notebook is given as attached. Students should turn in this notebook the same day the final report is turned in. Grading of laboratory performance is based on the quality of the results and the contents of the notebook.
Exams will be given during the semester along with a comprehensive final. Notify me prior to the exam if you cannot take the exam due to illness or other special circumstances. If you miss an exam without prior notice, please be aware, no make-up exams can be given unless you have written medical authorization.

Forensic analysis will be included in the last part of the laboratory. This is to show you how similar molecular techniques are used in different fields, and how things you have learnt here can be applied in other disciplines. An unknown sample will be provided for the students to identify and compare with the known samples.

Weights for each Assessment Type. The grades for each of the different assignment/assessment type will be averaged by category, and the weights of each category will be used to calculate the final numerical grade. The weights for each category are:

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<tr>
<th>Assessment Type</th>
<th>Weight</th>
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<tr>
<td>Mid Term</td>
<td>20%</td>
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<tr>
<td>Final</td>
<td>30%</td>
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<tr>
<td>Laboratory Report</td>
<td>20%</td>
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<tr>
<td>Final Report</td>
<td>15%</td>
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<tr>
<td>Laboratory Performance</td>
<td>10%</td>
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<tr>
<td>Forensic</td>
<td>5%</td>
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<td><strong>Total</strong></td>
<td><strong>100%</strong></td>
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Final Letter Grade Determination. The final numerical grade will be converted to a letter grade according to the following table. This allows you to determine your final letter grade at any point in the semester and at the end of the semester.

<table>
<thead>
<tr>
<th>Percentage</th>
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<tbody>
<tr>
<td>94-100</td>
<td>A</td>
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<td>90-93</td>
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<tr>
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Week 1 1/20
Introduction to Microorganisms: taxonomy; cell structure and function; microbial growth; sterilization/disinfection.
Week 2 1/27
Microbial Molecular Genetics: DNA structure and replication; Gene expression and regulation; Proteins and Enzymes; rRNA technology

Week 3 2/3
Basic molecular techniques: DNA extraction and purification; DNA quantification; Gel electrophoresis; RNA extraction; Hybridization

Week 4 2/10
PCR techniques: PCR components and mechanisms; nested PCR; touchdown PCR; RT-PCR; Primer design

Week 5 2/17
DNA cloning; DNA sequencing; heteroduplex; chimera; DNA database

Week 6 2/24
Quantitative DNA techniques: competitive PCR; real-time PCR

Week 7 3/3
Microbial Ecology I: microbial diversity; DGGE/TGGE

Week 8 3/10 Mid-Term

Week 9 3/17 Spring Break

Week 10 3/24
Microbial Ecology II: RFLP; tRFLP; fingerprint analysis; dendograms

Week 11 3/31
Microbial Ecology III: Single-Strand Conformation Polymorphism (SSCP)

Week 12 4/7
Microbial Ecology IV: FISH; Flow cytometry

Week 13 4/14
Microbial Ecology V: Biochemical methods: PLFA

Week 14 4/21
Emerging techniques: microarray; proteomics; LAMP; community RNA ratio determination

Week 15 4/28
Case study

Week 16 Final
Laboratory

Week 1 1/20
Introduction to a molecular laboratory; preparation of sterile supplies; discussion of sample preparations for the semester.

Week 2 1/27
Cultural media preparation; autoclaving; plate count (spread plate/strip plate); membrane filtration; MPN

Week 3 2/3
DNA extraction I: traditional method

Week 4 2/10
DNA extraction II: commercial kit

Week 5 2/17
DNA quantification

Week 6 2/24
Competitive PCR

Week 7 3/3
Hybridization probe preparation and purification

Week 8 3/10
Mid-Term

Week 9 3/17 Spring Break

Week 10 3/24
Hybridization

Week 11 3/31
DGGE I: PCR and electrophoresis

Week 12 4/7
DGGE II: fingerprints and preparation for DNA sequencing

Week 13 4/14
Potentially tRFLP or DNA cloning

Week 14-15 4/21-4/28
Just for fun: forensic identification – a test of knowledge.

Week 16 Final
Suggestions for keeping a laboratory notebook - scientific notebook instructions

Under U. S. law a patent is granted to the first to conceive the idea for the invention, not the first to apply for the patent. So a laboratory notebook is essential evidence of the date of conception.

When properly kept, a laboratory notebook permanently records, for future proof, what was done on a project, and particularly what inventions were made and when. Use a book with permanently bound pages. Spiral or comb bound books are not suitable for use in court. Make all entries with ink.

The intent of all entries is proving in court such facts, as the idea conception, model tests, and the test results.

If an invention is made the dates of "conception" and "reduction to practice" are essential. The record must show there no abandonment between these dates. Avoid making negative notes such as "No good", "Doesn't work " which might be later construed as indicating you were abandoning the idea.

Generally a sketch and a brief written description are enough to establish conception. Reduction to practice needs construction and successful testing of a device incorporating the invention. At any time before or after a patent has been issued, another applicant for a patent on the same invention may start a contest called an "interference", to determine who was the first inventor. Each party can offer depositions and documents to prove their dates of conception and reduction to practice. The patent for the invention is then awarded in accordance with the facts proven by this evidence.

Even if a patent application is not made on the subject matter of a research project it may become important to prove what was done. Others may obtain a patent on subject matter reduced to practice during the project and sue for patent infringement. The earlier notebook record would provide a defense. Sometimes it may be desirable to prove whether or not an invention occurred in the course of a specific research project.

Entries should show which project the work applies to. Include all formulae or diagrams, sketches of circuits and equipment which were considered during the project, including the ones actually built and tested. Diagrams and sketches should have information to identify and explain the subject matter. Another investigator, by examining these entries, should be able to determine the nature of the project, when it started, what ideas were considered what compounds made or circuits and equipment actually built and tested, the test results, the dates with respect to all of the above, and the final conclusions.

Sign and date pages when full. Don't leave blank areas on a page. At least one other worker, who is competent to understand the work, should regularly examine and witness the entries by signing and dating each page examined. This person should not be a co-worker or joint inventor.
All letters, sketches, photos, charts or computer printouts pertinent to the project should be permanently put in the notebook with your initials and date.

Notations should be made of the progress and completion of compounds, assemblies or models which are being prepared for testing. These entries should make clear, as by reference to a previous sketch, as to how the compound or equipment is being made.

The date of successful testing of a compound or particular setup or piece of equipment i.e.; "reduction to practice", is of utmost importance. Notations of such tests should be made, with the compound or equipment being identified, and with comments concerning the results of the test. Make tables of the test data if possible.

Don't erase. Cross out errors and make a new entry. Entries should not be changed at a later date. Make a new entry, pointing out any change.