Biol 4590C

Research Project Laboratory

Recombinant DNA technology

Fall Semester, Year 2015

Lectures – Tuesday 12:05 pm - 12:55 pm, Room 320 CE

Labs – Tuesday 1:05 pm - 3:55 pm and Thursday 12:05 pm - 2:55 pm, Room D104 CE

Professors:

Francesca Storici (Biology)

Room 5017, EBB1, 404-385-3339, storici@gatech.edu

Office hours – Thursday 3-4 PM

Teaching Assistants:

Chance Meers

Room 5116B-D, EBB1; (404) 385-3338; chancemeers@gmail.com

Textbooks (all required material from these sources will be provided):

- *Getting Started with Yeast* by Fred Sherman. Modified from: F. Sherman, Getting started with yeast, Methods Enzymol. 350, 3-41, 2002.

- *An Introduction to the Genetics and Molecular Biology of the Yeast Saccharomyces cerevisiae* by Fred Sherman, 1998.

- *Gene Correction: Methods and Protocols – Methods in Molecular Biology*; Ed. Storici, F.; Humana Press, New York, NY; Vol. 1114, 2014.

- *Biometry: The Principles and Practice of Statistics in Biological Research* 3rd edn. Eds. Sokal, R.R. & Rohlf, F.J.; W.H. Freeman, New York, 1995.

- Additional text sources will be provided during the semester.

Tentative Schedule.

Week 1, 08/18-20	Organizational meeting, Lecture: Introduction to the class topics and get to know yeast as a model eukaryote Lab: Experiment I – Media preparation, Growing yeast, Replica plating; Plasmid preparation from bacterial cells
Week 2, 08/25-08/27	Lecture: Recombinant DNA techniques, Polymerase Chain Reaction (PCR), and yeast cell transformation using DNA Lab: Experiment II – Restriction analysis gel electrophoresis, Polymerase Chain Reaction (PCR), yeast plasmid transformation
Week 3, 09/01-09/03	Lecture: Site-directed mutagenesis, and gene disruption in yeast Lab: Experiment III – Site-directed *in vitro* mutagenesis, yeast gene disruption, yeast phenotype testing
Week 4, 09/08-09/10	Lecture: Molecular analysis of gene disruption (colony PCR), cell Counting, and analysis of *in vitro* mutagenesis results Experiment IV – Colony PCR, microscopy and cell counting and culture dilutions minipreps and sequencing of mutants of *in vitro* mutagenesis Notebooks to be turned to the Professor for comments Quiz 1
Week 5, 09/15-17	Lecture: CRISPR/Cas9 system Lab: Experiment V – Random mutagenesis screen; Test of the CRISPR/Cas9 system in yeast.
Week 6, 09/22-24	Lecture: Gene targeting approaches and *delitto perfetto* method for *in vivo* site-directed mutagenesis. Lab: Experiment VI – Mutant screening by pronging technique and *delitto perfetto in vivo* site-directed mutagenesis.
Week 7, 09/29-10/1	Lecture: Recombinant DNA vectors, yeast gene libraries. Lab: Experiment VII – Phenotype testing and molecular testing of yeast transformants, statistical analysis of data, colony PCR, PCR purification and sequencing.
Week 8, 10/06-08	Lecture: DNA repair and DNA recombination Lab: Experiment VIII – Scoring of pronging results and sequencing results Notebooks to be turned to the Professor for comments Quiz 2
Week 9, 10/13	No lecture, no lab

10/15	Discussion of individual projects Practice Project Report to be turned to Professor for Comments

Week 10, 10/20-22	Individual Project
Week 11, 10/27-29	Individual Project
Week 12, 11/03-05	Individual Project
Week 13, 11/10-12	Individual Project
Week 14, 11/17-19	Individual Project
Week 15, 11/24	Individual Project (to be completed)
Week 16, 11/30	Individual Project materials to be turned to TA
	Notebooks to be turned to Professor for Grading
	Individual Project Reports to be turned to Professor for Grading

RULES AND REQUIREMENTS

Students should organize into groups, with 2 students per team.

