MOLECULAR BIOLOGY LAB  
(CELL 312 –01, -02, -03)  
Fall 2007

LAB SUPERVISOR:  Dr. Nancy Eddy Hopkins, Room 4009, Stern Hall, 862-3162, nhopkin@tulane.edu Office hours- Tuesday 10-11:30 am or by appointment (make by e-mail)

Class meetings: Stern Hall, Room 3000  
CELL 312-03 Monday, 1-5 pm  
CELL 312-01 Tuesday, 1-5 pm  
CELL 312-02 Wednesday, 1-5 pm

Required texts:  A Photographic Atlas for the Molecular Biology Laboratory by Patrick Guilfoile.  A three ring binder (1-2 inches) is also needed.  Additional readings may be assigned from “Molecular Biology, 4th Edition” by Robert F. Weaver.  This is the text for CELL 311.

OBJECTIVES  
CELL 312 Molecular Biology Laboratory is a one semester, one credit laboratory.  This course is designed to introduce you to the basic techniques and principles used in a modern molecular biology lab.  It is impossible to cover all the technology that is available to research scientists.  The techniques you will learn are fundamental to molecular biology research and form a basis for understanding many more complex technologies.

We will complete four projects in this course.  Weeks 2-4 compose a complete RFLP analysis with Southern blotting.  During weeks 5-7 and 9 we will perform two PCR methods and demonstrate RNA isolation.  Weeks 6-11 introduce cloning.  During weeks 10 and 11, we will discuss concepts of DNA sequencing.  Each of these experiments has several parts and it will be necessary for you to be able to coordinate several tasks at the same time.

SCHEDULE  
Meeting 1  Sept 10, 11, 12  Use of Equipment  
Running a Gel  
Lab Safety  
Atlas Chapters 1 & 2

*Meeting 2  Sept 17, 18, 19  Restriction Digest/ 
RFLP  
Isolation of DNA for Probe  
Atlas Chapters 3 & 4

*Meeting 3  Sept 24, 25, 26  RFLP Analysis  
Southern Blotting  
Labeling Probe DNA  
Atlas Chapter 4

Meeting 3B  Sept 25, 26, 27  Complete Southern Blot
| Meeting 4A | Sept 30, Oct 1, 2 (day before regular lab) | Prehybridization and Hybridization of Blot | Atlas Chapter 4 |
| *Meeting 4B | Oct 1, 2, 3 | Washing and Developing Southern Blot | Atlas Chapter 4 |
| Meeting 5 | Oct. 8, 9, 10 | PCR mRNA Isolation | Atlas Chapter 5 & 6 |
| *Meeting 6 | Oct. 15, 16, 17 | PCR Gel Transformation Blue/White Screening | Atlas Chapter 5 & 7 |
| Meeting 7A | Oct. 21, 22, 23 | Plasmid Cultures | |
| *Meeting 7B | Oct. 22, 23, 24 | Quantitation of mRNA, Calculation of Transformation Efficiency Isolation of Plasmid DNA | Atlas Chapter 6 & 8 |
| *Meeting 8 | Oct. 29, 30, 31 | Linker Based Mutagenesis, Preparation of Linker Ligation | Atlas Chapter 9 & 10 |
| *Meeting 9 | Nov. 5, 6, 7 | Transformation With Ligated Plasmid RT-PCR | Atlas Chapter 7 & 9 |
| Meeting 10A | Nov. 11, 12, 13 (day before regular lab) | Plasmid Cultures | |
| Meeting 10B | Nov. 12, 13, 14 | Isolating Plasmids Luciferase Assay Computer Analysis | Atlas Chapter 8, 9 & 11 |
| Thanksgiving Break | Nov. 19, 20, 21 | No Labs | |
**Meeting 11**  Nov. 26, 27, 28
*Plasmid Prep Gel*  Atlas Chapters
RT-PCR gel  6, 8 & 11
DNA Sequencing
*(Concepts only)*

**Meeting 12**  Dec. 3, 4, 5
*Final Exam*

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**GRADING**
Two mid-term exams will be given over the course of the semester, a final exam. Each mid-term exam is worth 30 points and the final exam is worth 60. In addition, 8 scheduled quizzes (see meetings marked with an “*”) will be given, each worth 10 points. You will keep a notebook and will be given written assignments that will count for 120 points. The remaining 80 points will come from class participation as deemed by your TA and instructor. Thus, your final grade will be based on a total of 400 points. Please note that there will be **NO MAKE-UP EXAMS OR QUIZZES**!

