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BCMB 301A Laboratory Schedule Fall 2013

Week	Date	Lab(s)	Day 1	Day 2	Due Dates
1	Sept. 9 – 13	Introduction Lab 1: Bioinformatics Lab 2: pH & Buffers Lab 3: Determination of Protein Concentration Calculation Exercise	Introduction Lab 1: Bioinformatics Lab 3: Buffer Calculations	Safety Talk Lab 2: Buffer calculations Lab 3: Lowry solution prep Calculation Exercise	Day 1: Lab 3 Calculations Day 2: Lab 2 Calculations Academic Integrity Assignment (complete by Sun, Sept. 15, by 11:59 pm)
2	Sept. 16 – 20	Literature Exercise Lab 2: Buffer and pH	Lab 2: pH & Buffers	Literature Exercise (In Library Classroom 130)	Day 1: Lab 1 Summary Day 2: Calculation Exercise
3	Sept. 23 – 27	Lab 3: Determination of Protein Concentration	Lab 3: Biuret, Lowry, Bradford, A_{280}		Day 1: Lab 2 Summary Day 2: Literature Exercise
4	Sept.30 – Oct.4	Lab 4: Purification of ß-galactosidase	Lab 4: AS precip, GPC, IEC		Day 1: Lab 3 Summary
5	Oct. 7 – 11	Lab 4: Purification of ß-galactosidase	Lab 4: Prepare & Run SDS-PAGE	Lab 4: Destain gel **Quiz on Labs 1-3**	
6	Oct. 14 – 18	Thanksgiving – no labs			
7	Oct. 21 – 25	Lab 4: Purification of ß-galactosidase Lab 5: Hybridomas & Immunodetection	Lab 4: Lowry assay & ß-gal assay	Lab 5: Coat ELISA plate, Subculture hybridoma cells	
8	Oct. 28 – Nov.1	Lab 5: Hybridomas & Immunodetection	Lab 5: Harvest Secreted Antibody, ELISA		Day 1: Lab 4 Summary
9	Nov. 4 – 8	Lab 5: Hybridomas & Immunodetection	Lab 5: SDS-PAGE & Transfer	Lab 5: Image gel & Develop blot	Day 1: Antibody Titre Graph
10	Nov. 11 – 15	Reading Break – No labs this week			Lab 6 Group Work Contract due Friday 15th by 10:30 a.m.
11	Nov. 18 – 22	Lab 6: Reversible Enzyme Inhibition	Lab 6: Reversible Inhibition **Quiz on Labs 4-5**	Lab 6: Revesible Inhibition	Day 1: Lab 5 Summary
12	Nov. 25 – 29	Hand-in Lab 6 Report			Day 2: Lab 6 Summary
Final Exam – in scheduled exam period					

Safety Regulations

Work in both biochemistry and microbiology laboratories, involves exposure to hazardous chemicals and living microorganisms, many of which are potential pathogens. Your attitude and actions in the laboratory determine your own safety, and that of your colleagues and the community. Laboratory equipment and design can contribute to safety only if they are used properly. Personal recognition of safety and the acceptance of certain precautions are therefore necessary prerequisites to working in the laboratory. The following safety regulations are designed to reduce the risks inherent in the use of potentially dangerous materials and they must be observed when working in the BCMB 301 laboratory.

The following are excerpts from Laboratory Biosafety Guidelines published by Health Canada, 3rd Edition.

Biohazard Containment Level 2

Judgments of the inherent risks of a pathogen are made on the basis of the severity of the disease it causes, the route of infection and its virulence and infectivity. Also taken into account are factors such as antibiotic resistance, immunization, the presence or absence of vectors, quantity of agent being used, whether the pathogen is indigenous to Canada, and possible effects on other species including plants and animals.

Biological agents classified as Risk Group 2 are pathogens that pose moderate individual risk and limited community risk. Included are pathogens that can cause human or animal disease but, under normal circumstances, are unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease. Effective treatment and preventative measures are available and the risk of spread is limited.

Containment level 2 is suitable for work with Risk Group 2 pathogens. The physical requirements in addition to a containment level 1 laboratory include a location apart from the public and general office, a biohazard sign with appropriate information, work surfaces which are impervious and readily cleanable, self-closing doors, coat hooks for lab coats and a nearby autoclave.

