Biology 466/689 session/4-Winter 2012. Advanced Laboratory In Molecular Biology

Tutorial location/time: CC-101, Mondays 11:40-13:10

Instructor: Michael Sacher, SP-457.01

Tel: 848-2424 ext 5627

Email: msacher@alcor.concordia.ca

Web site (Class notes, lab manual, assignments and info): accessible via Moodle

Technician: Michel Harvey (tel. 848-2424 ext. 3818) (Room SP-375.29)

Lab location: SP-385.01

Lab times: Tue (01), Wed (02), Thurs (03), starting at 13:30

Lab schedule: Week of January 2 – Week of March 19.

A USB key is mandatory. All results will be in digital format and not printed.

T.A.	Email address	Office Number
Shaghaayegh Ostadjoo	shaghaayegh@gmail.com	SP-501.06
Yu Zhan	zhanyuzzyy@hotmail.com	SP-401.1
Hala Khalil	halakhalilconcordia@gmail.com	SP-501.09

1. **Office hours: Tuesdays and Wednesdays, 2pm-3:30pm.** You can also drop by at your convenience but there is no guarantee I will be in or available to answer your questions. Questions can be sent by email (I respond to email quickly) and you can always make an appointment with me by email before dropping by my office. Laboratory report grading questions should be directed towards the T.A.s.

2. **Course Description:** The purpose of this course is to introduce you to the theory and practice of experimental molecular biology. As much as possible, the course is intended to mimic the "real life" conditions that you would encounter in a university or industrial research laboratory. Therefore, the emphasis in the tutorials, assignments and exams will be on experimental design, analysis and interpretation of experimental results, and reliable, informative record keeping**.**

3. **Marking Scheme: Note that the pass grade for this course is a 50%.**

Item	% of final grade	Notes
Quizzes	15	During regular tutorial period
Lab reports	30	Hand in to your TA
Lab work	5	Performance in lab
Project	15	Hand in to professor by 5pm March 30th
Final exam	35	During regular exam period

Note: If your final exam is ~10% or more above your semester average, then the final exam will count for 70% and the semester for 30%. In this case, the weight of the 30% semester mark will be as indicated in the table above. **This scenario only applies to students with a 50% or higher semester average and no more than 5 days of cumulative late lab reports.**

Laboratory experiments:		% of final grade:	Due week of:
Project # 1:	PCR	6 %	January 23
Project # 2:	Sequencing	6 %	February 13
Project # 3:	Gene expression	6 %	March 5
Project # 4:	Yeast 2-hybrid	6 %	March 19
Project # 5:	Mutagenesis	6%	**On** April 4 by 5pm

4. **Lecture notes:** Lecture notes will be posted in black and white as a PDF file prior to each class. There may be overlap in some of the slides depending on what was covered in the course of the lecture (e.g. if not all material was covered in the prior lecture, some of the slides will be duplicated for the subsequent lecture). **Students are encouraged to bring coloured markers/pens/pencils to highlight portions of the notes based on the lectures.**

5. **Lab Reports:** Reports are due at the beginning of the lab session, 1 week after a project is completed unless otherwise noted. Hand in (i) **a hard copy to your TA** and (ii) **upload an electronic version on the Moodle site**. Reports are considered late if either version is late. For the e-version, upload in **WORD format** and **do not include figures, gels or DNA sequences**. The file name should be: **last name_project X**. Reports should be brief and concise. It should be formatted as a scientific paper (see the course web site for helpful resources) with an Introduction, Methods, Results, Discussion and Reference section. Make sure to put the relevant material in each section (ie. do not discuss the results in the results section). Do not reiterate the procedure, state only the changes if any. The **results section must be worded**, not simply a series of figures, tables and calculations. Non-adherence to this requirement will result in a 10% "style" deduction. The **Intro and Discussion should be ~4 pages. The Results should be brief but, given that DNA sequences may have to be shown, is of no defined length.** It must be typed (no smaller than font 12). Failure to format the reports properly will result in stiff deductions, so if you are not sure how to do it ask your TA or the instructor. Late labs: -5%/day, a grade of 0 will be assigned after labs have been returned to other students. **Note:** for late work, it is your responsibility to make an appointment with the TA or instructor to hand in your work. Leaving a report under an office door is not acceptable. If it is not discarded by the janitorial crew, it will be marked as received the day it is found.

Lab reports should include the following:

Cover page: include name, ID #, section, date and project #/title.

Introduction: Summarize relevant theory. **Do not turn this into a materials and methods section**.

