

BCMB 406B

Laboratory Manual

Spring 2022

Student Name: _____ Section: _____

Email address: _____

Instructors and Contact Information

Lab	Instructor	Email	Phone	Office
1 & 2	Erika Wall	ewall@uvic.ca	250-472-5119	Petch 194
2	Stephen Redpath	sredpath@uvic.ca	250-721-7076	Petch 179a
3	Val Kerr	valk@uvic.ca	250-472-5119	Petch 194

Each instructor is responsible for a different portion of the course as indicated above.
Please address any concerns or questions to the appropriate instructor.

We acknowledge and respect the ləkʷəŋən peoples on whose traditional territory the university stands and the Songhees, Esquimalt and WSÁNEĆ peoples whose historical relationships with the land continue to this day.

Department of Biochemistry and Microbiology
University of Victoria

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Table of Contents

Introductory Information

Schedule	i
Course Description and Learning Objectives	ii
Health Measures	iii
Brightspace, Office Hours and Student Resources	iv
Evaluation and Assessment	v-vi
Laboratory Report Guidelines and Format	vii
Course Policies, CES and Department Information/Policies	viii-ix
University Policy on Academic Integrity	x-xi
Safety Regulations and Evacuation Procedures	xii-xiv

Laboratory Procedures

Lab 1 – Primer Design	1-1
Appendix 1A: Primer Design Characteristics	1-16
Appendix 1B: NetPrimer Interface	1-19
Appendix 1C: Using Primer3	1-20
Lab 2 – Site Directed Mutagenesis of a Carbohydrate Binding Module	2-1
Appendix 2A: Cloning of the Wild Type CBMs	2-33
Appendix 2B: Genotypes of <i>E.coli</i> Strains	2-34
Appendix 2C: Buffers and Solutions	2-35
Lab 3 – Purification and Characterization of a Mutant CBM	3-1
Appendix 3A: Use of a Flow Adaptor	3-35
Appendix 3B: Plate Reader Instructions	3-36
Appendix 3C: Pouring and Running a Polyacrylamide Gel	3-37
Appendix 3D: Recipes for SDS-Polyacrylamide Gels	3-42
Appendix 3E: Recipes for Non-Denaturing Polyacrylamide Gels	3-43
Appendix 3F: Drying Polyacrylamide Gels Using Cellophane	3-44

Laboratory Schedule

Week	Dates	Lab	Day 1 (5 hours)	Day 2 (2 hours)	Due Dates
1	Jan 10-14	Lab 1: Primer Design	Primer design and evaluation using web-based tools		
2	Jan 17-21	Lab 2: Site Directed Mutagenesis of CBM Proteins	<i>In silico</i> cloning		Lab 1 Report
3	Jan 24-28		Inverse PCR, agarose gels, DpnI digestion, electrocompetent cells	Electroporation	
4	Jan 31 - Feb 4		Plasmid purification, restriction digests, agarose gels	Plasmid preps, DNA quantification, sequencing	Electroporation controls
5	Feb 7-11		Lab 3: Purification and Characterization of a Mutant CBM	Lab 2 - Chemical transformation Lab 3 - Media prep	Lab 2 - Count plates Lab 3 - Starter culture
6	Feb 14-18	Inoculate/induce large culture, prep solutions, WT macroarray		Pellet cells, macroarray development	Day 1: Lab 2 Report
7	Feb 21-25	No Labs – Reading Break			
8	Feb 28 – Mar 4	Lab 3: Purification and Characterization of a Mutant CBM	Chemical lysis of cells, IMAC: nickel column chromatography	Identify protein-containing fractions	Bradford assay assignment
9	Mar 7-11		SDS-PAGE of fractions	Dry gels, pool fractions, dialysis	Midterm exam Thurs. Mar. 10 7-9 pm
10	Mar 14-18		Bradford assay and A ₂₈₀ , macroarray (mut vs. WT)	Macroarray development	Day 2: Protein conc. calc's
11	Mar 21-25		Affinity gels, lysozyme crystallization	Dry gels, visualize crystals	
12	Mar 28 - Apr 1		Pick up gels Exam preparation		
13	Apr 4-7				Day 1: Lab 3 Report
TBA – Lab 3 exam during regular exam schedule					

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.

