

MBG*3350
Laboratory Methods in Molecular Biology
Summer 2016

Department of Molecular and Cellular Biology
University of Guelph

COURSE OBJECTIVES

This course involves laboratory based instruction in the basic methodologies of Molecular Biology. Students will have the opportunity to develop technical skills and practical knowledge sufficient to perform basic procedures independently, and to diagnose and analyze experimental results obtained with these techniques.

COURSE PERSONNEL

INSTRUCTOR: Dr. David Josephy
Office: SSC 2253
Ext. 53833
Email: djosephy@uoguelph.ca

**LAB
COORDINATOR:** Amanda van der Vinne
Office: SSC 3519
Ext: 56189
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COURSE SCHEDULE

Laboratory: Tuesday & Wednesday
1:30 pm – 5:20 pm
SSC 4108

Lecture: Tuesday
Noon – 12:50 pm
(some weeks will include a second lecture session, 1:30 pm – 2:30 pm)
SSC 3317

LEARNING OUTCOMES

By the end of this course, students should be able to:

1. Explain the fundamental principles of practical molecular biology

2. Recognize and interpret experimental results
3. Implement the theoretical principles and apply them in the execution of lab experiments
4. Plan, design, monitor, troubleshoot, and optimize experiments
5. Use online tools to research a particular topic and read primary research articles in molecular genetics

COURSE RESOURCES

Lab Requirements:

MBG*3350 Laboratory Manual: purchased from **SSC 4480** the first three days of the semester

A bound Laboratory Notebook

Lab Coat

Indelible (“Sharpie”) marker: ultra-fine point

ImageLab (PC and Mac compatible) and **CFX Manager** (PC compatible): software provided by the lab demonstrator for download on your computer

Courselink:

This course will make use of the University of Guelph’s course website on D2L (via Courselink). Consequently, you are responsible for all information posted on the Courselink page for MBG*3350. Please check it regularly.

Undergraduate Calendar:

The Undergraduate Calendar is the source of information about the University of Guelph’s procedures, policies and regulations, which apply to undergraduate programs. It can be found at: <http://www.uoguelph.ca/registrar/calendars/undergraduate/current/>.

COURSE FORMAT

Lecture

One lecture per week: Tuesday 12:00pm – 12:50pm with some additional lectures scheduled from 1:30pm – 2:30pm in SSC 3317.

Laboratory

Two lab sessions per week: Tuesday and Wednesday 1:30pm – 5:20pm SSC 4108

Progress Reports

During the course of the semester you will be required to complete and hand in progress reports (see course outline for due dates). These reports are meant to assist you in continually monitoring the outcomes of your experiments. The reports are designed to have you analyze

your results and perform calculations so your formal lab reports are a compilation of results already obtained.

Literature Review

Before research is conducted one should have a good grasp of what is currently known for the topic/area of study. As such each student will be required to complete a literature review on His-tagged proteins and green fluorescent protein (GFP). Specific details will be presented in lab and on Courselink.

Formal Lab Report

You are required to write one formal lab report for this course, covering the cloning and analysis of GFP. Although the report will be written in the form of a scientific manuscript, you must remember that the **audience** and **purpose** of a formal report is somewhat different than that of a scientific paper. The aim is to show that you understand the **principles** and **significance** of the experiments you performed. Remember your data will have been marked already. What is of importance here is your ability to discuss and interpret **cumulative** data in a manner that demonstrates an **understanding** of what you have accomplished in the lab and the relevance of the experiments. Further information and guidelines for your lab report will be available in lab and on Courselink.

Assignments

One web-based assignment in the first week (to be completed during lab time) will have you use several web-based sequence analysis programs. Three other assignments will be due towards the end of the semester for the PCR optimization, qPCR GMO Analysis, and the qPCR *Arabidopsis* experiment. Instructions will be given in lab and posted to Courselink.

Lecture Topics and Schedule (*subject to modification*)

Lectures: Tuesday, noon-1 pm (1:30-2:30 pm as required)			
Class	Date	Length	Topic
1	May 17	120	nucleic acid structure, properties; plasmids; restriction enzymes; PCR (intro.)
2	May 24	120	DNA analysis: spectrophotometry; electrophoresis
3	May 31	120	plasmid "mini-preps"; transformation; DNA sequence analysis
4	June 7	60	molecular cloning; restriction enzymes; DNA polymerase
5	June 14	45	plasmid vectors
6	June 21	60	Class test 1
7	June 28	60	protein expression in <i>E. coli</i>
8	July 5	60	<i>E. coli</i> strains; protein purification; electrophoresis
9	July 12	60	SDS-PAGE and Western blotting
10	July 19	60	PCR methods; presentations re. 4th-year projects & graduate studies
11	July 26	45	advanced PCR techniques: qPCR; RT-PCR
12	August 2	60	Class test 2 spare

Laboratory Schedule

Week 1 (May 17-18)

Day 1

- Introduction to the lab
- Lab safety
- Molecular Biology Review
- PubMed Search
- Molecular Biology Web Tools

Day 2

- Web exercise assignment (bring your computer!)

