

# **BCMB 406A**

# **Laboratory Manual**

# **Fall 2019**

Name: \_\_\_\_\_ Section: \_\_\_\_\_

Email address: \_\_\_\_\_

The Copyright for this material is owned by the Department of Biochemistry and Microbiology. This material must not be reprinted, amended, or redistributed in any way without written permission.

Instructors: Stephen Redpath (Lab 1), Erika Wall (Lab 2), Val Kerr (Lab 3)

Department of Biochemistry and Microbiology

University of Victoria



## Table of Contents

<b>Introductory Information</b>	
Schedule	ii
Instructor Contact Information	iii
Evaluation and Assessment	iv
Laboratory Report Guidelines and Format	vi
CES and Course Policies	vii
Department Information and Policies	viii
University Policy on Academic Integrity	ix
Safety Regulations	xi
Fire and Earthquake Evacuation Procedures	xiii
<b>Laboratory Procedures</b>	
Lab 1 – Isolation and Identification of Peptides and Proteins	1-1
Lab 2 – Immune Recognition of Tumour Cells	2-1
Appendix 2 – Flow cytometry theory	2-27
Lab 3 – Chromatin Immunoprecipitation (ChIP) Analysis of YEF3	3-1
Appendix 3 – Glossary of Terms	3-26
<b>Equipment Usage and Instructions</b>	
Appendix A – Fumehood Guidelines	A-1
Appendix B – Laminar Flow Hoods and Biological Safety Cabinets	B-1
Appendix C – Working in a Biological Safety Cabinet	C-1
Appendix D – Gen5 & KCJunior Software Instructions	D-1

## BCMB 406A Laboratory Schedule

Week	Dates	Lab(s)	Day 1	Day 2	Due Dates	
1	Sep 9-13	<b>Introduction and Safety talk</b>	Bradford assay	1 <sup>st</sup> dimension of 2D-PAGE		
2	Sep 16-20					
3	Sep 23-27	<b>Lab 1: Isolation &amp; Identification of Peptides &amp; Proteins</b>	Lab 1: Tryptic digestion & HPLC Lab 2: Prelab talk	Lab 1: Zip-tip plate spotting	Lab 2 calc. problem set	
4	Sep 30 - Oct 5		Lab 1: Mass spectrometry Lab 2: Cell culture & cell staining	Lab 2: Complete cell staining		
5	Oct 7-11		<b>Lab 2: Immunological Characterization of Cancer Cell Lines</b>	Cell culture & T cell assay set-up	T cell assay development	Lab 1 Report (Day 2)
6	Oct 14-18			<b>No labs</b>	FlowJo tutorial	FlowJo exercise
7	Oct 21-25	Data analysis		Data analysis		
8	Oct 28- Nov 1	<b>Lab 3: Chromatin Immunoprecipitation Analysis of YEF3</b>	Chromatin prep, MNase digestion & reverse cross-linking	DNA purification & agarose gel	Lab 2 Report (Day 2)	
9	Nov 4-8		Immunoprecipitation (IP)	IP & reverse cross-linking		
10	Nov 11-15	<b>Reading Break – No Labs</b>			Lab 2 Midterm Nov. 14	
11	Nov 18-22	<b>Lab 3: Chromatin Immunoprecipitation Analysis of YEF3</b>	IP DNA purification, conventional PCR & QPCR	Agarose gel & QPCR data analysis		
12	Nov 25-29				Lab 3 Report (Day 1)	
<b>TBA – Lab 3 final during regular exam schedule</b>						

## Instructor Contact Information

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

Each instructor is responsible for a different lab as indicated above. Please make sure that you address any concerns or questions to the appropriate instructor.

### CourseSpaces

<http://coursespaces.uvic.ca/my/>

Enter: NetLink-ID and Password

Select: 201909 BCMB 406A B01/B02/B03/B04 X

Important information for the course and each lab is posted on CourseSpaces. Announcements are frequently sent to students, so please make sure that you check the email you have on record.