Operationally, a containment level 2 facility requires that workers (including students) understand procedures for handling spills of infectious materials, and wear a laboratory coat at all times. Windows and doors of the facility must be closed when working with Risk Group 2 organisms, and contaminated glassware must not leave the facility before being decontaminated by autoclaving.

BCMB 301 Safety Practices

1. Safety Glasses are to be worn in the BCMB 301 labs.

2. Long hair must be adequately tied back to protect against burning, falling into stains, or bacterial contamination.

3. A protective laboratory coat must be worn and properly fastened by all persons working in the laboratory. The laboratory coat must remain in the laboratory.

4. Bare legs or open-toed shoes are not acceptable when working in the laboratory. Suitable footwear with closed toes and heels and preferably non-slip soles is recommended.

5. Gloves are to be worn for all procedures that might involve direct skin contact with toxins, blood, corrosive or infectious materials. Do not touch communal objects, such as taps, door handles, etc., with gloved hands, since others will assume that these objects are being contaminated with the substance(s) on your gloves. There are absolutely no gloves allowed outside of the lab. Dispose of contaminated gloves in an appropriate manner.

6. Hands must be washed with hand disinfectant* before beginning and after completing lab work, and at any time after handling materials that are known or suspected to be contaminated, even when gloves have been worn.

7. Open cuts and abrasions should be adequately covered to prevent infection.

8. Eating, drinking, smoking, storing food or utensils, applying cosmetics and inserting or removing contact lenses is not permitted in the laboratory. Leave your lunches and water bottles outside the lab. Paper, pencils, fingers, and other objects should be kept out of the mouth.

9. Lab notebooks, coats, purses, bags, etc. should be kept away from the working area. Sitting on any lab counters is not permitted.

10. Work surfaces must be cleaned and decontaminated with bench disinfectant* before beginning and after completing lab work, and after any spill of potentially infectious or dangerous material.

11. Mouth pipetting is prohibited. A safety bulb or pipettor must be used. When using a pipettor, the tips must be discarded by using the tip ejector if possible, or carefully removed by hand, touching only the uncontaminated upper part of the tip.

12. Inoculating needles or loops, used to transfer cultures, should be sterilized in the flame to red hot before and after use. Flame the wire vertically, not horizontally. If it is covered with viscous material, dry it at the side of the flame before sterilizing, to avoid scattering any culture.

13. Bunsen burners should remain off when not being used.

14. All accidents such as cuts or burns must be reported to the laboratory instructor as soon as possible. Wash under running water any skin that comes into contact with chemicals.

Note the locations of the nearest safety shower and eye wash station and know how to operate them.

15. All spills must be reported to the laboratory instructor as soon as possible. In the event of a bacterial spill, add an equal volume of bench disinfectant to the spill, and wait for five minutes; clean up using a no-touch technique, and discard waste in a biohazard container. Wash your hands using hand disinfectant if skin contact occurred. To avoid spills, keep tubes vertical, do not pick tubes up by their caps, do not shake tubes violently, and vortex carefully.

16. No liquids used in the lab may be poured down the sink. Liquid bacterial cultures must be sent for autoclaving. All other liquids used in the lab, including buffers, stains, reagents etc. must be decanted into the appropriate hazardous waste container.

17. All technical procedures should be performed in a manner that minimizes the creation of aerosols.

18. All infectious solid wastes, such as contaminated Petri plates, must be placed in the yellow biohazard buckets provided for this purpose.

19. Under no circumstances are bacterial cultures or other contaminated materials to be removed from the laboratory.

20. To avoid possible injury or infection, do not pick up broken glass; notify the instructor. Broken glassware will be placed in the designated container.

21. Note the locations of fire extinguishers and familiarize yourself with their use.

22. Access to the laboratory is limited to instructors and students of BCMB 301.

23. Cell phones, computers and other electronic devices must be turned off at all times unless being used for a purpose relevant to the class (e.g. note taking).

24. Before leaving the laboratory:
    - Place all cultures and other contaminated materials to be discarded in the appropriate containers for sterilization in the autoclave.
    - Remove all labels from test tubes and other glassware.
    - Put experimental materials (labelled) in the appropriate containers for incubation.
    - Check that gas is turned off.
    - Clean microscopes and put them in their designated storage areas.
    - Clean up any water or spills on your bench, around the sinks or on the floor.
    - Put away all apparatus and wash the bench top with bench disinfectant*.
    - Hang up lab coat; push stool under bench so that the walkways are clear.
    - Wash your hands thoroughly with hand disinfectant*.

