BCMB 406B

## **Table of Contents**

Introductory Information		
Schedule	ii	
Evaluation and Assessment		
Course Policies		
Formal Laboratory Report Format	vi	
University Policy on Academic Integrity		
Safety Regulations		
Building and Earthquake Evacuation Procedures		
Laboratory Acknowledgement Form	хi	
Laboratory Procedures		
Lab 1 – Primer Design	1-1	
Appendix 1A: Primer Design Characteristics	1-15	
Appendix 1B: Using Net Primer	1-18	
Appendix 1C: Using IDT SciTools Oligo Analyzer	1-20	
Appendix 1D: Using Primer3	1-21	
Lab 2 – Site Directed Mutagenesis of a Carbohydrate Binding Module	2-1	
Appendix 2A: Cloning of the Wild Type CBMs	2-30	
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	3-40	
Appendix 3E: Recipes for Non-Denaturing Polyacrylamide Gels	3-41	
Appendix 3F: Drying a Polyacrylamide Gel Using Cellophane	3-42	

BCMB 406B ii

# **BCMB 406B Laboratory Schedule, 2014**

Week	Dates	Lab(s)	Day 1	Day 2	Due Dates
1	Jan. 6-10	Introduction and Safety talk  Lab 1:Primer Design	Primer design & evaluation using web-based tools		
2	Jan. 13-17	Lab 2: Site Directed Mutagenesis of CBM Proteins	In silico cloning using Vector NTI Advance		Lab 1 Report
3	Jan. 20-24	Lab 2: Site Directed Mutagenesis of CBM Proteins	Inverse PCR, agarose gels, DpnI digestion, electrocompetent cells	Electroporation of E.coli DH5α with PCR product	
4	Jan. 27-31	Lab 2: Site Directed Mutagenesis of CBM Proteins	Plasmid DNA purification, restriction digests, agarose gels	Plasmid preps, DNA quantification, automated sequencing	
5	Feb. 3-7	Lab 2: Site Directed Mutagenesis of CBM Proteins	Transformation of <i>E.coli</i> BL21 with CBM mutant plasmid	Counting plates	
6	Feb. 10-14	Reading Break - No Labs			
7	Feb. 17-21	Lab 3: Purification of a Mutant Carbohydrate Binding Module	Preparation of solutions, starter culture of mutant CBM, macroarray of WT	Inoculate large culture, IPTG induction, development of macroarray blot	Lab 2 Report
8	Feb. 24-28	Lab 3: Purification of a Mutant Carbohydrate Binding Module	Chemical lysis of cells, IMAC: nickel column chromatography	Identify the fractions containing protein using A <sub>280</sub>	Midterm exam
9	Mar. 3-7	<b>Lab 3:</b> Purification of a Mutant Carbohydrate Binding Module	SDS-PAGE of column fractions	Dry gels, pool fractions, dialysis of purified mutant CBM	Bradford assay assignment
10	Mar. 10-14	Lab 3: Purification of a Mutant Carbohydrate Binding Module	Bradford assay and A <sub>280</sub> , macroarray (mut vs. WT)	Development of macroarray blot	
11	Mar. 17-21	Lab 3: Purification of a Mutant Carbohydrate Binding Module	Affinity gels, Lysozyme crystallization	Dry gels, visualize crystals	
12	Mar. 24-28	Lab 3: Purification of a Mutant Carbohydrate Binding Module	Pick up gels Exam preparation		Crystallization assignment
13	Mar. 31-Apr. 4				Lab 3 Report

## **Evaluation and Assessment**

## Percentage Breakdown for the Course:

Lab Reports	35 %	see below and page vi for more details
Exams	40 %	see below for more details
Practical Assessment	15 %	see page iv for more details
Laboratory Journal	10 %	see page iv for more details

## Final Percentages will be assigned Letter Grades as follows:

(see CourseSpaces for a written description what each letter grade represents)

90.00 - 100	Α+
85.00 - 89.99	Α
80.00 - 84.99	A-
77.00 - 79.99	B+
73.00 - 76.99	В
70.00 - 72.99	B-
65.00 - 69.99	C+
60.00 - 64.99	С
50.00 - 59.99	D
0 - 49.99	F (or N)

Lab Reports (35%): Marks for the lab reports will be assigned as indicated below.