### Departmental Web Page

<http://www.uvic.ca/science/biochem>

### Occupational Health and Safety

250-721-8971

<http://ohs.uvic.ca>

**Police, Fire, Ambulance** 911

### Campus Security

250-721-7599

<http://www.uvic.ca/security>

### University Health Services

250-721-8492

<http://www.uvic.ca/services/health>

## Evaluation and Assessment

### Percentage Breakdown for the Course:

Exams	<b>40 %</b>	see page v for more details
Lab Reports	<b>30 %</b>	see page v and vi for more details
Practical Assessment	<b>20 %</b>	see page v for more details
Laboratory Journal	<b>10 %</b>	see page v for more details

### Final Course Percentages:

A final percentage will be calculated for the course based on the above criteria. All percentages will be rounded to the nearest whole number. For example, a calculated percentage of 79.49% will be recorded as 79% whereas 79.50% will be recorded as 80%.

### Letter Grades will be assigned as follows:

90 - 100	A+
85 - 89	A
80 - 84	A-
77 - 79	B+
73 - 76	B
70 - 72	B-
65 - 69	C+
60 - 64	C
50 - 59	D
0 - 49	F (or N*)

#### \* N grades

Students who have completed the following course requirements will be considered to have completed the course and will be assigned a final percentage and letter grade.

- In class lab work (all Day 1 and Day 2 practical components must be performed)
- Lab 1 Report (full lab report)
- Midterm and final exams

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.

**Lab Exams (40%):**

There will two exams in this course. Each exam will only cover material from either Lab 2 or Lab 3.

Exam	Date	Lab covered	Percent
Midterm	Evening of Nov. 14th	Lab 2	20 %
Final	TBA (during exam schedule)	Lab 3	20 %

**Lab Reports (30%):** Marks for the lab reports will be assigned as indicated below.

	Percent
Lab 1	15 %
Lab 2	7.5 %
Lab 3	7.5 %

**Practical Evaluation (20%):**

Practical assessments will be done for each student by laboratory instructors and teaching assistants and will consist of:

- Frequent pre-lab quizzes to assess preparedness
- Pre-lab and in-lab assignments, calculations and problem sets
- Frequent evaluation of experimental results to assess technique
- Thoroughness of clean-up at each bench after the lab

Weighting of these assignments will vary based on the discretion of the instructors.

**Maintenance of a Laboratory Journal (10%):**

A hard cover or spiral bound notebook to be used as a laboratory journal for recording raw data generated while performing the labs and processed data used to create figures and tables. Portions of lab reports will not be considered as lab journal entries. Journals will be marked periodically throughout the term, so the expectation is that they are kept up to date and brought to every lab session. If you do not have it available for marking, it must be brought in by 10 am the following morning and a 30 % penalty will be applied

Please write in ink and include all relevant information, such as:

- Date and title of the experiment
- Unknown numbers
- Pre-lab or in-lab calculations
- Detailed procedural steps used when not working directly from the lab manual
- All raw data you (and/or your partner) generate with important information included
- Experimental conditions (temperature, time, wavelengths, etc...)
- Loading order and volumes of samples put onto gels, etc...
- Changes to the procedure or mistakes/errors made
- Handouts of data and/or copies of student data posted on CourseSpaces
- **Observations made during the lab experiment**
- **Processed data in the form of tables, graphs or other important figures**
- **Interpretations of results – clearly labeled figures and/or written statements that summarize the findings**

## Laboratory Report Guidelines and Format

- **All laboratory reports must be written up individually.**
- Text should be typed and double-spaced, with margins no smaller than 1.9 cm (0.75")
- Use 12 point font of a standard style such as Arial or Times New Roman.
- All written text should be concise, well written and proofread for grammar and spelling.

Below is a general outline of what should be included in the following portions of a lab report in this course. Each lab report will have a different set of requirements so be sure to read the instructions for each submission carefully. Refer to CourseSpaces for a more detailed description of lab report expectations.