*__Disinfectants:__
Hand disinfectant: 50% ethanol, 0.13% zephiran chloride (benzalkonium chloride).

Bench disinfectant: 0.45% Microclean (octyl decyl dimethyl ammonium chloride/dioctyl dimethyl ammonium chloride/didecyl dimethyl ammonium chloride/alkyl dimethyl benzyl ammonium chloride).

Both disinfectants are quaternary ammonium salts (cationic detergents), and are antimicrobial through their disruption of the bacterial plasma membrane and inactivation of enzymes and other cellular proteins.

Fume Hood Guidelines

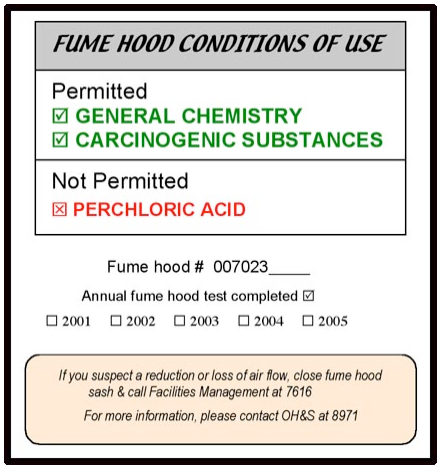
1. Before using a fume hood, ensure you will be provided with an appropriate level of protection and that the fume hood is appropriate for your work. Refer to the conditions of use, including any restrictions, noted on the fume hood label.

    a) All operations, which generate airborne chemical contaminants, must be performed in a fume hood with either a "General Chemistry" and/or "Carcinogens" designation.

    b) Radioisotopes must be used in a fume hood designated exclusively for its use.

    c) Perchloric acid must be used in a hood designated exclusively for its use, having appropriate wash down capabilities.

    d) Biohazardous materials must be used in a certified biological safety cabinet.



2. Keep all apparatus at least 15cm (6 in) from the front face of the fume hood and the back damper to ensure air is adequately drawn into the hood. Items stored at the back of the fume hood and larger items should be elevated on a shelf.

3. Do not use a fume hood that does not have the maximum working sash height clearly labeled. Always keep the sash at or below this level, since increasing the sash height reduces the air flow at the face of the hood.

4. All fume hoods should have an "Air Flow Indicator" attached to the sash. Prior to starting work in a fume hood, check the ribbon to make sure air is flowing into the hood (ribbon should be angled towards the hood).

5. To prevent a reduction in airflow at the face of the hood, limit the number of individuals standing close to the fume hood and ensure that open doors and windows are not creating a cross draft.

6. Clearly WHMIS-label all chemicals and long-term experiments including the user's name and date.

7. Do not use a hood for storing chemicals unless it has been designated for storage.

8. If a power outage occurs, fume hood function will likely be compromised. In this situation, take precautions to ensure adequate protection.

9. Ensure that you are aware of the nearest emergency eyewash and shower. A lab coat, gloves, eye protection and appropriate footwear must be worn.

10. Do not place electrical apparatus or other ignition sources inside the fume hood when flammable liquids or gases are present in the hood.

11. Fume hoods with an on/off switch have been labeled. Ensure the hood is turned on before starting your work. Before turning the hood off, ensure that any vented chemical storage will not be affected.

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 an 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 (Collection Site A: in front of the Cunningham building).
- 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

- Get away from windows and heavy objects.
- Duck, cover and hold. (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

- After the shaking stops wait 60 seconds then evacuate the building. Do not use elevators.
- Meet at your designated Emergency Evacuation Site (Collection Site A: in front of the Cunningham building). Keep way from power lines and buildings to avoid falling debris.
- Report any injuries to Campus Security Services.

