

BCMB 406B

Laboratory Manual

2018

Name: _____

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: Erika Wall (Lab 1 and 2), Glen Pryhitka (Lab 2), Val Kerr (Lab 3)

Department of Biochemistry and Microbiology

University of Victoria

Table of Contents

Introductory Information	
Schedule	i
Instructor Contact Information	ii
Evaluation and Assessment	iii
Laboratory Report Guidelines and Format	vi
Course Policies / Department Information and Policies	vii
University Policy on Academic Integrity	ix
Safety Regulations	x
Building and Earthquake Evacuation Procedures	xii
Laboratory Procedures	
Lab 1 – Primer Design	1-1
Appendix 1A: Primer Design Characteristics	1-18
Appendix 1B: Using Net Primer	1-21
Appendix 1C: Using Primer3	1-23
Lab 2 – Site Directed Mutagenesis of a Carbohydrate Binding Module	2-1
Appendix 2A: Cloning of the Wild Type CBMs	2-32
Appendix 2B: Genotypes of <i>E.coli</i> Strains	2-33
Lab 3 – Purification and Characterization of a Mutant CBM	3-1
Appendix 3A: Use of a Flow Adaptor	3-33
Appendix 3B: Plate Reader Instructions	3-34
Appendix 3C: Pouring and Running a Polyacrylamide Gel	3-36
Appendix 3D: Recipes for SDS-Polyacrylamide Gels	3-41
Appendix 3E: Recipes for Non-Denaturing Polyacrylamide Gels	3-42

BCMB 406B 2018 Laboratory Schedule

Week	Dates	Lab(s)	Day 1	Day 2	Due Dates	
1	Jan. 8-12	Introduction and Safety talk Lab 1: Primer Design	Primer design & evaluation using web-based tools			
2	Jan. 15-19	Lab 2: Site Directed Mutagenesis of CBM Proteins	<i>In silico</i> cloning using Vector NTI Advance		Lab 1 Report	
3	Jan. 22-26	Lab 2: Site Directed Mutagenesis of CBM Proteins	Inverse PCR, agarose gels, DpnI digestion, electrocompetent cells	Electroporation		
4	Jan. 29-Feb. 2	Lab 2: Site Directed Mutagenesis of CBM Proteins	Plasmid DNA purification, restriction digests, agarose gels	Plasmid preps, DNA quantification, automated sequencing		
5	Feb. 5-9	Lab 2: Site Directed Mutagenesis of CBM Proteins	Chemical transformation	Counting plates	Electroporation control assignment	
6	Feb. 12-16	Reading Break - No Labs				
7	Feb. 19-23	Lab 3: Purification of a Mutant CBM	Preparation of solutions, starter culture of mutant CBM, macroarray of WT	Inoculate & induce culture, macroarray development	Lab 2 Report	
8	Feb. 26-Mar. 2	Lab 3: Purification of a Mutant CBM	Chemical lysis of cells, IMAC: nickel column chromatography	Identify protein-containing fractions	Bradford assay assignment	
9	Mar.5-9	Lab 3: Purification of a Mutant CBM	SDS-PAGE of fractions	Dry gels, pool fractions, dialysis	Midterm exam (Lab 1 and 2)	
10	Mar. 12-16	Lab 3: Purification of a Mutant CBM	Bradford assay and A ₂₈₀ , macroarray (mut vs. WT)	Macroarray development	Protein conc. calculations	
11	Mar. 19-23	Lab 3: Purification of a Mutant CBM	Affinity gels, Lysozyme crystallization	Dry gels, visualize crystals		
12	Mar.26-30	Lab 3: Purification of a Mutant CBM	Pick up gels Exam preparation		Crystallization assignment	
13	Apr. 3-6				Lab 3 Report	

Instructor Contact Information

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

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

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

Enter: NetLink-ID and Password

Select: 201801 BCMB 406B 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://www.uvic.ca/ohse/>

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	50 %	see page v for more details
Lab Reports	20 %	see page v and vi for more details
Practical Assessment	20 %	see page v for more details
Laboratory Journal	10 %	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 components of the practical work 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.