- Abstract:** State the purpose of the experiment(s) and put it in context. Summarize the methods, results and conclusions of the research.
- Introduction:** In a few well written paragraphs, state the purpose and introduce the main concepts of the lab by defining important terms and explaining new ideas. As well, briefly describe and indicate the purpose of performing the individual techniques or experiments.
- Methods:** Refer to the source of the procedure in proper citation format (most of the time this will be the lab manual). For procedures that you have designed, briefly summarize techniques and materials used so that someone could repeat the experiment (minor details are not necessary). Include any *significant* modifications that were communicated to you either verbally or in written form. Also, include mistakes that were made by you, your partner or other individuals that may have affected your results.
- Results:** Organize data in the form of fully labeled tables, graphs or figures. State the results in written form in a *Results Summary* placed before the figures, drawing attention to the key results. Reserve *all* interpretation for the discussion. Tables, graphs and figures should be numbered in the order in which they are cited in the text.
- Discussion:** The discussion should provide an interpretation of your results. Be concise. Keep discussion relevant to the data generated from your own experiment (and that of your classmates when appropriate). Incorporate into your discussion the answers to any relevant questions that appear in the lab manual. In a couple of sentences, draw a conclusion based on the results of the experiment.
- Appendix:** Include raw data, calculations and other information that is relevant. Remember to number your appendices and include a basic title for each.
- References:** Online links to all relevant reference papers can be found on CourseSpaces. Laboratory reports must be referenced following the format of the *Journal of Molecular Biology*. This is the same format we use in the lab manual.

**Please note:** A lab report submission consists of both a hard copy and electronic copy. Hard copies of reports are to be submitted to the instructor, whereas electronic copies are to be uploaded to CourseSpaces. Both must be submitted by the designated date and time, otherwise late penalties will apply.



## 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.

## Course Policies

### ***Attendance***

Laboratory attendance is compulsory. Failure to attend a lab without a written medical excuse will result in a mark of **'N' (incomplete)** for the course. A change of lab section must be arranged with the lab instructor **prior** to the lab period. Students who miss a lab for medical reasons are responsible for maintaining their lab journal and for obtaining the data needed to write up the lab report.

It is important to arrive on time. Students who arrive after a pre-lab quiz has begun will not be given extra time to complete the quiz. No makeup quizzes will be given for students who arrive after a quiz is over. The instructor reserves the right to refuse late arrivals or withhold practical marks associated with that lab if a student shows up late without a legitimate reason.

### ***Late Assignments***

Late assignments (either the hard copy or the electronic copy in the case of reports) will be penalized 10% per day and will not be accepted after one week (7 days) following the designated due date. Late assignments can be emailed to the instructor on days when the university is closed but a hard copy must be submitted by noon on the first working day after the emailed submission.

### ***Re-marking Policy***

Request to have assignments (lab reports, quizzes, exams, journal entries, practical assessments) reviewed or remarked must be made within one week of the assignments being made available. Students are expected to promptly pick up assignments after marking is completed, either in lab or from the instructor. Any resubmissions will involve remarking of the entire assignment and students will be given the grade assigned after the second review.

### ***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>

## 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 unless being used for a purpose relevant to the class. Students having a cell phone, tablet, or computer on their person during an exam will be assumed to have it for the purpose of cheating.
3. Any recordings of lectures may only be performed with written permission of the instructor, and are for personal use only. The instructor retains copyright to such recordings and all lecture materials provided for the class (electronic and otherwise); these materials must not be shared or reposted on the Internet.
4. 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.
5. Students are expected to be present for the midterm and final exams. Instructors may grant deferrals for midterm examinations for illness, accident, or family affliction, and students must provide appropriate documentation 48 hours after the midterm exam. 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 a deferred examination are considered to be in violation of the University of Victoria policy on academic integrity (see current University Calendar). 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.
6. Multiple choice scan sheets for machine scoring (bubble sheets) are considered the authentic exam answer paper and will be retained by the department for 1 year.
7. Professors may refuse to review/remark exams not written in indelible ink. In addition, requests for review/remark of a midterm exam must be made within one week of the exam being returned. Students are expected to promptly pick up midterm exams after marking has been completed, either in class or from the instructor.
8. Examination papers that have pages removed, or are mutilated will not be marked.
9. The instructor reserves the right to use plagiarism detection software or other platforms to assess the integrity of student work.
10. Supplemental exams or assignments will not be offered to students wishing to upgrade their final mark.

## **University Policy on Academic Integrity**

Suspected cases of plagiarism or cheating will be documented and submitted to the department chair for penalty assessment as described in the UVic calendar (2019-2020).