Evaluation

The final mark will be based on:

lab summaries	30%
lab journal	10%
practical assessment	10%
quizzes	15%
final exam	35%

Final grades will be strictly determined as follows:

90.00 – 100%	A+
85.00 – 89.99%	A
80.00 – 84.99%	A^-
77.00 – 79.99%	B+
73.00 – 76.99%	B
70.00 – 72.99%	B^-
65.00 – 69.99%	C+
60.00 – 64.99%	C
50.00 – 59.99%	D
≤ 49.99%	F (or N)

Attendance

Laboratory attendance and punctuality is compulsory. Failure to attend a lab or to arrive on time for a lab without prior arrangement or a written medical excuse may 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 are responsible for maintaining their lab journal and for obtaining the data in order to write up the lab report. This may involve a student performing the lab once they have recovered.

Lab Summaries (30%)

Lab summaries require that you present the results of your experiment and answer the posed questions in a numbered format (not essay format).

Summaries are to be written independently; **collaboration on written work is strictly prohibited** (refer to p. xiv). They are to be type written using 12 point font and double-spaced. Double-sided printing is acceptable. Please check your work for grammatical and spelling errors.

Summaries are due at 4:30 p.m. for due dates that fall on a Day 1 and 10:30 a.m. for due dates that follow on a Day 2, unless otherwise stated by the instructor. Late lab reports are penalized **ten percent per day** (24h), and **fifteen percent** for the weekend. Late summaries are to be submitted directly to the lab instructor. No credit will be given for lab reports more than six days late.

An electronic copy of your summary must be submitted to Turnitin on the due date by 4:30 p.m. Turnitin is a plagiarism prevention and detection service employed by the University. It compares your document to other reports submitted to Turnitin as well as to text found on the web. Turnitin then generates a report which the instructor reviews to identify any cases of non-original work.

Grading queries will not be considered later than one week following the return of the marked report or summary. Any requests for reconsideration of the grade will involve remarking the entire submission. Students will receive the grade assigned upon remarking.

Lab Journal (10%)

Keeping a detailed, accurate, and legible record is essential for recording and documenting experimental procedures and results. An accurate account of the steps you performed and the data you generated is necessary to duplicate results, communicate your discoveries, and patent new products. To give you practice in this skill, you will be required to maintain a lab journal. The journal will be marked weekly by a teaching assistant (TA) who will look for specific data entries from the previous week(s)/day. Lab journal entries must be entered into a **bound** book, written in **ink** and should include data such as:

- date and title of the experiment
- pre-lab or in-lab calculations
- raw data
- observations
- experimental conditions (e.g., % gel, absorbance wavelengths, incubation conditions…)
- changes to the procedure
- any errors
- unknown numbers and bacterial strains used

Data and calculations must be presented in a manner so that what appears on the page will need no further clarification e.g. variables are clearly labelled with units, and data tables are titled. The TA marking your journal will be justified in taking off marks if they cannot read or interpret your writing, or if they have to search excessively for required data.

Practical Assessment (10%)

The practical assessment will be based on your preparation for labs, attendance, organization, calculations, experimental results, and general lab practice including clean up. Your Calculation Exercise (p. xvi) and Literature Search Assignment (p. xvii) will also be included in this mark.

Guidelines for Lab Summaries

Lab summaries must include results, discussion, reference, and when required, appendix sections. Like all your written work, your summary should be concise. Abbreviations must be defined in brackets after the first use of the term. Any uncommon non-English words must be italicised, e.g., genus and species, genetic loci, names of Greek letters. When submitting the hard-copy of your summary, include your name and student number, your partner's name, the date, and the title of the report. Submit summaries as a stapled document, not in a folder. When submitting an electronic copy to Turnitin, do not include any identifying information.

Results

This section includes all your data organized into figures, graphs, and tables (unless otherwise stated). Interpretations of the data or conclusions are not to be included in this section but should be reserved for the discussion section.

Tables and figures are always to be numbered, properly titled, and presented in the order in which you address them in the discussion. Any standards used, or supplied data, needs to be referenced.

There are two major parts to every figure in a BCMB 301 lab report, the **figure title** and the **figure** itself. The **figure title** describes the experiment that created the data, and provides enough information so that the figure can be understood without frequently having to refer to the text of the report. The title should include:

- The type of figure (e.g., Agarose gel, Western blot, graph, SDS-PAGE).
- What the figure is showing (e.g., isolated *E.coli* CSH66 DNA).
- The experimental treatment (e.g., *E. coli* culture grown overnight in the presence of lactose, gel was run for 45 min at 20 mAmps, absorbance was taken at 750nm).
    - How long was the reaction incubated?
    - How did you detect the molecule?
    - What percentage was your gel?
    - What wavelength was the absorbance read at? etc.

