

BIOL-UA 130 At The Bench: Chemistry Genomes to Molecular Machines

Instructor:

Michael Carrozza

Course Description:

This is an upper-level elective lab course for students majoring in Biology and those seeking to fulfill requirements for entrance into advanced degree programs. Using biochemical and genetic approaches with the yeast *Saccharomyces cerevisiae*, students will characterize a large multisubunit protein complex that modifies chromatin and is involved in gene regulation. *S. cerevisiae* is a unicellular eukaryote better known as baker's yeast that is a widely used biochemical and genetic model organism. Affinity chromatography will be used to produce purified preparations of wild-type and mutant protein complexes. The purified protein complexes will be compared using a wide variety of biochemical techniques, including Sypro Red-stained SDS-PAGE, western blot, enzymatic assay, and protein interaction assays. Yeast expressing the same mutants will be used in genetic experiments to evaluate the importance of the protein complex in cell growth and gene regulation in the cell.

Pre-requisites:

Principles of Biology I (BIOL-UA 21).
Organic Chemistry I (CHEM-UA 225)
Organic Chemistry II (CHEM-UA 226)

Textbook and Required Materials:

Boyer, Rodney. Biochemistry Laboratory: Modern Theory and Techniques. 2nd ed. Upper Saddle River, NJ: Pearson, 2012.

Grading:

Midterm Exam	25%
Final Exam	25%
Lab Report 1	15%
Lab Report 2	15%
Oral Presentation	10%
Lab Participation	5%
Lab Performance	5%

Topics:

Model organisms for genetics and biochemistry
Chromatin Structure
Whole cell extract preparation/Centrifugation (glass bead breakage; salt extraction; high speed centrifugation)
Amino acid properties and protein structure
Chromatographic separation of proteins
Tandem Affinity purification of proteins
Immunoglobulin affinity purification (Modified TAP purification handout)
Electrophoretic separation of macromolecules and Immunochemistry
Calmodulin affinity purification
SDS-PAGE of complexes and Sypro Red staining
Assay of Acetyltransferase enzymatic activity

Western blot analysis of Acetyltransferase assay

Develop western blot and analyze data

Protein interactions

Protein-Protein Pulldown SAGA complex interaction with Gal4 transcription factor

Develop pulldown western and analyze data

Catabolism of sugars/The galactose regulon

Growth of SAGA mutants using galactose as sole carbon source

Approaches to analyzing transcription

Measuring RNA abundance transcription in the cell by RT-PCR

Isotopes in biochemistry experiments

Measuring effect of SAGA mutants on a galactose inducible gene (GAL1) RT-PCR: Preparation of RNA and cDNA

Basic statistics of experimental data

Measuring effect of SAGA mutants on a galactose inducible gene (GAL1) RT-PCR: PCR analysis of cDNA

RT-PCR: Agarose gel analysis of PCR samples