BMB402

Principals of Experimental Biochemistry and Molecular Biology

Basic Course Information

Instructor: Dr. Flavia Fontanesi
Department of Biochemistry and Molecular Biology
Office: UM Medical Campus 1420 NW 9th Avenue NRB room #103
Phone: 305-2437215
E-mail: ffontanesi@med.miami.edu
Office hours: By appointment

Class meeting time and location: Spring semester 2016.
Tuesday and Thursday 9.00 am – 11.50 am
Cox Science Building room #261

Credits: 2.

Prerequisite: CHM202 or CHM221. Co-requisite: BMB401

Course Overview

The course provides students with a hands-on laboratory experience that introduces them to several standard biochemical and molecular biology techniques, such as spectrophotometry, protein purification, electrophoretic analysis, PCR, site-directed mutagenesis, etc. Emphasis is placed on the analytic and quantitative aspects of experimental biochemistry, both at the experimental design and data collection and analysis levels. Moreover, the course is aimed at promoting the interest of the students in pursuing a training experience in a biochemical/biomedical research laboratory and at developing the basic skills and proper laboratory habits instrumental for such experience.

Course Learning Outcomes

By the end of the course you should be able to:
- Maintain a useful and appropriate laboratory notebook.
- Organize your lab bench space, material and work efficiently.
- Prepare common solutions, buffers and reagents used in a biochemistry lab.
- Be familiar with the principles of some basic techniques of experimental biochemistry and molecular biology such as chromatography, spectrophotometry, electrophoresis and PCR methods.
- Be able to follow several common laboratory protocols autonomously.
- Present your results in a clear, logic and critical manner.
**Course Format**

This is a lecture-lab course in which topics are presented by the instructor and experiments are conducted by the students individually or in groups of 2-4 people during lab hours. However, laboratory notebooks and reports will be always evaluated for each student individually.

**Assessments and Evaluation**

Your performance will be assessed though multiple activities, including classroom and homework. You will receive regular feedback on your work to help you monitor and improve your performance.

Your final grade will be based on the following:
- Attendance and participation 25%
- Laboratory Notebook 25%
- Midterm Test 25%
- Final Laboratory Report 25%

Attendance is expected at all the classes and more than two absences will affect your final grade.

You will be asked to keep a laboratory notebook, which will be reviewed by the instructor to provide you with feedbacks. You will receive a grade for your notebook at the midterm and at the end of the course.

**Tentative Course Schedule (2016)**

Note: changes in the schedule may occur throughout the semester. You will be promptly informed of any changes.

(January 11 to January 15, Preparation of materials. No classes.)

**Tuesday January 19**, Introduction to the course, lab safety, lab notebook, lab rudiments.

**Thursday January 21**, Experiment 1, Preparation of buffers.

**Tuesday January 26**, Experiment 2 Part 1, Spectrophotometry: absorbance spectra, pKₐ and extinction coefficient of p-nitrophenol.

**Thursday January 28**, Experiment 2 Part 2, Spectrophotometry: absorbance spectra, pKₐ and extinction coefficient of p-nitrophenol.

**Tuesday February 2**, Experiment 2 Part 3, Spectrophotometry: absorbance spectra, pKₐ and extinction coefficient of p-nitrophenol.

**Thursday February 4**, Experiment 2 Part 4, Spectrophotometry: absorbance spectra, pKₐ and extinction coefficient of p-nitrophenol.
**Tuesday February 9**, Experiment 3 Part 1, Protein purification: β-galactosidase purification by affinity chromatography.

**Thursday February 11**, Experiment 3 Part 2, SDS-PAGE and Coomassie blue staining.

**Tuesday February 16**, Experiment 3 Part 3, Protein quantification.


**Tuesday February 23**, Experiment 4 Part 2, β-galactosidase enzymatic activity, calculation of $K_M$ and $V_{max}$.


**Tuesday March 1**, Review of material. Evaluation of lab notebook.

**Thursday March 3**, Midterm Test.

Spring break (March 5-13)

**Tuesday March 15**, PCR technology and its application to identify point mutations in genes. Experiment 5 Part 1, PCR reactions.

**Thursday March 17**, Experiment 5 Part 2, PCR product analysis.

**Tuesday March 22**, Use of model organisms to analyze the effect of human disease-associated mutations. Experiment 6 Part 1, Bioinformatics analysis and primers design. Mutagenesis PCR

**Thursday March 24**, Experiment 6 Part 2, DpnI digestion and reagents preparation.

**Tuesday March 29**, Experiment 6 Part 3, E. coli transformation.

**Thursday March 31**, Experiment 6 Part 4, Plasmid extraction from E. coli (Minipreps).

**Tuesday April 5**, Experiment 6 Part 5, Miniprep analysis.

**Thursday April 7**, Semi-quantitative analysis of enzymatic activity. Experiment 7 Part 1, Yeast transformation.

**Tuesday April 12**, Experiment 7 Part 2, Analysis of transformation efficiency. Preparation of cell extracts.

**Thursday April 14**, Experiment 7 Part 3, Electrophoresis and SOD1 in gel activity.

**Tuesday April 19**, Experiment 7 Part 4, Phenotypic analysis of yeast resistance to oxidative stress.

**Thursday April 21**, Revision. Final laboratory report due.