Experimental Biochemistry 11:115:414 Spring

Meeting days/time and location will be shared as the semester begins. Students may also look at the class schedule.

TEACHING TEAM CONTACT INFORMATION:

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COURSE WEBSITE, RESOURCES AND MATERIALS:

- Laboratory protocols, weekly presentations, and other relevant materials are posted on the Canvas course site under course content
- Safety goggles /lab coat (required, provided). Everyone is required to wear closed-toe shoes, long pants, goggles, and a lab coat while performing laboratory work
- All students are required to maintain a laboratory notebook. Laboratory electronic notebook (LabArchives platform) will be used in this course. Open LabArchives account at the URL shared later on the canvas course site. Note each section has specific URL.

To access the course, please visit https://tlt.rutgers.edu/canvas. For more information about course access, support or technological assistance with the Canvas site contact the Canvas Help Desk via email at 877-361-1134 immediately upon having an issue. Do not ask the teaching team to solve technical issues you are experiencing in Canvas or LabArchives. Instead, please call or email the respective company and follow up with them to resolve your technical issues. To protect yourself from possibly missing a deadline because of a technical issue, we ask that you work in advance of deadlines to ensure timely completion of assignments. Please have a backup plan in place in case your computer or your internet go down during the semester as make-ups will not be given due to technical difficulties.

COURSE DESCRIPTION:

This second course in the two-course experimental biochemistry sequence is intended to provide a comprehensive understanding of recombinant protein expression and purification. The course project is Purification and characterization of *Streptomyces lividans* endoglucanase CelB2 expressed in *Escherichia coli*.

LEARNING GOALS:

1) Describe theoretical considerations of recombinant protein expression in *E.coli*
2) Express recombinant wild type endoglucanase CelB2 in *E.coli*
3) Describe strategies for protein purification protocol development
4) Develop CelB2 purification protocol and purify the protein
5) Keep laboratory notebook: define objectives, record data, analyze results, draw conclusions
6) Present data in the laboratory report

RESPONSIBILITIES & ASSESSMENT:
Weekly assignments, two exams, final laboratory report, laboratory e-notebook, laboratory performance evaluation.

Individual weekly assignments (due at the start of lab the following week)
Weekly assignments in the course aim on getting you to think about the project and prepare you to write final laboratory report due at the end of the semester. Feedback on the assignments will help with your final laboratory report. Details for each assignment will be discussed in class. LATE WORK WILL NOT BE ACCEPTED.

Individual Final Laboratory Report (due at the time of the final exam for this course)
Final laboratory report on your semester's work in this class will be due at the time of designated final exam time for the course. The laboratory report serves as an evaluation of your understanding of the project, your ability to analyze your results into a broader context, your ability to think critically and creatively about the project and future experiments. The report should be written in the format of a manuscript submitted to the *Biochemistry* with a title, abstract, introduction, materials and methods, results and discussion, acknowledgements, references, and, if needed, supplemental information.

Notebook
All students are required to maintain a laboratory notebook. A neat and well-organized notebook is primary record of the performed experiments in the course. A protocol for each experiment should be written in the notebook before you begin and edited as you do the experiment. Only your laboratory protocol in your laboratory notebook will be used in the laboratory to guide you through lab procedure. Do not upload lecture power point slides and posted protocols into your notebook. You can judge your record keeping by how easily another scientist will be able to replicate experiment by using only information in your notebook. Notebooks will be evaluated each laboratory session.
The record of every experiment should contain date, title, aim, materials, and procedures. Your observations, results, and data should be recorded for each experiment. Record your calculations in the notebook (concentrations, dilutions, molecular weights, molarities, etc.).
Laboratory notebook grade is based on three major requirements:
1) Before lab component: weekly protocol preparation before lab session starts.
2) In lab component: in class data entry, comments, notes, protocol edits, observations.
3) After lab component: data analysis, calculations, summary. Must be completed weekly before start of the next lab.

Exams
The average of two exams is included in the final grade for the course. Exams test students' ability to draw inferences from experimental evidence, carry out relevant calculations, and concentrate on understanding of the biochemical principles underlying experimental procedures.

ATTENDANCE
Students are expected to attend and participate in all lectures.
Attendance of all laboratory sessions is mandatory. Attendance means arriving on time to lab session, prepared, and ready to carry out experiment in a safe manner.
There will be no makeup labs. There will be no makeup exams.

Partnerships

You will work in pairs in the laboratory. It is imperative that everyone shares equally in the work. Everyone in group should exchange copies of all data before leaving at the end of a lab period. It is appropriate (and encouraged) to consult and discuss background science, your data, and the preparation of lab assignments. However, you must write up **individual lab assignments**, write in **your own words** and prepare all figures/graphs/tables individually.

All assignments should be submitted through the Canvas course site. All assignments will be graded using Turnin.com anti-plagiarism software.