  Do not include detailed experimental methods in the figure title. Procedural steps are best described/referenced in the materials and methods section and not in the figure title.

Data in **the figure** itself should relate directly to the question that was addressed in the experiment. Always include the original figure when presenting gels and membranes. Unless specifically requested, raw data is not usually included in the Results but rather in the Appendix.

Correct formatting requires that both the figure and the title must appear on the same page, *i.e.* not split over multiple pages.

Discussion

In the discussion section of the summary, the questions posed address how your results relate to the purpose of the experiment. Simply number each of your answers so that they correspond to the questions asked. However, be sure to discuss any anomalous results and why they might have occurred. Apply the following checklist to your discussion:

- Have you written in the third person?
- Were you concise?
- Did you refer to and discuss all the data presented in the results section?
- Did you provide specific examples from your results to support your conclusions?
- Did you relate the relevant theory to your experimental results?
- Did you compare your results (values, observations) to literature values or class data?
    - Explain any significant (use your own judgement) differences.
    - When making a comparison to published values or standards, be sure to include both your result and the data to which you are referring.
    - Similarities and differences should be expressed numerically wherever possible.
- Is your data consistent and error free?
- Did you cite your references and put the information into your own words?
- Did you compare the duplicate tests or results of tests measuring the same values in all relevant combinations?
- Did you answer all questions assigned in the lab manual or by the lab instructor?
- Did you draw conclusions based on your data and address the purpose(s) of the lab as outlined in the introduction?
- Did you proofread your work and check for spelling errors?
- Is your work formatted in 12-point font and double-spaced?

Listed below are common terms that you will encounter in the discussion questions:

Compare: Examine qualities or characteristics that resemble each other. Emphasize similarities, but don't forget to address important differences. Compare/contrast strategies are often useful for questions that ask about whether results are consistent, or not.

Define: Clearly state the meaning of the word or term. Relate the meaning specifically to the way it is used in the subject under discussion. Sometimes an example may be required.

Diagram: Give a drawing, chart or some other graphic answer. Labels are often required. In some cases, a brief explanation or description is also required.

Discuss: This requires the most complete and detailed answered. Answers should, however, still be concise and to the point. Examine and analyze pros and cons, positive and negative controls etc. To "discuss briefly" requires (typically) 2-3 sentences of the critical factors.

Explain: Explanatory answers must clarify the cause(s) or reason(s) for something. This is a "how" and "why" type of question. Give reasons for differences between results (e.g. your result vs group result or literature result), or give reasons for your opinion on something.

Justify: Prove or provide solid reasons for decisions or conclusions, i.e. cite evidence or give clear logical reasons.

List Present an itemized (point form) series of information. Be concise.

Relate: Show how things are connected with each other, or how one affects the other, correlates with something else etc.

Summarize or State: Present the main points or facts in **brief** condensed form. Omit details, illustrations and examples.

References

You are required to read published literature and incorporate the relevant material into the discussion of your lab report. Most required reference material can be found on the course's Moodle site. The information source **must always** be cited in the text of your report by number, and in the reference section in the corresponding order. References are to be written according to style of The Journal of Bacteriology.

Authors. Year. Title. Journal. **Volume**: Pages.

Any standards used in your Results section as well as the complete citation for the BCMB 301 Lab Manual should be included in this section. In addition, the source of any supplied data must be cited. Please note that Wikipedia is not to be used as an academic reference.

Appendix

In this section include your work for all mathematical calculations (can be handwritten). Any extra information, results, and/or raw data are presented here, i.e. data borrowed (and referenced!) from other students.

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. The following is an excerpt taken from the UVic Undergraduate Calendar, 2013-2014, p. 32-34.

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
- resubmits their own work that has been used in an identical or similar form to fulfill an academic requirement

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

Note: 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.

Aiding Others to Cheat

- helping or attempting to help others to engage in any of conduct described above is an offence

Academic Integrity Assignment

Please read the UVic Libraries' webpage on plagiarism and UVic's Policy on Academic Integrity. Both can be found at: http://library.uvic.ca/instruction/cite/plagiarism.html.

