

**BIOLOGY 515: Biotechnology and Genomics
Laboratory Manual**

Fall 2016

Lab schedule:

Standard lab exercises/techniques

Run PCR reactions on gel

Sept. 12

Sept. 19

Module 1: Real Time Quantitative RT-PCR

RNA extraction

Sept. 19

Total RNA quantification, determination of quality, Bioanalyzer, gel

Sept. 26

Real time quantitative PCR (long day)

Oct. 3

Thanksgiving. No Lab

Oct. 10

Module 2: Protein/Protein interactions:

A. Affinity purification of TAP-tagged protein, Mass spectrometry

Protein extraction, quantification

Oct. 17

1st step affinity purification

Oct. 24

2nd step affinity purification

Oct. 31

SDS PAGE gel, stain Coomassie Blue and Silver (separate gels)

Nov. 7

Processing of coomassie-stained gel pieces for mass spectrometry

Nov. 14

Processing continued-zip-tipping

Nov. 21

LTQ MS/MS

Nov. 28

B. Co-immunoprecipitation

One step affinity-purification

Oct. 24

Western Blot: SDS PAGE gel, transfer

Oct. 31

Incubate blot with antibodies, chemiluminescence

Nov. 14

Stripping, blot incubation with antibodies, chemiluminescence

Nov. 21

Biol 515: Biotechnology and Genomics Laboratory Course Outline

Lab	SP 385.1, Monday, 11:00 am-3:00 pm (times may vary***)
Tutorial	CJ 1.129 Loyola Wed, 10:15-11:30
Course web site	Log in to https://www.myconcordia.ca Lab protocols and tutorial information will be posted for downloading/printing
Course Instructor	Dr. Amandeep K. Randhawa Glory, Ph.D. amandeep.glory@concordia.ca
Technical Officer	Dr. Marc Champagne Marc.Champagne@Concordia.ca
Teaching Assistant	Raha Omran raha.parvizi.o@gmail.com
Office hours	available during lab, or email for appointments (Office #: SP375.23)

Evaluation (subject to change)

Basic lab skills (prelab 1):	5 marks
Report on Standard lab exercises and PCR	5 marks
Lab report 1 Module1:	15 marks
Lab report 2 Modules 2 (A and B):	25 marks
Assignment for Module 2:	5 marks
Quizzes:	5 marks
Final exam:	30 marks
General Lab Performance	5 marks
Tutorial participation	5 marks

Grading scheme

A+ ≥ 90	C+ = 67-70
A = 85-90	C = 64-67
A- = 80-85	C - = 60-64
B+ = 77-80	D+ = 57-60
B = 74-77	D = 54-57
B- = 70-74	D- = 50-54
	F <50

Course content/objectives:

Biol 515 is a lab course designed to provide hands-on experience with several genomic/biotechnology approaches, and provides the opportunity to apply some of the theoretical knowledge obtained in first term course of the Diploma Program in Biotechnology and Genomics. The lab is composed of 2 main modules, which take several weeks each. There will be some overlap between modules during certain weeks. The modules involve qRT-PCR, affinity purification of TAP-tagged proteins, mass spectrometry and co-immunoprecipitation. Although it normally takes several trials in order to become independently competent with certain experimental techniques, the lab course provides “hands-on” experience with a diversity of molecular and biochemical approaches at the genome-wide level, which in turn contributes a solid base for future work in these areas.

Protocols:

Experimental protocols obtained from experts in various fields have been adapted to fit roughly 4 h time slots. Since many of the techniques require numerous steps or lengthy incubations, some labs require that you stay longer than the allotted 4 h. Other lab slots may take less than 4 h, so the timing will balance out. Reagents are expensive; for this and other reasons, including time constraints, you will be following protocols and using stock reagents prepared for you rather than developing/making your own. Similarly, researchers that wish to employ new techniques in their lab often start by obtaining established protocols, and include trouble-shooting to adapt to their specific organism/experimental question. Thus, you will be learning current and core techniques employed by most molecular/biochemical researchers.

The lab protocols will be posted on the web site for you to **download and print** (following the first tutorial, hard copies will **not** be provided). You must print the material, keep it in a binder and bring to the tutorial and lab. You can also include loose sheets such as your flow charts. It is important to keep detailed notes of what you do in the lab, including any changes made to the outlined procedure, mistakes that you may make, and the results that you obtain.

Tutorials:**The tutorial sessions are mandatory:**

They are designed to provide theoretical background for the 2 modules, and review the protocol for the up-coming lab. **It is not a lecture-your participation is mandatory** (see below). A brief overview and outline will be provided at the beginning of each lab, but the majority of this information will be covered in the tutorials. You should be prepared to discuss the lab module during the tutorial. **Read the protocols before each tutorial**, make any flow charts or other tables that will assist you during the lab, and ask questions ahead of time so that you are aware of the general lab procedures and background information. *There will be some quizzes during the tutorials.*

Laboratory:**Labs are mandatory:**

If you miss one lab, there will be 5% penalty. If you miss 2 labs, you will be un-enrolled and will no longer get a credit for the course.

