Biol 4590C

Research Project Laboratory

Genetic engineering applied to study DNA metabolism

Fall Semester, Year 2014

Lectures – Tuesday 12:05 pm - 12:55 pm, Room 322 CE

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

Professors:

Kirill Lobachev (Biology)

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Office hours – Tuesday 4-5 PM

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Office hours – Thursday 3-4 PM

Teaching Assistants:

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Textbook:
D.A. Miklos and G.A. Feyer. DNA Science. A First Course. 2d edition, Cold Spring Harbor Laboratory Press, 2003.

Additional sources:

Watson J. D., Myers R. M., Caudy A. A., Witkowski J.A. Recombinant DNA, W H Freeman & Co., Cold Spring Harbor.; 3rd edition (2007)

Sambrook, J. and Russel DW. (2001) Molecular Cloning: A Laboratory Manual, 3d ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Getting Started with Yeast, 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

Tentative Schedule.

Week 1, 08/19-21	Organizational meeting, Lecture: Restriction Analysis; Lab: Experiment I – Restriction Analysis
Week 2, 08/26-08/28	Lecture: Gene Cloning Lab: Experiment II – Ligation and bacterial transformation
Week 3, 09/02-09/04	Lecture: Polymerase Chain Reaction, Site-Directed Mutagenesis, Lab: Experiment III – polymerase chain reaction
Week 4, 09/9-09/11	Lecture : Identification of cloned genes and gene modifications Experiment IV – Plasmid extraction Notebooks to be turned to the Professor for comments Quiz 1
Week 5, 09/16-18	Lecture: Introduction to yeast genetics, yeast as a model eukaryote Lab: Experiment V – Media preparation, get to know yeast, and yeast transformation, gene disruption.
Week 6, 09/23-25	Lecture: Recombinant DNA vectors, yeast cell transformation using DNA. Lab: Experiment VI – Phenotype testing and molecular testing of yeast transformants; random mutagenesis and cell plating technique.
Week 7, 09/30-10/2	Lecture: Gene targeting approaches and *delitto perfetto* method for *in vivo* site-directed mutagenesis. Lab: Experiment VII – Mutant screening by pronging technique and *delitto perfetto in vivo* site-directed mutagenesis.
Week 8, 10/7-9	Lecture: DNA repair and DNA recombination Lab: Experiment VIII – Colony PCR and scoring of pronging results. Notebooks to be turned to the Professor for comments Quiz 2
Week 9, 10/14 10/16	No lecture, no lab Discussion of individual projects

Practice Project Report to be turned to Professor for Comments

Week 10, 10/21-23	Individual Project
Week 11, 10/28-30	Individual Project
Week 12, 11/4-6	Individual Project
Week 13, 11/11-13	Individual Project
Week 14, 11/18-20	Individual Project
Week 15, 11/25	Individual Project (to be completed)
Week 16, 12/01	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, 09/13 by 3 PM. You will receive them back with critical comments. Final versions of notebooks are to be turned in

on 10/11 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, 12/04. 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.