MICR 3449 - Prokaryotic Molecular Genetics - Spring 2014

Instructor: Dr. Rosa A. Maldonado, PhD
Office: Biosciences Bldg room 5132
Office Hours: Monday and Wednesday, 10:00-11:00 am
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E-mail: ramaldonado@utep.edu

Course Objectives (Learning Outcomes):

The course will cover structure and organization of the prokaryotes genome, transfer of gene information, regulation of gene expression and genetic engineering. The student will able to understand the basic principles of molecular biology, analyze sequences, design primer, transcribe, translate gene sequences and to improve the students oral skills. The laboratory is designed to provide research experience using molecular biology techniques. These include manipulation of microorganisms, transfer of genetic material and molecular engineering techniques (p.e. transfection, sequencing, PCR). They will learn how to write a grant to NIH.


Lectures’ days and time: Mon/Wed/Fri, 8:30-9:20 am; Quinn Hall 212.
Lectures’ room: TBA
Labs days and time: Section 21789, Mon, 3:00 - 5:50 pm, room BIOL 412
Section 21790, Tue, 7:30 - 10:20 am, room BIOL 426
Section 21791, Wed, 3:00 - 5:50 pm, room BIOL 412
Section 21792, Thurs, 3:00 - 5:50 pm, room BIOL 412

*Note: Labs will start in the week of January 27 - 30, 2013

Grading:

Three 100-point exams will be given during the course, and they are worth 60% of the final grade. One seminar (20% of the final grade) must be presented by the students in groups of maximum 4 students (see Seminar schedule below). The lab is worth 20% of the final grade.

No make-up exams will be given. The final exam grade will replace any missing exam or the lowest grade on Exams 1, 2 or 3. Quizzes will be given every week and it will add a maximum of 10 extra-points to the exams average grade. *Attention: No make-up quizzes and exams will be given.

Exam #1 (Feb 13, Mon 8:30 am): 100 pts
Exam #2: (March 11, Mon 8:30 am): 100 pts
Exam #3 (April 3, Wed 8:30 am): 100 pts
Group Seminar: 100 pts
Lab: 100 pts
Total: 500 pts

Final Exam (May 12 Mon, 10:00 am-12:45 pm): 100 pts
Quizzes: 10 extra-points

Final Grades:
448–500 pts = A 398–447 pts = B 348–397 pts = C
298–347 pts = D <297 pts = F
Course Program

1. Structure and function of DNA (identification of genetic material in bacteria and phages; double-helix model; semi-conservative replication) Replication (DNA polymerases; synthesis of DNA). Replication in bacteria.
2. Structure and function of RNA. Transcription: initiation, promoter recognition, repression, activation, RNA polymerase structure/catalysis, elongation and termination.
3. Translation: tRNA and genetic code; ribosome structure, initiation, elongation, termination and frame shifting; regulation; mRNA stability; RNA processing; introns
4. Mutation; types of mutation; selection of mutants
5. Repair of DNA and mutagenesis; Specific and General Repair
6. Plasmids; Definition, properties
7. Transfer of genetic information; F-plasmid and conjugation, transformation, transduction;
8. Bacteriophages genetics, general transduction; Phage \( \lambda \), lysogeny, specialized transduction; lysogenic phages and bacterial pathogenesis.
9. Transposons: structure and mapping applications
10. Recombination; definition; molecular models; recombination in bacteria and phage
11. Gene regulation in bacteria; Types of regulation: a) Transcriptional, b) negative, c) positive, d) by attenuation of transcription and f) feedback inhibition.

EXAM I (Feb 12)

SPEARING BREAK 10-14 MARCH

13. Molecular cloning; cloning vectors (plasmids, phages, cosmids, and artificial chromosomes –YACs). Construction and screening of Genomic and cDNA libraries. Analysis of the cloned gene by restriction mapping,
14. Expression of cloned genes in heterologous systems;
15. Applications of recombinant DNA technology
16. Genomics, post-genomics and bioinformatics

EXAM III (April 4, 2014)

PRESENTATIONS: Start 04/07/14.
Two groups will be presenting each day; 20 min seminar and 5 min. for questions. The topics will be assigned randomly in the first day of class.

1) History of molecular biology and biotechnology
2) Recombinant DNA technology, bioethics and legislation
3) Genetic manipulation and biosafety
4) Biodefense and bioterrorism
5) Implications and mechanism of bacterial drug resistance
Lab Description: This lab is designed to teach you a few techniques of molecular biology and will not necessarily correspond to concepts being reviewed in lecture. You will conduct this lab like a research team. Each team will undertake the design and execution of experiments (under guidance) to address a novel question in biomedical research.

Lab Goals:
- To participate in the scientific process by designing experiments and writing a research proposal
- To conduct experimental assays
- To articulate your findings in the form of a written and oral presentations

Attendance in labs is required. You CAN NOT attend another lab section so do not schedule appointments during your assigned lab period (no excuses whatsoever will be accepted). You should arrive on time.

Laboratory Grading: Your lab grade is dependent on your conduct and performance during lab activities and on your participation within the team. Thus, it is to your advantage to attend and participate. The lab component of the course will constitute 20% of your overall grade for PMG.

<table>
<thead>
<tr>
<th>Drafts</th>
<th>5 points (1.5pts Intro, 1pts Hypothesis, 2.5 pts Res. Design)</th>
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<tbody>
<tr>
<td>Proposal</td>
<td>20 points</td>
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<tr>
<td>Weekly Lab Reports</td>
<td>15 points (5 reports/3 pts each)</td>
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<tr>
<td>Notebook</td>
<td>5 points</td>
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<tr>
<td>Final Report</td>
<td>15 points</td>
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<tr>
<td>Oral Presentation</td>
<td>15 points</td>
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<tr>
<td>Conduct/Performance</td>
<td>12.5 points</td>
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<tr>
<td>Team Participation</td>
<td>12.5 points</td>
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100 points total

The class will be divided into teams. As a team, you will meet every week during the scheduled lab time. The purpose of this lab is for you to actually conduct a research project throughout the semester. We will meet for the first three weeks to go over background material, potential projects/questions that you might want to address, and potential approaches you might use to address your questions. This component of the lab will have to be written up as a Research Proposal. A proposal is submitted by every member of your team. You can work on the proposal together but each student must submit an original document. More details as to what this entails will be provided in class.

