BCMB 406A

Laboratory Manual

Summer 2021

The lab will be conducted in rooms 141 and 159 of the Petch Building.

Student Name: _____ Section: _____

Email address: _____

Instructors and Contact Information

Lab	Instructor	Email	Phone	Office
1	Ellen Busby	erbusby@uvic.ca	250-721-6504	Petch 186
2	Erika Wall	ewall@uvic.ca	250-472-5119	Petch 190
3	Val Kerr	valk@uvic.ca	250-472-5119	Petch 190

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 with respect the Lekwungen peoples on whose traditional territory the university stands and the Songhees, Esquimalt and W̱SÁNEĆ peoples whose historical relationships with the land continue to this day.

Department of Biochemistry and Microbiology
University of Victoria

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Laboratory Schedule

Week	Dates	Lab(s)	Day 1 (5 hours)	Day 2 (2 hours)	Due Dates
1	May 10-11	**Lab 1:** Isolation & Identification of Peptides & Proteins	Bradford assay	1st dimension of 2D-PAGE	
	May 12-13		2nd dimension of 2D-PAGE	Gel imaging & spot excision	
2	May 17-18		Lab 1: Tryptic digestion	Lab 1: Zip-tip plate spotting	Lab 2 calc. problem set
	May 19-20		Lab 2: Cell culture & cell staining	Lab 2: Complete cell staining	
3	May 24-25	**Lab 2:** Immunological Characterization of Cancer Cell Lines	**Victoria Day - No labs**	FlowJo tutorial	**Day 2: Lab 1 Report** FlowJo ex.
	May 26-27		Cell culture & T cell assay set-up	T cell assay development	
4	May 31-June 1		Data analysis	Data analysis	
	June 2-3	**Lab 3:** ChIP Analysis of YEF3	Chromatin prep, MNase digestion & reverse cross-linking	DNA purification & agarose gel	**Day 2: Lab 2 Report**
5	June 7-8		**Lab 2 Review**	**Lab 2 Midterm**	
	June 9-10		Immunoprecipitation (IP)	IP & reverse cross-linking	
6	June 14-15		IP DNA purification, PCR & QPCR	Agarose gel & QPCR data analysis	Day 2: QPCR Calculations
	June 16-17				**Day 2: Lab 3 Report**
7	June 21-22		**Lab 3 Review**	**Lab 3 Midterm**	
	June 23-24				

Introduction Quiz - Due Mon. May. 10 at 12:00 pm

The quiz is posted on Brightspace at the top of the Introduction page. 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. This is particularly important with new COVID-19 procedures and limited face-to-face interactions. You must obtain a mark of 100% to successfully complete the quiz. However, you may repeat it as many times as necessary to achieve this.

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, 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 BCMB 406A 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

COVID-19 Safety Measures

Due to the COVID-19 pandemic we are implementing some new procedures to keep everyone safe while working in the lab. These procedures are in addition to the general safety procedures outlined on pages xii-xiii. Please ensure that you read and understand the following:

- Physical distancing measures are in place throughout the Petch Building. This includes limited occupancy in washrooms, uni-directional staircases and hallways.
- We are using both Petch 141 (3rd year lab) and Petch 159 (4th year lab) for 406 students to perform their labs. There will be a maximum of 12 students in each lab to allow for physical distancing.
- You will be assigned to a designated lab bench in either Petch 141 or 159 which will be finalized the Friday before labs start.
- Please do not congregate in the hallways prior to the start of the lab. The lab will be opened 15 mins prior to the start of the class. For Petch 141, use the entrance closest to the Petch breezeway, and for Petch 159, use the foyer entrance (closest to the sitting area). Keep 6 ft away from others and do not block the hallway when lining up.
- When entering the lab, please sanitize your hands and put on a disposable mask that we will supply. Disposable masks must be worn the whole time you are in the lab. When you leave the lab, you must remove your mask and throw it in the waste bin.
- Floor signs will designate the correct direction you should move around the lab.
- We will provide plastic bins at your workstation to store your personal items.
- Sanitize your workspace before and after use with bench disinfectant using only one paper towel if possible. Place used paper towel in the general waste bin.
- During the class, remain within your designated area at your workstation as much as possible. Only approach communal areas if they are vacant. Raise your hand if you need to get the instructor's attention.
- You must provide your own lab coat and safety goggles. These will be stored in Ziploc bags in the lab for the duration of the semester.
- Sanitize or wash your hands with soap and water for 20 seconds before leaving.
- To exit either Petch 141 or 159 follow the directional flow signs and use the designated exit door.

Importantly, do a daily self-assessment before coming to campus. If you are experiencing any symptoms of COVID-19 such as fever, chills, cough, headache, shortness of breath, sore throat and painful swallowing, and you know they are not the result of a pre-exiting condition, you must:

- Stay home and self-isolate for a minimum of 10 days
- Consult the COVID-19 self-assessment tool at bc.thrive.health/covid19
- Contact 811 for medical advice
- Inform your lab instructor

Due to the pandemic and the potential for travel restrictions, students are strongly advised to live within the Greater Victoria area while taking a face-to-face lab course in the BCMB department. Students unable to attend labs due to travel restrictions may be unable to complete the course requirements.