Methods and miscellaneous information: keep this section brief but categorized (see any scientific paper for ideas; do not simply regurgitate the lab manual). **Changes must be stated.**

Results: **worded** to show you understand what the point of each overall step is. DNA sequences can be shown in this section. **Full sequences should be shown with the relevant portions or boundaries and restriction sites highlighted**. Include plasmid maps, strains, etc.

Discussion: In this section you must explain the results that you expected to get, and why you expected to get them (i.e. the biological and/or technical basis for the experiments). You must also explain the results that you actually got, as well as a reasonable explanation for the differences (if any) between your results and the expected results. Lastly, you must clearly answer any questions asked in the lab manual or in the tutorial.

References: **Do not reference Wikipedia**. You may use Wikipedia but always check

original references as there are often mistakes in Wikipedia. Remember, quotes without appropriate references will be considered as plagiarism resulting in severe disciplinary action. Do note overuse quotes; put it in your own words.

Questions concerning the corrected lab reports should be directed towards the TA. If you are unsatisfied with the conclusion of the TA, put in writing what the issue is, what the response of the TA was and submit that to the instructor along with the original lab report. **Penalties for missed labs:** A 2 week project (# 1) will incur 50% penalty for each missed week; a 4 week project (#s 2, 4 and 5) will incur 25% penalty for each missed week; a 6 week project (# 3) will incur 17% penalty for each missed week. Keep in mind that, due to overlaps in the projects, one missed week could incur a penalty on as many as three different projects. These penalties will be voided for documented cases of family or medical problems.

6. **Quizzes:** There will be four 20 minute quizzes, generally focusing on each of the five projects that you do in the lab. The dates of each quiz will be announced during the preceding tutorial and posted on the web site. **They will be based on the tutorials and labs up to that point**. There are no make-ups.

7. **Assignments:** On occasion, assignments designed to give you experience with web-based tools may be given to complement the lectures. Assignments will be announced in the preceding tutorial and posted on the web site. **Follow the instructions carefully and submit in the proper format**.

8. **Term project:** See page 6 for details. NOTE: You have ~11 weeks to do the project. Use your time wisely and do not save it until the final weekend. It is **due in my office by 5pm, Thursday March 29th.** There will be no extensions, except in documented cases of family or medical problems and late submissions will be docked 5 marks per day late. Keep in mind there is a **deadline to hand in gene names by 5pm, Monday January 30th**. Late deductions also apply to that deadline.

9. **Final exam:** During final exam period and covering all concepts (both theoretical and practical) dealt with during the tutorials and laboratories. Calculators only will be permitted (ie. a calculator on a cell phone is not permitted). **There is no supplemental exam for this course.**

10. **Computer skills:** You should have an email address that you check frequently and have internet access. You should also be comfortable using a word processor (Word) and a spread sheet (Excel). For help, contact IITS since they regularly provide free training sessions (http://iits.concordia.ca/services/training/).

11. **Books:** The lab manual is available on line. There are no assigned text books for this class, but you are expected to go back to reference books to refresh yourself on theory you may have forgotten. There are catalogs in the lab, and the information that they contain is also available on the company web sites. There is a copy of "Molecular Cloning: A laboratory manual. 3rd edition. Sambrook & Russell, Cold Spring Harbor Laboratory Press" (earlier editions are referred to as Maniatis) in the lab and on reserve at the Vanier library. This three-volume book provides basic and alternative protocols commonly used in molecular biology labs and provides background/practical information. You are welcome to browse the

one in the lab. However, please do not take it out of the lab without prior permission since the set is very expensive.

"Molecular Cell Biology" by Lodish, et al. (WH Freeman & Co) has a chapter dedicated to recombinant DNA technology. If you do not own the textbook, you can access the content through NCBI Bookshelf: http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mcb.TOC. Other relevant reading materials will be posted on the BIOL466 homepage as needed. "Molecular Biology" by Weaver, used for Biol367, is also an excellent book to reference for some of the subject material.

A copy of Benjamin Lewin's "Genes XIII" is also available in the lab. There are many chapters in this book that complement some of the lecture material.

Articles will be added to the course web site. They cover aspects of what was discussed in the lecture and should be read. You will not be responsible for any portion of the article that was not covered in the lectures. However, it would certainly be beneficial to you to read those sections.

12. **Safety:** There is a section on laboratory safety in the beginning of the lab manual (pp. 4-12). This must be read before entering the laboratory. Within this section is a safety sheet (p. 10). **The signed safety sheet must be handed in before the first lab.** There will be a one week grace period. If it is not handed in before the second lab session, then you will not be permitted to enter the lab (see above for penalties for a missed lab).