The following 11 weeks will be utilized for you to actually conduct the experiments. A new teaching lab, to be used only by PMG and MCB, has been created for this specific purpose. It houses cutting-edge instrumentation for you to use to conduct your experiments. A lab technician (Berenice Arriaga) will be available during normal working hours to help you with the instrumentation. Your TA and perhaps me will meet with the teams every week to discuss the experiments that each team has conducted and those to be
conducted the following week. It is up to the teams to go into the Teaching Research Lab to **conduct your experiments** on your own time.

**Lab Progress Report** will be required from each team member every week. This report should discuss the experiments that have been conducted, what outcomes were achieved, what the data means, and what is planned for the next week. A more detailed description of the Lab Progress Reports will be provided.

**Final Research Report** (April 30- May 3). One lab session has been set aside to discuss your findings and to determine what conclusions can be drawn from the data. A report is turned in by every student. Details as to how to write the report will be provide later.

**Oral Presentation** This is similar to your final report but is given in an oral format.

**Conduct/Performance** will be assessed by your TA and the lab technician. You will automatically start the semester with all the possible “Conduct and Performance” points. If any misconduct or lack of performance is observed, points will be deducted. Poor conduct/performance includes not following lab safety requirements, goofing off in lab, not coming to lab prepared, not contributing to the team experiments, wearing inappropriate attire, etc.

**Participation** within the group (in terms of conducting experiments, contributing to the proposal, final research report, and oral presentation) will be assessed. Your team mates will be responsible for providing this component of the grade since your team knows how each member actually contributed.

**Notebook.** Each student should maintain a detailed lab notebook (**please use a composition notebook**). This lab book is intended to serve as a detailed diary of the protocols, discussions, experiments and results pertaining to each of the lab exercises performed during the semester. Legibility, neatness, and organization are important. Handouts do not need to be recopied into the lab book but may be cut and pasted or taped into the appropriate section. Work with lab partners to put together findings and other materials but **write up each exercise in your own words. Plagiarism will not be tolerated. Do not loan out your finished product for others to copy.** Lab books will be checked at midterm. Completed lab books are due in class on the last day.

*Use a composition notebook as laboratory notebook.*

**Read this section carefully.**

*For each lab exercise your notebook should contain the following:*

**I. Title**
Brief and descriptive. Example: Lab Exercise # 1 - Basic genetic techniques I

**II. Introduction**
Describes the purpose of the lab exercise and includes background information

**III. Materials and Methods**
Describes the supplies used and how the experiment or technique was performed. It should be sufficiently detailed to permit another person to duplicate the work.

**IV. Results**
A written account of observations, findings, and raw data. (An excellent Results section also presents data in the form of drawings, graphs or tables where appropriate. Drawings, tables, and graphs are numbered and have their own brief titles.)

**V. Discussion**
A summary of what was learned from both methodological and principles standpoints. Also describes problems encountered and possible solutions.
<table>
<thead>
<tr>
<th>WEEK</th>
<th>DATE</th>
<th>LAB EXERCISE</th>
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<tbody>
<tr>
<td>1</td>
<td>Jan. 27-30</td>
<td>General Lab Safety, Discuss Syllabus, Create Teams, Instrument Orientation</td>
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<tr>
<td>2</td>
<td>Feb 3-6</td>
<td>Overview of How to Research a Topic; discuss format of Research Proposal</td>
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<td>3</td>
<td>Feb 10-13</td>
<td>Turn in draft of Intro – Edit Intro; Develop a Hypothesis.</td>
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<td>4</td>
<td>Feb. 20-23</td>
<td>Turn in draft of Hypothesis – Edit Hypothesis</td>
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<td>Develop Research Design/Approach</td>
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<td>5</td>
<td>Feb. 24-27</td>
<td>Turn in draft of Research Design – Edit Research Design</td>
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<td>Transformation</td>
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<td>6</td>
<td>March 3-6</td>
<td>Microplusin Expression in the vector pRSET A</td>
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<td>7</td>
<td>March 10-14</td>
<td><strong>Spring Break</strong></td>
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<td>8</td>
<td>March 17-20</td>
<td>Research Proposal Due</td>
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<td>Lab Progress Report 1 Due</td>
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<td>SDS-PAGE of the recombinant microplusin</td>
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<td>9</td>
<td>March 24-27</td>
<td>Purification of the recombinant microplusin</td>
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<td>10</td>
<td>March 30 - April 3</td>
<td>Lab Progress Report 2 Due</td>
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<td>Desalt of the recombinant microplusin</td>
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<td>11</td>
<td>April 7 - 10</td>
<td>Quantification of the recombinant microplusin</td>
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<td>12</td>
<td>April 17-20</td>
<td>NO LAB</td>
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<td>13</td>
<td>April 24-27</td>
<td>Lab Progress Report 3 Due</td>
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<td>Microplusin analysis in the MALDI</td>
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<td>14</td>
<td>April 30- May 3</td>
<td>Lab Progress Report 4 Due</td>
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<td>Bioactivity assay</td>
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<td>14</td>
<td>May 5- 8</td>
<td>Lab Progress Report 5 Due</td>
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<td>Final Report</td>
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<td>Oral Presentations</td>
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