- Labs 1 and 3 will be informal lab report write ups.
- Lab 2 will require a formal lab report write up.

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

## Lab Exams (40%):

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 (20%)

Final Exam (TBA)

• covers lab 3 (20%)

BCMB 406B iv

## **Practical Evaluation (15%):**

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 calculation and problem sets
- Frequent evaluation of experimental results to assess technique
- Thoroughness of clean-up at each bench after the lab

#### 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 raw data to be used for writing lab reports and must be brought to every lab session. Data will be marked periodically throughout the course and will be submitted in April for final evaluation.

Please write in ink and include the following:

- Date and title of the experiment
- Unknown numbers
- Pre-lab or in-lab calculations
- All raw data you (or your partner) produce
- Experimental conditions (temperature, time, wavelengths, etc...)
- Gel loading order and volumes
- Changes to the procedure or mistakes/errors made
- Observations and interpretations of results (this is new compared to expectations in second and third year lab courses in this department)

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

BCMB 406B

#### **Course Policies**

#### Attendance

Laboratory attendance is compulsory. Failure to attend a lab without a written medical excuse will result in the forfeit of all marks associated with the lab. 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 in order 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.

## Re-marking Policy

Students have one week to review marked assignments, lab reports, quizzes, journal entries, practical assessments, etc... and resubmit them. Any resubmissions will involve remarking of the entire assignment and students will be given the grade assigned after this second review.

## Accessibility Statement

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, please feel free to approach an instructor and/or the Resource Centre for Students with a Disability (RCSD) as soon as possible. The RCSD staff are available by appointment to assess specific needs, provide referrals and arrange appropriate accommodations <a href="http://rcsd.uvic.ca/">http://rcsd.uvic.ca/</a>. The sooner you let us know your needs the quicker we can assist you in achieving your learning goals in this course.

BCMB 406B vi

## **Formal Laboratory Report Format**

- All laboratory reports must be written up individually.
- Text should be type written and double-spaced.
- Use 12 point font of a standard style such as Arial or Times New Roman.
- Refer to CourseSpaces for a more detailed description of lab report expectations.

Laboratory reports should be *brief and concise* and should include the following:

**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 by referencing the lab manual in proper

citation format. 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. Only include

theory that relates directly to the lab experiments. 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:** 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.

<u>Please note:</u> A lab report submission consists of both a hard copy and electronic copy. Hard copies of reports are to be submitted to the instructor by **4:00 pm** on the designated due date. The electronic copy is to be uploaded to Turnitin by **11:59 pm** on the due date.

Late lab reports (either the hard copy or the electronic copy):

• will be penalized 10% per day and 15% over the weekend

BCMB 406B vii

 received following a long weekend will be penalized 25% (15% for Saturday and Sunday plus 10% for the additional day)

will not be accepted after one week following the designated due date

## **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 (2013-2014).

## <u>Plagiarism</u>

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

## <u>Collaborative Work (Penalties)</u> \* 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.

<sup>\*</sup> 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

BCMB 406B viii

with other students but all written work must be original. Be sure to submit work that is entirely your own.

# BCMB 406B ix 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, drinking or smoking 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.

BCMB 406B x

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

BCMB 406B xi

## **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.
- Make your way to the UVic Campus Assembly Area (playing fields) via safe routes for further information. Keep away from power lines and buildings to avoid falling debris.
- Report any injuries to Campus Security Services.

BCMB 406B xii

# Biochemistry and Microbiology Laboratory Acknowledgement Form

NAME	
COURSE	
LAB INSTRUCTOR	
Experiments conducted in a Microbiology and Biocher pathogenic organisms. Failure to handle and dispose to infection, injury or even serious illness. For the safe understand and follow the appropriate laboratory procinstructor.	of these organisms correctly may lead ety of everyone, it is required that you
Your signature below is your acknowledgement that y agree to abide by them.	ou have read the safety regulations and
STUDENT DA	ATE