13. **Prelab and lab performance:** You are expected to have a prelab write-up before each lab session which the TA will review. Among other things, it should include any formulae that will be needed for that week. If it is deemed illegible or plagiarized, you will not be permitted to enter the laboratory for that week and the penalties outlined above will be in effect. If the TA feels it is incomplete, you will be informed and, if an incomplete/unacceptable prelab is found again, the TA may prevent you from entering the laboratory for that week. The technician will make brief prelab announcements promptly at the beginning of each lab session that are essential to the smooth running of the session. The lab door will be locked and latecomers will be asked to wait in the hallway until after the announcements. Latecomers will be asked to sign a late sheet. Should your name appear multiple times on the late sheet, marks will be deducted from the "lab work" portion of the grade. In addition, should the technician or TA deem a student to be tardy in their work for reasons not beyond their control (ie. excessive talking, shmoozing, lateness), or if students leave a messy work space after the lab session, marks will also be deducted from the "lab work" portion of the grade. **There is no admittance to the lab after 2pm.** Keep meticulous notes in a laboratory notebook and clearly label tubes that are saved for future weeks. The location and position in the common storage boxes in the freezer should be noted in your lab notebook.

Students must obtain permission from the instructor to perform the lab on a day that is different from their registered lab day. Such permission will only be granted for valid reasons. Since expensive reagents are prepared for each lab, not showing up at your scheduled time results in unnecessary waste of money and preparation time. Changing lab days without prior permission will result in steep deductions since you will not be permitted to enter the laboratory (see section 4).

Welcome to Biology 466. I hope that you enjoy this course and trust you will gain valuable research experience over the coming months.

M. Sacher

Code of conduct (taken from University Academic Policies)

the full version is available at:
http://www.concordia.ca/vpirsg/documents/policies/AcademicCodeConduct2008.pdf

III Offences

14. Any form of cheating, plagiarism, personation, falsification of a document as well as any other form of dishonest behaviour related to the obtention of academic gain or the avoidance of evaluative exercises committed by a student is an academic offence under this Code.
15. Any attempt at or participation related in any way to an academic offence is also an offence under this Code and shall be dealt with in accordance with the procedures set out in this Code.
16. Without limiting, or restricting, the generality of article 14 above and with the understanding that articles 16 a)-l) are to be considered examples only, academic offences include, the carrying out, or attempting to carry out or participating in:
    a. plagiarism - the presentation of the work of another person, in whatever form, as one's own or without proper acknowledgement;
    b. the contribution by one student to another student of work with the knowledge that the latter may submit the work in part or in whole as his or her own;
    c. unauthorized collaboration between students;
    d. tearing or mutilating an examination booklet, inserting pages into a booklet or taking a booklet from the examination room;
    e. multiple submission - the submission of a piece of work for evaluative purposes when that work has been or is currently being submitted for evaluative purposes in another course at the University or in another teaching institution without the knowledge and permission of the instructor or instructors involved;
    f. the obtention by theft or any other means of the questions and/or answers of an examination or of any other University-related resource that one is not authorized to possess;
    g.the possession or use during an examination of any non-authorized documents or materials or possessing a device allowing access to or use of any non-authorized documents or materials;
    h. the use of another person's examination during an examination;
    i . communication with anyone other than an invigilator during an examination or the obtention of any non-authorized assistance during an examination;
    j. personation - assuming the identity of another person or having another person assume one's own identity;
    k. the falsification of a document, in particular a document transmitted to the University or a document of the University, whether transmitted or not to a third party, whatever the circumstances;
    l. the falsification of a fact or research data in a work including a reference to a source, which has been fabricated. Falsification shall not include those factors intrinsic to the process of academic research such as honest error, conflicting data or differences in interpretation or judgment of data or of experimental design.

Sanctions

50. Within ten (10) days from the conclusion of the hearing, the AHP shall write to the student and the Dean, with a copy to the Registrar, indicating its decision to dismiss the charge against the student or, in the case of upholding the charge, to impose one or more of the following sanctions:
    a. Reprimand the student;
    b. Direct that a piece of work be re-submitted;

c. Enter a grade of “0” for the piece of work in question;
d. Enter a grade reduction in the course;
e. Enter a failing grade for the course;
f. Enter a failing grade and ineligibility for a supplemental examination or any other evaluative exercise for the course;
g.Impose the obligation to take and pass courses of up to twenty-four (24) credits in addition to the total number of credits required for the student’s program as specified by the Dean. If the student is registered as an Independent student, the sanction will be imposed only if he or she applies and is accepted into a program.
h. Impose a suspension for a period not to exceed six (6) academic terms. Suspensions shall entail the withdrawal of all University privileges, including the right to enter and be upon University premises;
i . Expulsion from the University. Expulsion entails the permanent termination of all University privileges.