Complete the Academic Integrity Assignment on the BCMB301A Moodle website.

- Click on the "Quizzes" link under "Course Menu" on the left hand side of the page to find the assignment.

- Exercise is due **Sunday, September 15th by 11:59 p.m**.

- You must obtain a grade of 100% to successfully complete the assignment. However, you may repeat the assignment as many times as necessary in order to achieve this.

Calculation Exercise – Fall 2013

Refer to Appendix B for a Review of Calculations and Dilutions. Unless otherwise instructed, give your answers to 3 significant figures.

1. Convert the concentration of the reagents shown in column #1 to the units indicated in columns #2 and #3.

Reagent	MW or Density	#1	#2	#3
EDTA	372.20 g/mol	6.75% (w/v)	mM	mg/dL
Glycerol	92.09 g/mol D = 1.25 g/ml	169 mg/mL	%(v/v)	M
□-Mercaptoethanol	78.13 g/mol D = 1.1143 g/ml	1.25 M	g/L	%(v/v)

2. How would you prepare 10 ml of a 1/64 dilution of an antibody diluted with buffer (a single step, NOT a serial dilution)? Dilution is prepared in a test tube.
3. How would you prepare 600 ml of 1X TAE Buffer (40.0mM TRIS Base, 20.0mM acetic acid, 1.00mM EDTA) from a 25X stock solution? Solution prepared in a graduated cylinder.
4. How would you prepare 250 ml of 75.0% (v/v) ethanol from 95.0% (v/v) stock ethanol? Solution is prepared in a graduated cylinder.
5. In a single well of a 96 well plate, 45.0 □l of a 1.50 M protease inhibitor stock was added and brought up to a total volume of 0.20 ml with buffer. Calculate the final concentration of a protease inhibitor in the single well.
6. An isolated DNA sample has a concentration of 333 ng/μl. If you want to run 500 ng of the DNA in one well of an agarose gel, what volumes of DNA sample, 6X AGE sample buffer, and dH_2O are required to load the DNA sample in a total volume of 12 μl in one lane?
7. You wish to calculate the original protein concentration in your crude lysate sample. You've save an aliquot at a dilution of 1/50. You use 0.5 ml of this diluted lysate in the assay tube and make it up to 2.0 ml with dH_2O. To this you add 1.0 ml alkaline copper solution and 0.5 ml of Folin-Phenol reagent. For the standards, 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of 0.1mg/ml BSA are added to separate tubes. They are likewise made up to 2.0 ml with dH_2O, and 1.0 ml alkaline copper solution and 0.5 ml of Folin-Phenol reagent are added. You interpolate from the resulting standard curve a mass of 0.045 mg protein for your lysate sample. What was the concentration of protein in the original crude lysate?
8. Diagram the preparation of a 4.0×10^{-5} serial dilution of a bacterial culture using no more than 3 tubes, and a maximum volume of 1 ml per tube. Your volumes must be within the range of standard pipettors (10.0 μl to 1 ml).
9. Describe how you would prepare 250 ml of Transfer buffer: 192 mM glycine (MW= 75.07), 20% (v/v) methanol, 22 mM TRIS base (MW = 121.14), pH 8.3. (Refer to page 2-7 in the manual, making buffers with "the conjugate acid or conjugate base method").

Literature Search Assignment

PubMed, a database developed at the National Library of Medicine (NLM), provides access to over 19 million citations, from as far back as 1950. For more information on PubMed, see www.ncbi.nlm.nih.gov/entrez/query/static/overview.html.

The following exercise is to be completed and handed-in for grading. The purpose of the exercise is to familiarize you with finding information through the library and with the concept of properly citing works to avoid plagiarism. Each student will be assigned both a faculty member and a subject (not related) to use for the assignment. Be sure to clearly indicate your assigned faculty member and subject on your assignment.

Searching for Journals Subscribed to by UVic

1. To determine if the library subscribes to a particular journal, either online or in print:

   - On the library homepage (http://library.uvic.ca), click on the "Journal Titles" tab.
   - Enter the full title of the journal (abbreviated titles are not recognized) in the box, and click "search".
   - Check the resulting list for the desired journal title. If that journal title is displayed, click on it to determine the specifics of the library's holdings for this journal. Try this with the Journal of Bacteriology and answer the following questions:

     a. Does the library have a print subscription? If so, for what years?
     b. Does the library have an online subscription? If so, for what years?