Lab performance mark:

Your mark for lab performance will be based on how well-prepared you are for the lab, your conduct in the lab, and your attendance and participation in the tutorials. Organization, timing, and competence with techniques will also be evaluated. You will need to be careful yet efficient, which requires adequate preparation.

Lab reports due dates: To be announced in the tutorials at beginning of class. Tentative dates are given below, however they are subject to change.

Prelab exercise 1: Sept. 19

Report on Standard lab techniques and PCR: Sept 26

Module 1: Oct 11

Module 2 part A: Nov 28 (excluding mass spec results)

Module 2 part B: Dec 2 (include mass spec results along with this report)

Final exam: Dec. 9

Late lab reports: 5% off for each day. Submit hard copy by 4:00 p.m for the schedule day. You will be also required to upload electronic copy without figures on moodle. This will be checked for plagiarism. The electronic copies will be scanned for plagiarism only at the end of the term (after all the lab reports have been submitted).

Graded reports can not be handed back until all reports have been handed in.

Additional Resources:

Any genomics-related text will be useful, but there is no required text for the course. General background information on qRT-PCR, affinity purification and mass spectrometry can be obtained from any updated molecular biology textbook (eg. *Molecular Biology of the Cell*, Alberts, and *Molecular Biology of the Gene*, Watson. Specific papers on the various topics are included in each of the modules to provide additional background/technical information.

Lab Reports:

The lab report will be written in a manuscript format, with an Abstract, Introduction, Materials and Methods, Results, and Discussion sections, according to the instructions to authors provided by a leading journal (*Molecular Biology of the Cell*, for example). In this way, you will also gain experience in writing and organizing a manuscript to be submitted to a journal. Details on the format will be discussed in an up-coming tutorial.

Experiments often do not work due a variety of reasons. If the results are not what you expected, be prepared to explain potential reasons in the lab reports. In the end, even if you do not obtain the expected results, you will have obtained experience with the technology.

Plagiarism will not be tolerated!

Important notes on academic misconduct

Plagiarism is the most common act of academic misconduct. Plagiarism can be defined as handing in a work that is not your own. This includes copying materials from published or unpublished sources without citing the source, or copying from another student's work.

All lab reports will be scanned by plagiarism software at the end of the term. If suspicious activities are detected, the instructor must file an incident report to the Code Administrator and the sanction will be determined by the Code Administrator. The procedure is in place to identify students who repeat misconducts. Students may not be notified individually by the instructor or TA until the incident report is submitted. Since scanning is done at the end of the term, you may receive notification only at the end of the term. In that case, you will temporarily receive a grade of NR. NR grade will be changed to appropriate grade later on.

If Student A provides her/his assignment to Student B and Student B ends up copying the assignment, both Student A and B are guilty of misconduct. Please safe guard your assignments and assignment files. It is for your best interest. Lab partners may have the same results to work with. However, each report must be written in the individual's own words.

The following statements are taken from The Academic Integrity Website (<http://www.concordia.ca/students/academic-integrity/plagiarism.html>).

“Plagiarism:

The most common offense under the Academic Code of Conduct is plagiarism which the Code defines as "the presentation of the work of another person as one's own or without proper acknowledgement."

This could be material copied word for word from books, journals, internet sites, professor's course notes, etc. It could be material that is paraphrased but closely resembles the original source. It could be the work of a fellow student, for example, an answer on a quiz, data for a lab report, a paper or assignment completed by another student. It might be a paper purchased through one of the many available sources. Plagiarism does not refer to words alone - it can also refer to copying images, graphs, tables, and ideas. "Presentation" is not limited to written work. It also includes oral presentations, computer assignments and artistic works. Finally, if you translate the work of another person into French or English and do not cite the source, this is also plagiarism.

In Simple Words:

DO NOT COPY, PARAPHRASE OR TRANSLATE ANYTHING FROM ANYWHERE WITHOUT SAYING FROM WHERE YOU OBTAINED IT!"

If you are not sure how to paraphrase without plagiarizing, please refer to this example given by the Academic Integrity information: <http://www.concordia.ca/students/academic-integrity/plagiarism.html> . Examples are shown near the end of the web page.

Lab safety:

Safety is the most important aspect of the lab, so it is essential that you read the Biology Department Safety Guidelines included in this manual and sign and hand in the accompanying consent form. Being prepared for the lab decreases the chance of any accident.

Always concentrate on whatever you are doing, and if you are ever unclear on anything, ASK.

For each lab session, you will have to bring the following items:

(**MANDATORY**-if you don't have these, you will not be able to be in the lab).