Term Project: Due: March 29th, 2012, by 5pm

In order to study a protein, one of the first steps is to express a recombinant form of the protein in bacteria and test its function. Your assignment is to design an experimental strategy that will allow you to:

1) Isolate a gene of your choice using PCR. **The gene should be important for a human disease process.**
2) Clone the gene into an *E. coli* expression vector using restriction enzyme cloning.
3) Introduce the cloned gene into *E. coli*
4) Show that the transformed cells are making the correct protein, purify it, and test it for function.

Each student must have a separate gene. As soon as you have chosen one, let me know by email (msacher@alcor.concordia.ca). First come, first serve. In the unlikely event that two students have chosen the same gene, the student who submitted the gene last will be asked to identify another gene. All students must submit their gene names by 5pm on January 30th, 2012. Standard late penalties (5%/day) will apply if your gene names are not in on time. Put some thought into your choice since you will eventually need to assay the function of the purified protein (how easy is it to assay, what type of assay will you devise).

Details: You should hand in the following material, in this order (see note below):

1) The name of your organism where the gene originates.

2) The name of your gene

3) The Genbank accession # for the gene and the cDNA. Make sure the organisms are the same (ie. Don't give me the accession for the human gene and the rat cDNA).

4) The name of the protein product

5) The name of the disease in which it is involved

6) The function of the protein product (how it contributes to the disease). This should be a summary of the pathway or process that the protein is involved in (no more than 2 pages). Attach a copy of all the articles you used for this question.

7) The original sequence of your **gene** (from Genbank, must include the heading information for your gene and the pages with the start and stop codons). Also include a page with just the coding sequence. You **must** indicate on all sequence pages submitted the location of:
   - **a) The start codon.**
   - **b) The stop codon.**
   - **c) The position of the two PCR primers** that you will use to isolate the gene. (Note: The sequence of primers that are not localized on the sequence will not be corrected). If there is an intron, discuss how you would handle that (bacteria cannot process introns).

8) A restriction enzyme analysis of the unmodified sequence to show that the restriction enzyme sites that you are going to use for cloning do not occur within the piece of DNA you are working with. **Highlight (on the table/printout)** the two enzymes that you have selected.

9) A map of the vector including the sequence of the MCS, sufficient to indicate reading frame. **Indicate on the map** and the MCS what restriction sites you are using for cloning. Note: use a bacterial expression vector. List, in your own words, the main characteristics of the vector that led you to your choice.

10) The sequence of your two PCR primers, written 5' to 3'. **Highlight on the primer the location of the restriction enzyme cut sites** that you will use for the cloning.

11) The complete sequence of the recombinant plasmid, showing the location of:
- **a) The insert.**
- **b) The restriction enzyme sites used for cloning.**

12) A generated map of the recombinant plasmid with relevant sites indicated.

13) A brief explanation of the techniques, strains and reagents that you will use, **from beginning to end**, to verify that you have successfully cloned your gene, it is in the proper orientation, that the transformed cells are actually making the desired protein, that the protein has been purified and how you would test the function of that purified protein. Basically, think about the overall objectives and explain all steps required to meet that objective.

Note: This is NOT an essay or term paper. Hand in only the material listed above. Well organized material is easier to mark and therefore tends to get a better grade.

Point for clarification: Each semester, I receive the following question so I want to make my answer clear to everyone. The question is: "I have found a paper where my gene was cloned, expressed and assayed. Can I simply do what they did?".

In short the answer is NO. All you would be doing is copying someone else's work with no indication that you know what was done or why.

You must choose a different vector for cloning in this case. You must also include a copy of the paper that you found. This is the first thing I check for when grading these projects and, if I find the paper and see that you copied what was done, you will receive a zero on the project and be brought up on plagiarism charges. To avoid the latter, more serious, consequence, you should be up front and include the paper you found. You should then include a short note on the paper stating that you saw it and avoided doing exactly what they did. This will allow you to avoid the appearance of plagiarism.

Keep in mind there is "more than one way to skin a cat". That is, you should be able to come up with your own strategy (i.e. different vector, different restriction enzyme sites). It also means that several strategies may be found in the literature. So don't show me one such paper while copying another. Show all papers that you found that include something similar to what you are required to do in order to avoid the appearance of plagiarism.

I hope you find this term project to be interesting to research and write, and that you use it for the educational purpose that it was intended.