It is imperative that you come to class prepared. You should read the experimental procedure in the lab manual and in handouts and the corresponding chapters in the Atlas. Many of the texts used in CELL core courses contain background material that will help you to understand the experiments. I will provide citations in these texts for your use. These citations are not exhaustive and you may find other material in that text that will be helpful to you. The weekly quizzes will cover both the material from the previous lab and questions (up to 5 pts) on the experiment for that week. You should read the laboratory procedure and the Atlas to be prepared for these questions.

You will be required to keep a laboratory notebook. This notebook will be somewhat informal and kept like a research notebook. Part of the notebook will consist of the data and question sheets in your lab handouts. Your lab handouts and additional paper to record data should be placed in a three ring binder. There is no lab manual for this course. You will be given handouts with the various procedures and information about reagents that we use which should be placed in your notebook. Each packet of handouts will include datasheets to complete and questions to answer. You should date the entries and take notes complete enough that you and your TA can read them. Some examples of data that you will record are loading sequences for gels, photographs of gels and blots, etc. Tape and a 3-hole punch will be provided in the lab so that you will be able to easily add these items to your notebook. Your TAs will check the notebooks on a regular basis so they should be kept up to date. If the teaching assistants feel that you are coming to lab unprepared then they may give written prelab assignments as a part of your participation and notebook grades.

The quizzes will be relatively short and will cover information that was previously covered (since the last quiz) as well as information pertaining to that day’s lab. The 2 midterms will cover all of the material and labs as indicated and the final will be comprehensive.

**ALWAYS** read the “Photographic Atlas Reference” and other texts cited for that day’s assignment. This is an excellent source of background information (think quiz, exams).
Handouts for the labs will be given at least one week before they are used. You should place those in your notebook and read them before you come to lab. Handouts will also be posted on Blackboard. It is your responsibility to be sure that you have the handouts and have read them before lab and, therefore, before the quiz. All handouts should be hole-punched and placed in your lab binder.

**ATTENDANCE**

Attendance is mandatory and there are NO MAKEUP labs. You must be on time. Most of the labs will not require the entire four hour period if you are prepared and are attentive to the lab lecture. You will be working with a lab partner. You should plan your time and share the work. In the molecular biology lab, it is often necessary to plan ahead so that solutions and gels are ready when you need them. Also, often it is necessary to be able to monitor two tasks at the same time. Occasionally, you will need to come in on the day before the lab to prepare solutions or begin an experiment. Also, you will sometimes need to return the day after lab to look at a result or place materials in the refrigerator. If you must miss a lab, you should notify both Dr. Hopkins and your teaching assistant before the lab. This may be done by e-mail providing there is sufficient time for them to receive the e-mail. Otherwise you must call. If you are excused, you may obtain any data that you need from your lab partner. You should indicate in your notebook the name of the individual that collected the data. Use of data other than your own without permission of the instructor or TA is a violation of the Honor Code and failure to acknowledge the source of the data in your notebook is a violation of the Honor Code. This means that if your experiment fails and you are told to use data from another group, it is your responsibility to record this in your notebook. Additional written work may be required in order that the missed material is mastered.

**LAB MAINTANCE AND CLEANLINESS**

In order to complete the experiments in the lab period, many of the reagents will be made for you by the teaching assistants. Some reagents will be used on a continuing basis. The first time that you receive these reagents, you will be given storage conditions and told where the reagent is to be stored. You should label the container with your group number, day of lab meeting and both partners’ initials. Other reagents will be available at the side bench either in bulk or as aliquots. Expensive reagents such as enzymes, enzyme buffers and markers will be aliquotted for each lab group. Your TA will instruct you in proper handling and disposal for all reagents. You are expected to properly label and store reaction products that are needed for later experiments.

You will be assigned a permanent lab station for the semester. Each group will have a set of equipment assigned to your group. Each group will be responsible for returning this equipment to its proper place at the end of each lab. Some equipment, i.e. centrifuges, will be shared by two groups at the same bench. Report any malfunctioning or broken equipment to your TA.

You will be instructed as to the proper manner of disposal of reagents and plasticware for each lab. You are responsible for cleaning your area, storing reagents and equipment and disposing of waste. Failure to properly store your reagents, maintain your area and maintain the common areas of the laboratory will result in loss of participation points.
BLACKBOARD
We will be using Blackboard (http://blackboard.tulane.edu) for this course this semester. Check it often for announcements and information. Handouts will be posted on Blackboard. Blackboard requires that you use your Tulane e-mail address. If you do not regularly check your Tulane account, forward the Tulane account to one you check regularly as the TAs and I will use e-mail to contact you.