1) Safety goggles; 2) Lab coat; 3) Lab protocol, outlines, flow sheets; 4) Calculator

*Bags, coats are not allowed in the lab. Arrange for a locker ahead of time.

List of Services:

- Concordia Counseling and Development (<http://cdev.concordia.ca>)
- Concordia Library Citation guide: <http://library.concordia.ca/help/howto/citations.html>)
- Advocacy and Support Services: <http://supportservices.concordia.ca>
- Student transition center: <http://stc.concordia.ca>
- New student program: <http://newstudent.concordia.ca>
- Access center for students with disabilities: <http://supportservices.concordia.ca/disabilities>
- Student success center: <http://studentsuccess.concordia.ca>
- The academic integrity website: <http://provost.concordia.ca/academicintegrity>
- Financial aid and awards: <http://web2.concordia.ca/financialaid/>
- Health services: <http://www-health.concordia.ca/>

CONCORDIA UNIVERSITY BIOLOGY DEPARTMENT LABORATORY SAFETY REGULATIONS

Safety is everybody's responsibility. The teaching laboratory will be a safe workplace if you are aware of the hazards, exercise good judgement, and follow correct operating procedures. If you are unsure about a certain protocol or have a specific safety concern, don't hesitate to ask your demonstrator, teaching assistant, technician, or professor about it. Your concerns may lead to the development of safer work practices for yourself and future students.

The following regulations are for your own protection and failure to observe them may result in dismissal from the laboratory.

1. Safe Work Procedures

General Precautions

- Get to know the laboratory you are working in. Check out the location of all exits and evacuation routes from the building.
- Familiarize yourself with the location of safety showers, eyewashes, fire extinguishers, safety blankets (if present), and first aid kits.
- Eating and drinking are **prohibited** in the lab.
- Coats, hats, briefcases, backpacks, purses or books that are not required for the lab exercise are not permitted into the laboratory. The labs are small and there is no storage space for your personal effects, it is therefore strongly recommended that you get a locker. Extra personal effects will have to be left in the hallway, at your own risk, outside the laboratory.
- Wearing sandals and open footwear in the lab should be avoided due to the danger of chemical exposure and blunt trauma.
- Long hair should be tied back.
- Baseball caps are not permitted since chemical fumes can be trapped underneath the brim.
- Only authorized experiments may be performed. The professor, technician, or teaching assistant must authorize any changes in procedure as given in the lab manual.
- Concentrate on the task at hand. Do not leave your experiment unattended.
- Notify your demonstrator, teaching assistant, technician, or professor about malfunctioning equipment or instruments. Do not attempt to fix it yourself.
- **Never** work alone in the laboratory. A demonstrator, teaching assistant, technician or professor must be present at all times.

- Before leaving the laboratory, clean your work area. This includes properly disposing of contaminated materials, glass slides and sharps, cleaning the lenses of your microscope, and wiping the bench top with disinfectant if necessary.
- All accidents, regardless of how minor, must be reported immediately to a demonstrator, teaching assistant, technician or professor. The technician assigned to the course has been trained to react appropriately.
- An *Injury (Accident)/Incident/Occupational Disease Form* must be completed within 24 hrs of the event.

Chemical Safety

- All chemical spills must be reported immediately to a demonstrator, teaching assistant, technician or professor. The technician assigned to the laboratory is trained to deal with spills. Do not attempt to clean up without proper supervision.
- Read the label of a chemical stock bottle or solution bottle carefully. Many chemicals have similar names and formulae but may have different properties. Note the precautions on the label.
- A Material Safety Data Sheets for the chemicals used in the laboratory are available for detailed hazards, toxicological, precautionary, and first aid information.
- **Never** return chemicals to stock bottles. Consult your demonstrator, teaching assistant, technician, or professor on waste disposal.
- Always pour acid into water, **never** the reverse. Pour slowly while stirring constantly.
- **Never** use mouth suction for filling pipettes. Devices such as suction bulbs or pumps must be used.
- **Never** use cracked or damaged glassware. The slightest flaw can cause an accident or chemical spill.
- Avoid exposing glassware to extremes of pressure or vacuum. Either one can cause the glassware to break, sending shards of glass flying.
- Do not force glass tubing into rubber stoppers. Lubricate the tubing with soapy water, glycerine, vacuum grease, or other suitable lubricant and protect your hands with a cloth or sturdy gloves before insertion.
- Wash your hands thoroughly, immediately after coming in contact with chemicals.

- In the event of a personal injury caused by chemical exposure, the treatment of personal injury must take precedence over chemical spill clean-up procedures.
- Minor injuries like scrapes, superficial cuts or burns can be treated on site. The technician responsible for your lab is trained in first-aid (see emergency response).
- More serious chemical injuries requiring more than first aid treatment must be immediately reported to Security **SGW: 3717** or **Loyola: 3707** (security will call 911 if necessary).
- All injuries related to chemical exposure must be reported to the demonstrator, teaching assistant, technician, or professor.
- An ***Injury (Accident)/Incident/Occupational Disease Form*** must be completed within 24 hrs of the event.