Academic integrity violations covered by this policy can take a number of forms, including the following:

### **Plagiarism**

A student commits plagiarism when he or she:

- submits the work of another person in whole or in part 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 acknowledgment as described above

The university reserves the right to use plagiarism detection software programs to detect plagiarism in essays, term papers and other work.

### **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.

Review by fellow students and tutoring that do not include editing are normally permitted. In addition to consulting with their instructors, students are encouraged to seek review of and feedback on their work that prompts them to evaluate the work and make changes themselves.

### **Falsifying Materials Subject to Academic Evaluation**

Falsifying materials subject to academic evaluation includes, but is not limited to:

- fraudulently manipulating laboratory processes, electronic data or research data in order to achieve desired results
- using work prepared in whole or in part by someone else (e.g., commercially prepared essays) and submitting it as one's own
- citing a source from which material was not obtained
- using a quoted reference from a non-original source while implying reference to the original source

- submitting false records, information or data, in writing or orally

### **Cheating on Work, Tests and Examinations**

Cheating includes, but is not limited to:

- copying the answers or other work of another person
- sharing information or answers when doing take-home assignments, tests or examinations except where the instructor has authorized collaborative work
- having in an examination or test any materials or equipment other than those authorized by the examiners
- accessing unauthorized information when doing take-home assignments, tests or examinations
- impersonating a student on an examination or test, or being assigned the results of such impersonation
- accessing or attempting to access examinations or tests before it is permitted to do so

Students found communicating with one another in any way or having unauthorized books, papers, notes or electronic devices in their possession during a test or examination will be considered to be in violation of this policy.

### **Aiding Others to Cheat**

It is a violation to help others or attempt to help others to engage in any of the conduct described above.

**\* In BCMB 406A, all assignments and lab reports must be written up independently.** Collaborative work is only allowed in specific situations when students are directed to share data by the instructor. Students can discuss thoughts and ideas with other students but all written work, including figures and figure titles, must be original. Be sure to submit work that is entirely your own.

## 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 prerequisites to working in the laboratory.

1. **Access to the laboratory is limited to instructors and students.**
2. **No eating, drinking or smoking in the laboratory.** Keep paper, pencils, fingers, and other objects out of the mouth.
3. **It is strictly prohibited to attend labs while under the influence of an intoxicant (alcohol, cannabis, drugs, etc).** Students that do not comply will be asked to leave and all marks associated with that lab will be forfeit.
4. **Mouth pipetting is prohibited.** A safety bulb or pipettor must be used.
5. **Safety glasses must be worn at all times.**
6. **Laboratory coats must be worn and properly fastened by all personnel working in the laboratory and must not be worn in public places.** Laboratory coats will be provided for you and will be shared between sections. If you would prefer a lab coat of your own, you will need to bring one to your first lab section. It will be kept in the lab until the end of the course, at which time it will be autoclaved and available for pick-up.
7. **Open-toed shoes or sandals should not be worn and bare legs are not allowed.** Capri pants, skirts and shorts are only permitted if they cover the knees when you are sitting down.
8. **Long hair must be tied back** to protect against burning and falling into stains, chemicals or bacterial cultures.
9. **Personal items (coats, bags, etc...) must be kept away from the work area.**
10. **Sitting on laboratory work surfaces is prohibited.**
11. **Use of cellular devices is not allowed in the lab.**
12. **Wash hands before and after completing all lab work.**
13. **Gloves must be worn in the following situations:**
  - Working with Level 2 organisms
  - Working with potentially harmful reagents
  - If you have open cuts or abrasions on your hands
14. **Gloves are not to be worn when working with communal equipment and computers or outside of the lab.**
15. **Lab doors and windows must be closed when working with Level 2 organisms.**

16. **Bunsen burners are to remain off when not in use.**
17. **Dispose of all liquids into the designated waste containers.** No liquids are to be poured down the sink (except water).
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 experimental materials (**labeled!**) 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)
  - 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 signature on the Biochemistry and Microbiology Lab Safety Form is your acknowledgement that you have read the safety regulations and agree to abide by them.

## Fire Evacuation Procedure

### 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 Procedure

### 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.
- Make your way to the UVic Campus Assembly Area to receive instructions and information. Keep away from power lines and buildings to avoid falling debris.
- Report any injuries to Campus Security Services.