Equipment.

Each group receives individual equipment. The equipment you receive is your responsibility and you have to make sure that it is returned to the TA at the end of the course. *Completing an equipment checklist is required at the end of the course.* The Course Coordinator of the School of Biology may come and see you for damaged or lost units of equipment.

Grading.
Quizes – 40%
Notebooks – 20%
Individual Project – 40%

Notebooks.

Your notebooks should be handwritten (not typed), and should include original notes you take during the experiment. They are graded individually (each student is required to present his/her *own* notebook). Your notebooks should contain description of the procedures you have performed, and actual/original data. It is not necessary to rewrite the protocols (you may attach them if you wish to) in your notebooks. However, you have to outline experimental steps so that an experienced person (including yourself) should be able to trace your experiments without frequent references to the original detailed procedures. In addition, it is required that you include all the changes made (comparing to the original protocols), as well as all calculations, measurements/observations, etc.

Notebooks are to be turned in for the first time on Thursday, 09/10 by 3 PM. You will receive them back with critical comments. Final versions of notebooks are to be turned in on 10/08 by 3 PM. These notebooks are to be graded on a 0-20 scale, with emphasis on the recording of events in the experiments (esp. critical and usual data/observations) and on the clarity of event-procedure correspondence.

Students who want their notebooks back should make requests no later than one month after the completion of the course. Notebooks remaining beyond that point will be regarded as unwanted and discarded.

Individual Projects (One per team; teammates will receive the same grade for the individual projects)

Examples for Experiment VI (Individual Project) will be given one week before the projects begin. You will have options to choose from. You may suggest your own mini-projects, but please remember that they have to use techniques, equipment and materials available at the Rec Lab. Projects using additional equipment or materials can be approved only if there is a research lab willing to provide the equipment, materials and guidance for such a project.

Project Reports: should be typed as double-spaced manuscripts according to the format of the specific scientific journal, for example, *Journal of Biological Chemistry (JBC).* It is recommended that you find a few examples of the papers published in this journal and use them as references. The first issue of each year usually has rules and instructions on preparation of manuscripts; they can be found on the Web site as the Information for Contributors, too. Please note that different journals may have slightly different rules on manuscripts, even though principles are similar. You may choose a different journal than JBC as an example but you have to define which journal's rules you followed, and follow them exactly. A report generally includes the following sections: *Abstract (or Summary), Introduction, Materials (*or *Experimental Procedures), Results, Discussion,* and *References Cited*. The Results and Discussions sections could be sometimes combined, especially for simple projects. Experimental data should be presented as figures or tables with appropriate legends.

Practice Reports: Each team turns in one report for the experiment II or IV (by the team's choice). The due date is by 3 PM on Thursday, 10/18. These reports are NOT to be graded. These reports won't affect final grades. Their meaning is to demonstrate major errors that have to be taken into consideration while the Individual Project Reports are prepared. Practice reports will be returned to the students with comments. Typical errors will be discussed in the class during lecture hours.

Final Individual Project Reports: Each team turns in one report for the Individual Project. These reports are due by 3 PM on Tuesday, 11/30. These reports will be graded and contribute 40% to your final score. If your experiment didn't yield expected results, you can still get a high score if you properly address possible reasons for the failure and present reasonable strategies for correcting the problems. Your working habits will be

observed in class and will be counted toward your report grades. NOTE: Individual project reports won't be returned to the students, although you may see them by appointment to learn about your errors.

Note that DNA samples you have obtained during individual projects (and by Professor's request, some of the samples obtained during Experiments I-V) should be properly marked (with sample names, dates, and your group numbers), referred to in the text and returned to TA at the same time when your reports are turned to Professor. Failure to turn in DNA samples or to properly mark them will result in severe subtractions.