Bio-Safety

- Biohazardous materials are considered to be any organism such as viruses, bacteria, fungi, and parasites, including their toxic metabolites believed to be harmful to humans, animals or plants. Blood and other body fluids are considered biohazardous due to the possible presence of infectious organisms.
- All biohazardous releases or spills must be reported immediately to a demonstrator, teaching assistant, technician or professor. The technician on duty is trained to deal with biohazardous spills. Do not attempt to clean up without proper supervision.
- When dealing with biohazardous materials wash your hands frequently especially after completing experiments and before leaving the laboratory.
- Protective clothing and gloves, if required, must be removed before leaving the laboratory.
- Eating, drinking, applying make-up, or chewing gum in the laboratory is **prohibited**.
- Recommended procedures for decontamination of work surfaces and equipment should be followed.
- Always use needle-locking syringes or disposable syringe-needle units.
- All used syringes (complete unit), scalpels, razor blades, and Pasteur pipettes must be disposed of in an impervious container labelled "**BIOHAZARDOUS**".
- Broken glass that is **NOT** contaminated with hazardous materials must be disposed of in plastic containers or buckets clearly marked "**BROKEN GLASS**".

2. Personal Protection

Lab Coats

- Lab coats provide a barrier between you and the potential of a chemical splash
- The wearing of lab coats is **mandatory** at all times in the laboratory.
- All snaps /buttons must be fastened.
- It is **prohibited** to wear lab coats in the cafeteria or any other area where food is consumed.
- Eating, drinking or smoking while wearing a lab coat could lead to the inadvertent ingestion of chemicals and or biologicals.

Safety Eyewear

- Safety glasses should be worn during all laboratory procedures.
- Contact lenses must not be worn in the laboratory. If they are essential a letter from an optometrist or ophthalmologist must be submitted and safety glasses must be worn with them.

Protective Gloves

- Always use appropriate protective gloves if there is a potential for contact with a chemical or biohazardous agent.
- Gloves should be removed before leaving the immediate work area in order to prevent contamination of doorknobs, telephones, light switches, etc.
- Always remove gloves by pulling the cuff over the fingers.
- Rubber, plastic or latex gloves should **never** be used to pick up hot objects, as they can melt onto your fingers.

Special Health Concerns

- *If you are pregnant*, inform your professor. He or she will verify if the substances used in the lab pose a risk.

- ***If you are anaphylactic*** (hyper-allergic) inform your demonstrator, teaching assistant or technician. Extra care should be taken to avoid contact with chemical or biohazardous agents.
- ***If you have another medical condition*** that causes you concern in terms of the lab environment, discuss it with your professor and/or doctor

(particularly if you are immune-compromised-we will be using *C.albicans* in one of the labs).

Emergency Response

- In case of ***evacuation*** turn off all instruments and equipment. Shut off any open gas taps. Proceed directly to the nearest fire escape in an orderly manner. Fire monitors will be available to direct you to safety.
- If there is a small cut or break in the skin with coincident chemical or biohazardous exposure, treat the contaminated area immediately by rinsing with tap water for a ***minimum of 15 minutes***.
- If the chemical or biohazardous exposure is on the face, do not contaminate the eyes, nostrils or mouth. Wash the exposed area with mild soap and lukewarm water.
- Should chemicals or biohazardous material come into contact with your eyes, rinse with lots of water at the eyewash or tap water for ***at least 15 minutes***. Run temperate water gently enough to avoid damaging the eyes due to excess water pressure.
- If your clothes catch fire, **STOP** (where you are) **DROP** (to the floor) and **ROLL** (to smother the flames). Proceed to an emergency shower only when the flames are extinguished.
- Serious chemical or biohazardous injury related to ingestion, inhalation or skin/eye exposure requiring more than first aid treatment will be immediately reported to security **SGW: 3717** or **Loyola: 3707** (security will call 911 if necessary).

3. Last word

If you are ever unsure of what to do next in the lab or how to use a piece of equipment **STOP and ASK**. The technician and instructor will all be more than happy to assist you. This can prevent you from: ruining your experiment, damaging expensive equipment, or even hurting yourself.

TO BE COMPLETED AND HANDED IN AT THE FIRST LAB

I have read the Biology Departmental Safety Regulations and consent to observe them during my laboratory work.

DATE: _____ **NAME (print)** _____ **ID#** _____

COURSE _____ **Lab Sec.** _____

SIGNATURE _____

EMERGENCY CONTACT PERSON

NAME _____

RELATIONSHIP _____

TELEPHONE # _____

(Where this person may be contacted during the day)