Lab Exams (50%):

The lab exams are non-cumulative. The midterm will be a three hour exam and will cover material from labs 1 and 2. The final exam will be a three hour exam scheduled during the regular exam period in April and will cover material from lab 3 only.

Midterm (TBA)	• covers labs 1 and 2 (25%)
Final Exam (TBA)	• covers lab 3 (25%)

Lab Reports (20%): Marks for the lab reports will be assigned as indicated below.

	Marks
Lab 1 – Primer Design	15
Lab 2 – Site-Directed Mutagenesis of a CBM Protein	45
Lab 3 – Purification and Characterization of a Mutant CBM Protein	40
Total Marks	100

Practical Assessment (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%):

You are expected to provide a hard cover or spiral bound notebook to be used as a laboratory journal. This book is dedicated to recording both raw data generated while performing the labs and processed data used to create figures and tables. Your lab journal must be brought to every lab session. Data for each lab will be marked during the midterm exam.

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 and interpretations of results (this is new compared to expectations in second and third year lab courses in this department)**

Note: Portions of marked lab reports will not be considered as lab journal entries.

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 beginning of class on the designated due date otherwise late penalties will apply.

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 or Lab Reports

Late assignment or lab reports (either the hard copy or the electronic copy) will be penalized 10% per day and will not be accepted after one week (7 days) following the designated due date. Late lab reports or 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, etc.) reviewed/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. If you have a disability/health consideration that may require accommodations, please feel free to approach an instructor and/or the Centre for Accessible Learning (CAL) as soon as possible. The CAL staff is available by appointment to assess specific needs, provide referrals and arrange appropriate accommodations. The website is <http://www.uvic.ca/services/cal/index.php>. The sooner you let us know your needs, the quicker we can assist you in achieving your learning goals in this course.

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.

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.

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 (<http://web.uvic.ca/calendar2017-05/undergrad/info/regulations/academic-integrity.html>).

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 the inclusion of another individual's work
- paraphrases material from a source without sufficient acknowledgement as described above

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 by someone else 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 Assignments, 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 and 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

Collaborative Work (Penalties) * See below for details specific to this course.

In cases in which an instructor has provided clear written instructions prohibiting certain kinds of collaboration on group projects, instances of prohibited collaboration on a substantial part of the assignment should result in a grade of zero for the assignment, while instances of prohibited collaboration on the bulk of the assignment should result in a grade of F for the course.

*** In BCMB 406B, it is required that all assignments and lab reports 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 as 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 or drinking in the laboratory.** Keep paper, pencils, fingers, and other objects out of the mouth.
3. **Safety glasses must be worn at all times.**
4. **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.
5. **Open-toed shoes or sandals should not be worn and bare legs are not allowed.** Capri pants, skirts and shorts are only allowed if they cover the knees when you are sitting down.
6. **Lab doors and windows must be closed when working with Level 2 organisms.**
7. **Personal items (coats, bags, etc...) must be kept away from the work area.**
8. **Wash hands before and after completing all lab work.**
9. **Long hair must be tied back** to protect against burning and falling into stains, chemicals or bacterial cultures.
10. **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
11. **Gloves and lab coats are not to be worn outside of the lab.**
12. **Remove gloves when working with communal equipment and computers.**
13. **Bunsen burners are to remain off when not in use.**
14. **Dispose of all liquids into the designated waste containers.** No liquids are to be poured down the sink (except water).
15. **Contaminated liquid waste must be autoclaved prior to disposal.**

16. **Dispose of infectious solid waste in the yellow biohazard buckets for autoclaving.**
This includes pipette tips, agar plates, contaminated gloves or paper towels, etc...
17. **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.
18. **Mouth pipetting is prohibited.** A safety bulb or pipettor must be used.
19. **Sitting on laboratory work surfaces is prohibited.**
20. **Use of cellular devices is not allowed in the lab.**
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.

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.
- Meet at your designated Emergency Evacuation Site. Keep away from power lines and buildings to avoid falling debris.
- Report any injuries to Campus Security Services.

