

BCMB 406A

Laboratory Manual

Fall 2022

The lab will be conducted in Petch 159.

Student Name: _____ Section: _____

Email address: _____

Instructors and Contact Information

Lab	Instructor	Email	Phone	Office
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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 lək'wəŋən 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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Laboratory Procedures

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BCMB 406A Laboratory Schedule

Week	Dates	Lab(s)	Day 1 (5 hours)	Day 2 (2 hours)	Due Dates
1	Sep 12-16	Lab 1: Isolation & Identification of Peptides & Proteins	Bradford assay	1 st dimension of 2D-PAGE	
2	Sep 19-23		2 nd dimension of 2D-PAGE	Gel imaging & spot excision	
3	Sep 26-30		Tryptic digestion	Zip-tip plate spotting	Lab 2 calculations
4	Oct 3-7	Lab 2: Immunological Characterization of Cancer Cell Lines	Lab 1: Mass spec Lab 2: Cell culture & cell staining	Complete cell staining	
5	Oct 10-14		No labs	FlowJo tutorial	Lab 1 Report (Day 2)
6	Oct 17-21		Cell culture & T cell assay set-up	T cell assay development	
7	Oct 24-28		Data analysis	Data analysis	
8	Oct 31 – Nov 4	Lab 3: ChIP Analysis of YEF3	Chromatin prep, MNase digestion & reverse cross-linking	DNA purification & agarose gel	Lab 2 Report (Day 2)
9	Nov 7-11		Reading Break – No Labs		
10	Nov 14-18		Immunoprecipitation (IP)	IP & reverse cross-linking	Lab 2 Midterm Nov 17 6-8pm
11	Nov 21-25		IP DNA purification, PCR & QPCR	Agarose gel & QPCR data analysis	
12	Nov 28 – Dec 2			Lab 3 Report (Day 1)	
TBA – Lab 3 exam during regular exam schedule					

Course Description

In BCMB 406A, you will develop your practical skills to prepare samples for analysis using modern methods in laboratory research. In this process, you will become familiar with current techniques in biochemistry and microbiology; specifically you will get experience running samples on a mass spectrometer, HPLC, flow cytometer and QPCR machine. These 3-4 week experiments offer an introduction to project-based learning experiences where you will be given an opportunity to plan some logistical portions of your own experiment. Once samples have been processed, you will critically examine and interpret multiple sets of data to study complex problems.

In lab 1, you will learn how mass spectrometry and HPLC are used in the field of proteomics. In two parallel experiments, *E.coli* proteins will be separated by 2D gel electrophoresis and a Glu-C digest of insulin will be used to generate peptides that will be separated using HPLC. The resulting purified proteins/peptides will be identified by mass spectrometry.

The focus of Lab 2 is tumour immunology and flow cytometry. You will work with a tumour-derived cell line and characterize its phenotype by staining with fluorescently labeled antibodies and analyzing using flow cytometry. You will also evaluate the ability of these cells to elicit a T-cell response using a functional immune assay.

In lab 3, epigenetic theory is applied to data generated by performing QPCR on ChIP-enriched DNA. The first step of this experiment is isolating chromatin from yeast. This chromatin is then fragmented into smaller pieces using a micrococcal nuclease digestion in preparation for ChIP. After specific pieces of chromatin are immuno-precipitated, the resulting DNA will be analyzed using QPCR and conventional PCR.

Intended Learning Objectives:

Upon completion of this course you will be able to:

- Describe the theory and principles of mass spectrometry, HPLC, flow cytometry and QPCR
- Develop proficiency in practical skills used for protein and DNA sample preparation, cell culture, flow cytometry, epigenetics and ChIP
- Evaluate experimental controls
- Solve typical calculations used in a biochemistry/microbiology lab
- Generate a record of your experimental work and 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. Attendance and engagement in the classroom is an integral part of the learning process and cannot be substituted with recordings. It is at the instructor’s sole discretion whether they provide a recording or give permission to students to record a lecture. There is no obligation to do so nor is there any expectations about the quality of the recordings. Nor should students assume a lecture will be recorded as instructors may withdraw access to recordings or permission to record. It is the responsibility of students who miss lectures to catch up in the material through extra readings, and obtaining notes from fellow students. Students who miss several lectures due to illness should contact their instructors to discuss options.
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.