Search by Author

2. Using PubMed, find a peer reviewed journal article written by your assigned **faculty member** from the Department of Biochemistry and Microbiology.

   - Go to the UVic Libraries homepage (http://library.uvic.ca).
   - Click the "Databases" tab and then choose "All Databases A-Z". Search for the PubMed database and use it to find an article by your assigned faculty member.

Be sure to choose an article that has been published and is available through the library either in print or through on online subscription (i.e., perform a search using the **unabbreviated** title of the journal to ensure that the library subscribes to the journal that contains your chosen article).

Tip: Once you have found an article's abstract in Pubmed, you can find the full title of the journal by hovering the cursor over the abbreviated title. Or, you can use the Journals Database in PubMed to find the full title of a journal from the abbreviation – on the PubMed homepage, select "Journals Database" under "More Resources" at the right.

a. Is the article you chose available in print, online, or both?
b. Which years of the journal are available in each or either format?

3. Print (PDF format) or photocopy the first page of your article and hand it in with your assignment.

Citing Journal Articles

Referencing styles and formats can differ greatly from journal to journal. Specific citation requirements for any particular journal can be found on their webpage under "Instructions to Authors". The citation format for Journal of Bacteriology is as follows:

Authors. Year. Title. Journal. **Volume**: Pages.

The following is an example of how an abstract and citation for an article would be presented in Pubmed:

J Cell Biol. 2008 Feb 25;180(4):803-12.

Integration of Golgi trafficking and growth factor signaling by the lipid phosphatase SAC1.

Blagoveshchenskaya A, Cheong FY, Rohde HM, Glover G, Knödler A, Nicolson T, Boehmelt G, Mayinger P.

Division of Nephrology and Hypertension, Oregon Health and Science University, Portland, OR 97239.

Abstract

When a growing cell expands, lipids and proteins must be delivered to its periphery. Although this phenomenon has been observed for decades, it remains unknown how the secretory pathway responds to growth signaling. We demonstrate that control of Golgi phosphatidylinositol-4-phosphate (PI(4)P) is required for growth-dependent secretion. The phosphoinositide phosphatase SAC1 accumulates at the Golgi in quiescent cells and down-regulates anterograde trafficking by depleting Golgi PI(4)P. Golgi localization requires oligomerization of SAC1 and recruitment of the coat protein (COP) II complex. When quiescent cells are stimulated by mitogens, SAC1 rapidly shuttles back to the endoplasmic reticulum (ER), thus releasing the brake on Golgi secretion. The p38 mitogen-activated kinase (MAPK) pathway induces dissociation of SAC1 oligomers after mitogen stimulation, which triggers COP-I-mediated retrieval of SAC1 to the ER. Inhibition of p38 MAPK abolishes growth factor-induced Golgi-to-ER shuttling of SAC1 and slows secretion. These results suggest direct roles for p38 MAPK and SAC1 in transmitting growth signals to the secretory machinery.

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Grades	Grade Point Value	Percentage	Description
A+ A A-	9 8 7	90 – 100 85 – 89 80 – 84	**Exceptional**, **outstanding** and **excellent** performance. Normally achieved by a minority of students. These grades indicate a student who is self-initiating, exceeds expectation and has an insightful grasp of the subject matter.
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C+ C	3 2	65 – 69 60 – 64	**Satisfactory**, or **minimally satisfactory**. These grades indicate a satisfactory performance and knowledge of the subject matter.
D	1	50 – 59	**Marginal** Performance. A student receiving this grade demonstrated a superficial grasp of the subject matter.
F	0	0-49	**Unsatisfactory** performance. Wrote final examination and completed course requirements; no supplemental.
N	0	0-49	Did not write examination or complete course requirements by the end of term or session; no supplemental. Failure to complete one or more components of student evaluation 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 O. The maximum percentage that can accompany an N on a student’s transcript is 49

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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. 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.

5. Scan sheets for multiple choice exams (bubble sheets) will not be made available for review. Therefore, in addition to filling in answers on the scan sheet, students should also circle their answers in ink on their exam.

6. Professors may refuse to review/remark exams not written in 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.

7. Examination papers that have pages removed, or are mutilated will not be marked.