Intended Learning Objectives

Upon completion of this course you will be able to:

- Describe the theory and principles of primer design, site-directed mutagenesis and protein expression, purification and characterization
- Develop proficiency in practical skills and *in silico* techniques used for primer design, site-directed mutagenesis and protein expression, purification and characterization
- Solve typical calculations used in biochemistry and microbiology experiments
- Evaluate experimental controls
- Generate a written record of data in a lab journal
- Evaluate data generated and summarize findings in written lab reports

Compare and contrast data generated in the laboratory with that of relevant published research articles **UVic Grading Scheme**

A⁺	90 - 100	B⁺	77 - 79	C⁺	65 - 69	F	< 50
A	85 - 89	B	73 - 76	C	60 - 64	N **	< 50
A⁻	80 - 84	B⁻	70 - 72	D	50 - 59		

**** N grades**

Students who have completed the following elements will be considered to have completed the course and will be assigned a final grade:

- *In class lab work (all Day 1 and Day 2 practical components must be performed)*
- *Midterm exam*
- *Final exam*

Failure to complete one or more of these elements will result in a grade of “N” regardless of the cumulative percentage on other elements of the course. An N is a failing grade, and it factors into a student’s GPA as 0. The maximum percentage that can accompany an N on a student’s transcript is 49.

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 connecting and engaging with the class.
3. No recordings of live lectures are permitted without permission of the instructor. However, many courses will be recorded by the instructor for accessibility for students unable to attend. If you do not wish to be recorded, contact your instructor to determine if alternative arrangements can be made.
4. Students and instructors are expected to assess their health daily and avoid campus if they are ill.
5. Course materials, such as notes, problem sheets, quizzes, examinations, example sheets, or review sheets, may not be redistributed without the explicit written permission of the instructor.
6. Students are expected to be available for all exams. Instructors may grant deferrals for midterm examinations for illness, accident, or family affliction. Although students do not require documentation, students must contact their instructor and BCMB office (biocmicr@uvic.ca) with the reason for their absence within 48 hours after the midterm exam. The Department will keep a record of the absences. It is the responsibility of the student to ensure all required components are complete, and to arrange deferred exams/assignments with the instructor, which normally should occur within one week of the original exam date.
7. The Department of Biochemistry and Microbiology considers it a breach of academic integrity for a student taking a deferred examination to discuss the exam with classmates. Similarly, students who reveal the contents of an examination to students taking an examination are considered to be in violation of the University of Victoria policy on academic integrity (see

current University Calendar). Students must abide by UVic academic regulations and observe standards of scholarly integrity (no plagiarism or cheating). Online exams must be taken individually and not with a friend, classmate, or group, nor can you access notes, course materials, the internet, or other resources without the permission of the instructor. You are prohibited from sharing any information about the exam with others. Use of unauthorized electronic devices and accessing the internet and class material during exams is prohibited unless permission is granted by the instructor. Instructors may use Browser Lockdown Software to block access during classes and exams.

8. Deferral of a final exam must be requested with an Academic Concession form and submitted directly to Undergraduate Records. Deferred final exams for fall term courses will be arranged by the instructor. Deferred final exams for spring term courses will be arranged through Undergraduate Records and must be written before the end of the summer term as stipulated in the University Calendar.
9. Requests for review/remark of a midterm exam must be made within one week of the exam being returned.
10. The instructor reserves the right to use plagiarism detection software or other platforms to assess the integrity of student work.
11. Supplemental exams or assignments will not be offered to students wishing to upgrade their final mark.
12. Anonymous participation in online classes is not permitted without permission of the instructor.

Important note about COVID-related stress

The current pandemic is placing added stressors- financial, mental, and physical- on everyone. Your wellbeing is of foremost importance. If you are experiencing difficulties coping, the University has resources to help. Please reach out to Counselling Services, the Centre for Academic Communication, or Learning Assistance Program for assistance.

Centre for Accessible Learning

Students with diverse learning styles and needs are welcome in this course. In particular, if you have a disability/health consideration that may require accommodations, approach the Centre for Accessible Learning (CAL) as soon as possible in order to assess your specific needs.

<https://www.uvic.ca/services/cal/index.php>

Course Experience Survey (CES)

We value your feedback on this course. Towards the end of term you will have the opportunity to complete a confidential course experience survey (CES) regarding your learning experience. The survey is vital to providing feedback to us regarding the course and our teaching, as well as to help the department improve the overall program for students in the future. When it is time for you to complete the survey, you will receive an email inviting you to do so. If you do not receive an email invitation, you can go directly to your [CES dashboard](#). You will need to use your UVic NetLink ID to access the survey, which can be done on your laptop, tablet or mobile device. We will remind you nearer the time but please be thinking about this important activity.