COURSE GRADING SCALE

Grade	Range
A	89 – 100
B+	85 – 88
B	79 – 84
C+	75 – 78
C	69 – 74
D	59 – 68
F	Below 59

COURSE GRADE IS CALCULATED AS FOLLOWS:

Total	100%
Weekly assignments average	25%
Exams (average of 2)	25%
LabArchives notebook	20%
Final laboratory report	25%
Lab technique/participation	5%

ACCOMODATIONS FOR STUDENTS WITH DISABILITIES

Please follow the procedures outlined at https://ods.rutgers.edu/students/registration-form. Full policies and procedures are at https://ods.rutgers.edu/

ACADEMIC INTEGRITY

In this course you are bound by all the academic standards detailed in Rutgers University Academic Integrity Policy.

The university's policy on Academic Integrity is available at http://academicintegrity.rutgers.edu/academic-integrity-policy. The principles of academic integrity require that a student:

- properly acknowledge and cite all use of the ideas, results, or words of others.
- properly acknowledge all contributors to a given piece of work.

- make sure that all work submitted as his or her own in a course or other academic activity is produced without the aid of impermissible materials or impermissible collaboration.
- obtain all data or results by ethical means and report them accurately without suppressing any results inconsistent with his or her interpretation or conclusions.
- treat all other students in an ethical manner, respecting their integrity and right to pursue their educational goals without interference. This requires that a student neither facilitate academic dishonesty by others nor obstruct their academic progress.
- uphold the canons of the ethical or professional code of the profession for which he or she is preparing.

Adherence to these principles is necessary in order to ensure that

- everyone is given proper credit for his or her ideas, words, results, and other scholarly accomplishments.
- all student work is fairly evaluated and no student has an inappropriate advantage over others.
- the academic and ethical development of all students is fostered.
- the reputation of the University for integrity in its teaching, research, and scholarship is maintained and enhanced.

Failure to uphold these principles of academic integrity threatens both the reputation of the University and the value of the degrees awarded to its students. Every member of the University community therefore bears a responsibility for ensuring that the highest standards of academic integrity are upheld.

In this class we will take cheating very seriously. All suspected cases of cheating and plagiarism will be automatically referred to the Office of Judicial Affairs, and we will recommend penalties appropriate to the gravity of the infraction.

COURSE SCHEDULE

**Note: This schedule is subject to change as per the semester's date. All the changes will be posted on the canvas site.*

week	lecture	lab
1	1/25/22 Course introduction. Project description.	none
2	2/1/22 CelB2 cellulase. Recombinant protein expression in *E.coli*.	2/2/22 Wed 2/3/22 Thu CelB2 expression in *E.coli*: Plating transformed *E.coli* BL21(DE3) from glycerol stock. LB expression media preparation. **Assignment 1 is due.**
3	2/8/22 CelB2 expression in *E.coli*. Expression plasmid, pMAL: maltose binding protein (MBP)-CelB2 fusion, His_6 tag	2/9/22 Wed 2/10/22 Thu CelB2 expression in *E.coli*: Inoculation and expression (pre-induction, post-induction samples saved for SDS-PAGE). Expression plasmid isolation from *E.coli*.
4	2/15/22 Restriction enzymes. Restriction analysis.	2/16/22 Wed 2/17/22 Thu Expression plasmid restriction with EcoRI and HindIII restriction endonucleases. 1% agarose gel for restriction analysis. **Assignment 2 is due.**
5	2/22/22 Protein purification. Cell lysis.	2/23/22 Wed 2/24/22 Thu CelB2 purification. Cell lysis by sonication. Centrifugation. **Assignment 3 is due.**
6	3/1/22 Protein purification. Affinity chromatography. Purification table.	3/2/22 Wed 3/3/22 Thu Amylose affinity chromatography. Elutions: protein concentration, activity assay. Purification table. **Assignment 4 is due.**
7	3/8/22 Protein purification. **Lecture exam 1**	3/9/22 Wed 3/10/22 Thu SDS-PAGE: pre-induction, post-induction, lysate, flow through, wash, elutions. **Assignment 5 is due.**
8	3/22/22 Protein purification. Factor Xa protease digest. Buffer exchange.	3/23/22 Wed 3/24/22 Thu Factor Xa protease digest of MBP-CelB2 in combined elutions. Buffer exchange. Ion exchange chromatography column packing. **Assignment 6 is due.**
9	3/29/22 Anion exchange (DEAE) chromatography.	3/30/22 Wed 3/31/22 Thu DEAE chromatography, linear gradient Fractions: protein content A_{280nm} **Assignment 7 is due.**
10	4/5/22 CelB2	4/6/22 Wed 4/7/22 Thu SDS-PAGE: DEAE chromatography results Activity assay. **Assignment 8 is due.**

11	4/12/22 Pure CelB2: concentration and activity	4/13/22 Wed 4/14/22 Thu Buffer exchange. Sample concentration (volume reduction). SDS-PAGE: purified protein sample **Assignment 9 is due.**
12	4/19/22 Far UV (185-250 nm) circular dichroism (CD)	4/20/22 Wed 4/21/22 Thu CD **Assignment 10 is due.**
13	4/26/22 **Lecture exam 2**	4/27/22 Wed 4/28/22 Thu CD data analysis, loose ends
	Scheduled Final exam time	**Final laboratory report is due**