

BCMB 406B

Laboratory Manual

Spring 2023

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Each instructor is ON ³/₄ people on whose traditional territory the
 university stands and the 6 R Q J K H H V (V T X L P D O W D Q G : 6 È 1 (ù S H R S O H V Z K R V H K L
 relationships with the land continue to this day.

Department of Biochemistry and Microbiology
 University of Victoria

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Course Description

BCMB 406B is a project-based course that will build on research skills you have developed in previous lab courses. Unlike other lab courses that consist of several distinct labs, 406B has three labs that build on each other to create a continuous research project from start to finish. The overall aim of 406B is to create and characterize a mutant carbohydrate binding module (CBM). CBMs are accessory modules of glycoside hydrolases (GH) which are enzymes that hydrolyse the glycosidic bond between carbohydrates. As the name suggests, the CBM targets the enzyme to its substrate by binding to carbohydrates.

In lab 1, you will learn the principles of primer design and use a variety of web-based tools to design and evaluate a set of primers for site directed mutagenesis.

At the start of Lab 2, you will use molecular biology software to plan and predict the results of individual steps in the site-directed mutagenesis of a CBM gene. You will then use inverse PCR to generate the site directed mutant, and restriction digests and DNA sequencing to confirm the presence of the desired mutation. Once the correct mutation has been confirmed, you will move the newly created mutant CBM construct into an expression host.

Lab 3 focuses on the purification and characterization of the mutant protein. Initially, you will induce expression of the mutant CBM protein and purify the protein using Immobilized Metal Affinity Chromatography (IMAC). Once purified, you will assess the mutant CBM's ability to bind carbohydrate using two techniques that will allow you to compare the function of the mutant CBM to that of wild type. Finally, you will attempt to crystallize the mutant protein and use modelling software to compare and contrast the structures of the mutant and wild type CBMs.

In this course, emphasis is placed on experimental design, data analysis and problem solving with the intention of developing your ability to work independently in the lab.

Introduction Quiz: Due Fri. Jan. 13th at 11:59 pm:

The quiz is posted on Brightspace in the Introduction section. The purpose of it is to ensure that you have read and understood all the introductory information in the lab manual on pages ii-xiv prior to attending your first lab session. To successfully complete the quiz, you must obtain a mark of 100%. However, you m

Lab Exams (40%): There will be two non-

Laboratory Report Guidelines and Format

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Course Policies

Attendance

Department Information and Policies

1. The Department of Biochemistry and Microbiology upholds and enforces the University's policies on academic integrity. These policies are described in the current University Calendar. All students are advised to read this section.
2. Cell phones, computers, and other electronic devices must be turned off at all times during live class sessions unless being used for the purpose of engaging with the class.
3. No recordings of live lectures are permitted without permission of the instructor. Many online courses will be recorded by the instructor for as 9t.98 62.34 716.34 716.0 0 12 (m)3.4 (e1()0.6 (phon)5.1ofth)5.(ogy)3

University Policy on Academic Integrity

Maintaining a high level of academic integrity is essential in all aspects of the BCMB lab courses. You are required to abide by the University's policy on academic integrity in every aspect of this course whether it is an in-person or online assessment. Course materials provided to you by the instructors are the intellectual property of the Department of Biochemistry and Microbiology and must not be shared with anyone without the consent of the instructor.

Suspected cases of plagiarism or cheating will be documented and submitted to the department chair for penalty assessment as described in the current UVic calendar.

Academic integrity violations covered by this policy can take a number of forms such as:

Plagiarism

A student commits plagiarism when he or she:

- submits the work of another person as original work
- gives inadequate attribution to an author or creator whose work is incorporated into the student's work, including failing to indicate clearly (through accepted practices within the discipline, such as footnotes, internal references and the crediting of all verbatim passages through indentations of longer passages or the use of quotation marks) the inclusion of another individual's work
- paraphrases material from a source without sufficient acknowledgement

Multiple Submission

Multiple submission is the resubmission of work by a student that has been used in identical or similar form to fulfill any academic requirement at UVic or another institution. Students who do so without prior permission from their instructor are subject to penalty.

Unauthorized Use of an Editor

An editor is an individual or service, other than the instructor or supervisory committee, who manipulates, revises, corrects or alters a student's written or non-written work.

The use of an editor, whether paid or unpaid, is prohibited unless the instructor grants explicit written authorization. The instructor should specify the extent of editing that is being authorized.

Safety Regulations

Work in a microbiology laboratory involves exposure to living microorganisms, many of which must be considered potential pathogens. Personal recognition of safety and the acceptance of certain precautions are therefore necessary prerequ

18. Contaminated liquid waste must be autoclaved prior to disposal.
19. Dispose of infectious solid waste in the yellow biohazard buckets for autoclaving. This includes pipette tips, agar plates, contaminated gloves or paper towels, etc.
20. Report any accidents or safety concerns to an instructor immediately.
 - If skin comes into contact with chemicals, wash immediately with cold running water for at least 10 min.
 - In the event of a bacterial spill, pour an equal volume of bench disinfectant on top of the spill and allow it to sit for five minutes. Clean up the spill wearing gloves and using a no-touch technique. Discard all waste in a yellow biohazard bucket for autoclaving. WASH YOUR HANDS with hand disinfectant and soap.
 - Do not pick up broken glass. The instructor will do this.
 - If something has splashed in your eyes, rinse them at the eye wash station for at least 20 min.
21. Note the location of the following safety equipment:
 - Eye wash station
 - Safety shower
 - Fire extinguisher
 - Telephone
 - Fire alarm
22. Before leaving the laboratory:
 - Place all cultures and other contaminated materials to be discarded in the appropriate containers for sterilization in the autoclave
 - Put your labeled experimental materials in the appropriate bins or racks for incubation or storage
 - Place contents of “tip discard” and used microfuge tubes into yellow biohazard bucket
 - Rinse all glassware and place in the appropriate bin(s)
 - Remove all labels from shared glassware and equipment
 - Check that gas, water and microscope lights are turned off
 - Wash the bench top with bench disinfectant
 - Wash your hands thoroughly with hand disinfectant and/or soap

Experiments conducted in a microbiology laboratory involve the handling of pathogenic organisms. Failure to handle and dispose of these organisms correctly may lead to infection, injury or even serious illness. For the safety of everyone, it is required that you understand and follow the appropriate laboratory procedures as outlined by your laboratory instructor.

Your successful completion of the Intro ductory Quiz is your acknowledgement that you have read the safety regulations and agree to abide by them.

Building Evacuation in Case of Fire

If you discover a fire:

- Activate the nearest fire alarm pull station.
- Call 911 and Campus Security Services at 7599. State your name and location.
- Evacuate the building.

If you hear a fire alarm :

- If possible secure equipment and close windows and doors.
- Follow the established evacuation route. Do not use elevators.
- Meet at your designated Emergency Evacuation Site.
- Do not re-enter the building until permission is given by the Fire Department.

If you cannot evacuate:

- Close the doors between you and the fire.
- If possible call 911 and advise the Fire Department of your situation.
- Hang clothing or a cloth from a window to alert emergency response personnel.

Earthquake Evacuation Procedures

During an Earthquake:

- Get away from windows and heavy objects.
- Duck, cover and hold on. Crouch low to the ground; protect head with your arms; seek cover under and hold onto heavy furniture. Watch for moving objects.
- If you are in an interior hallway, stay there and crouch against the wall. Watch for swinging doors.

After an Earthquake:

- After the shaking stops wait 60 seconds then evacuate the building. Do not use elevators.
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