CHM 377 Experimental Biochemistry Spring 2023

Instructor

Dr. Poget: 6S-233, Thursday 11:30 am - 1 pm, or via Zoom (click here for appt), Sebastien.Poget@csi.cuny.edu

Dr. Cohen: 6S-226, Monday 9-10, Wed 12 pm - 1 pm or by appointment. Leah.Cohen@csi.cuny.edu

Text Books

No textbook is required and handouts will be provided via Blackboard. Consult your Biochemistry lecture textbook for background information. The following textbooks contain useful information on experimental techniques and would be helpful to have as a resource.

- Principles and Techniques of Biochemistry and Molecular Biology, 8th Edition

Keith Wilson and John Walker, Editors ISBN #: 9781107162273 (list price: ~\$121)

- Biochemistry Laboratory: Modern Theory and Techniques, 2nd Edition

Rodney F. Boyer ISBN #: 9780136043027 (list price ~\$75)

- Fundamental Laboratory Approaches for Biochemistry and Biotechnology, 2nd Edition

A.J. Ninfa, D.P. Ballou, M. Benore ISBN #: 9780470087664 (list price: ~\$75.00)

One book that we highly recommend is <u>How to Write a Scientific Paper: An Academic Self-Help Guide for PhD Students: Saramäki, Jari: 9781730784163: Amazon.com: Books</u>. There will be readings in this book that will help you to write your lab reports and, in the future, help you write papers for publication.

Course Hours: Friday, 8:00-4:25 PM, 6S-023

Course Outline

DATE	TOPICS AND EXPERIMENTS		
Cloning of the	E. <i>coli</i> β-hexosaminidase gene (<i>nagZ</i>)	Lab Report on days 1-4 due by 2/24	
1. Jan. 27	Isolation of E. coli genomic DNA and visualization by agarose gel electrophoresis.		
	Design of primers for PCR reaction.		
2. Feb. 3	PCR amplification of E. coli nagZ gene, digestion of plasmid and P	CR product.	
3. Feb. 10	Agarose gel purification of plasmid and insert, ligation, transformation.		
4. Feb. 17	Purification of nagZ expression plasmid and restriction digestion analysis.		
	Transformation into expression strain.		
Recombinant Expression and purification of <i>E. coli</i> β -hexosaminidase (NagZ)		Lab Report on days 5-7 due by 3/17	

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5. Feb. 24	Cell growth for optimization o	of protein expression conditions,	review of bioinformatics tools.

6. Mar. 3 Assessment of NagZ expression in each of tested conditions by Western blot analysis.

7. Mar. 10 Purification of recombinant protein (Transformation, growth and harvest of large *E. coli* cell culture for protein expression will need to be performed during the week ahead of the class).

Enzymatic analysis of NagZ

Lab Report on days 8-10 due by 4/14

8. Mar. 17 Optimization of enzymatic assay for recombinant NagZ.

9. Mar. 24 Determination of enzyme kinetics for NagZ, planning of independent research project.

10. Mar 31 Temperature denaturation assay of NagZ, mutagenesis primer design.

Independent research project: Mutational analysis of E. coli ß-hexosaminidase enzymatic mechanism

Apr. 14 Site-directed mutagenesis.

Apr. 21 Expression of mutant protein.

Apr. 28 Purification of mutant protein.

May 5 Enzymatic characterization of mutant protein.

Lab Report on Independent Study due by 5/23

Final Exams May 17-23 Symposium on independent study results will be held during Finals

Reading Outline

DATE	TOPICS AND EXPERIMENTS
1. Jan. 27	Part I: How to Choose The Key Point Of Your Paper
2. Feb. 3	Part I: How to Choose The Supporting Results
3. Feb. 10	Part I: How to Write The Abstract
4. Feb. 17	Part I: How to Choose The Title
5. Feb. 24	Part II: The Power of Outlining
6. Mar. 3	Part II: How to Write The Introduction, Part I: Structure and Part II: A Four-Paragraph Template
7. Mar. 10	Part II: How to Write The Introduction, Part III: The Lede and How to Write The Materials and Methods
8. Mar. 17	Part II: How to Write The Results, Part I: Figures and Part II: Text
9. Mar. 24	Part II: How to Write The Discussion
10. Mar 31	Part III Words

Grading

Lab Reports	45%	Quizzes	15%
Individual Research Project Report	25%	Seminar Presentation	15%

Grading of reports and presentations will be performed based on grading rubrics that you will be given in advance.

Learning Objectives

- You will learn concepts and advanced laboratory techniques of protein chemistry, enzymology and molecular biology.
- You will learn the strategy of designing research projects.
- You will learn how to effectively communicate research findings by writing papers and giving oral presentations.
- You will learn how to work safely in a biochemical laboratory and how to deal with biohazardous materials.

Blackboard

Relevant information to the course will be posted on Blackboard, and you should check the course home page before each lab for updates. Make sure to read any distributed literature before class.

General Notes

Photocopies of some of the background reading material and most of the experimental procedures will be provided or posted on Blackboard. In addition to submitting lab reports and answering short quizzes, you will be expected to carry out literature surveys on specific research topics and come up with and carry out an individual research proposal in the last part of the semester. You will present the results from that project during the mini-symposium day, which will be held instead of a final exam.

Attendance Policy

You are required to attend each class on time. The in-class quizzes will be held at the beginning of class.

Last day to drop the course

According to CSI's Spring 2023 Academic Calendar, the last day to withdraw with the grade of "W" without permission of an instructor or Chairperson is May 16, 2023.

Unexpected College Closure

Please check our course Blackboard page for announcements and instructions.

Center for Student Accessibility:

If you believe that you have a disability requiring an accommodation, please contact the Center for Student Accessibility at 718-982-2510. You can also check out their website at www.csi.cuny.edu/csa/. You must notify your instructor and lab technician about the accommodation at the beginning of the semester.

Academic Honesty

All students are expected to follow the CUNY policies related to academic integrity. You shouldn't copy any other person's work including any online resources as your own. Your reports have to be completely written in your own words, and any references to published material has to be paraphrased and the original work referred to in a citation. Students must work independently on all quizzes. Any forms of cheating or plagiarism on quizzes, presentations and reports will result in a zero point score for your assignment, which may result in an **F** grade for the course. Also, any academic dishonesty will be reported to the college authority.