Week 2 (May 24-25)

Day 1

- Micropipetting
- Plating Cultures
- Inoculating Cultures

Day 2

- Purification of Plasmid DNA
- Restriction Enzyme Digestion of pET-28a
- pET-28a Quantification

Week 3 (May 30-Jun 1)

Day 1

- Agarose Gel Electrophoresis of DNA products
- Polymerase Chain Reaction of *gfp*

Day 2

- Analysis of PCR products
- Purification of *gfp* PCR Product
- gfp* Quantification

Week 4 (June 7-8)

Day 1

- Preparing pET28a and *gfp* for Ligation
- Ligation of *gfp* into pET28a

Day 2

- Transformation of Ligation Reactions into *E. coli* DH5 α
- PCR Primer Design

Week 5 (June 14-15)

Day 1

- PCR to Determine Presence of Insert
- Isolation of pET28*gfp*

Day 2

- Isolation of pET28*gfp*
- Restriction Enzyme Digest and gel

Week 6 (June 21-22)

Day 1

Amplification of your *E. coli* gene (set up reaction)

Day 2

Isolation of His-GFP

His-GFP Purification Using Ni-NTA Column

Amplification of your *E. coli* gene (gel)

Week 7 (June 28-29)

Day 1

Amplification of your *E. coli* gene (continue until successful)

SDS-PAGE (make gels)

Day 2

SDS-PAGE and Coomassie Stain

Week 8 (Jul 5-6)

Day 1

SDS-PAGE and Western Immunoblotting

Day 2

SDS-PAGE and Western Immunoblotting

Week 9 (Jul 11-12)

Day 1

No Lab Scheduled

Day 2

No Lab Scheduled

Week 10 (Jul 19-20)

Day 1

qPCR – Isolation of DNA from a Soy sample

Day 2

qPCR – GMO detection

Week 11 (Jul 26-27)

Day 1

qPCR – RNA Isolation from *Arabidopsis*

Day 2

qPCR – *Arabidopsis* Gene Expression Assay

Week 12 (Aug 2-3)

Day 1 No Lab Scheduled

Day 2 No Lab Scheduled

Key Dates

May 16: Web exercise assignment
June 1: Literature Review
June 7: Progress Report #1
June 14: Test #1
June 14: Progress Report #2
June 21: Progress Report #3
July 5: Progress Report #4
July 8: 40th class day--Last day to drop the course
July 19: Progress Report #5
July 20: PCR Report
July 26: Test #2
July 28: Lab Report
August 3: qPCR GMO Assignment
August 3: qPCR *Arabidopsis* Assignment

METHODS OF ASSESSMENT

Assessment				
Form of Assessment	Weight of Assessment (% of final grade)	Due Date of Assessment	Course Content /Activity	Learning Outcome (see above)
Web-based Assignment	3%	May 18	Lab Weeks 1-11	3 and 5
Literature Review*	10%	June 1	Lab Weeks 1-11	1 and 5
Progress Reports (5)	20% (4% each)	Various (see key dates)	Lab Weeks 1-9	2, 3 and 4
Lab Report*	10%	July 28	Lab Weeks 1-9	1, 2, 3, 4 and 5
PCR Report*	4%	July 20	Lab Weeks 4-11	1, 2, 3 and 4
GMO Assign.*	4%	August 3	Lab Week 10	1, 2, 3, 4 and 5
<i>Arabidopsis</i> Assign.*	4%	August 3	Lab Week 11	1, 2, 3, 4 and 5
Lab Performance	15%	May 24 – July 27	Lab Weeks 2-11	2, 3 and 4
Test 1	15%	June 14	Lectures and Labs weeks 1-4	1, 2, 3 and 4
Test 2	15%	July 26	Lectures and Labs weeks 1-11	1,2,3, and 4

You will receive information for the week 1 Web-based assignment in the lab.

The three later Assignments are due at 1:30pm the scheduled dates above. Late assignments will be accepted without penalty only for medical or compassionate reasons with documentation.

Late assignments without documentation will be penalized 10% per day up to 50%. A grade of zero is assigned after 5 days late.

*Assignments must be typed, double-spaced, 12-point font.

Progress Reports are submitted on your first lab day of the week and are returned on the second lab day of the week. This is to provide you with immediate feedback as to whether your analysis, interpretation and conclusion of your experimental results are correct. Late Progress Reports will not be accepted. If medical or compassionate reasons are presented, the grade will be redistributed to the other Progress Reports.

All assignments must be completed or a grade of “INC” (incomplete) will be reported to the registrar.

The lab performance grade is determined by your performance in the lab. Of this, 10% is based on your actual results (success of your experiments). The other 5% is based on your day to day performance in the lab: punctuality, attendance, attitude, preparedness, independence etc.

Test 1 and 2 will be held during regular lecture time; if you fail to write Test 1 a grade of 0% will be assigned unless academic consideration is granted, in which case Test 2 will be adjusted to 30%. For missed Test 2 an Incomplete Grade will be submitted with a recommendation of 0% unless academic consideration is granted for a deferred exam.

COURSE AND UNIVERSITY POLICIES

You must come to lab prepared and ready to start working by 1:30

It is disrespectful to arrive late as this interrupts the TA, your partner and your fellow classmates. Additionally you will miss out on specific announcements for the day that the TA is not obligated to repeat. If you miss specific safety announcements you may be asked to leave. During the course of the lab there may be times where you can get a coffee as you have a gel running. Feel free to do so, however, if any announcements or discussions take place during your absence you will be responsible for obtaining the information from a fellow classmate.

Lab Attendance is mandatory

This is a **lab based** course where the majority of your final grade is assigned based on the laboratory component rather than the lecture component of the course. The lab exercises build on one another. As such, there is no opportunity for make-up labs. Lab absence is only acceptable for medical or compassionate reasons.

You must keep a lab notebook

Before coming to lab you must record in your lab notebook: What are you doing in lab today? What are the expected results? You must have completed all calculations that are required to carry out the experiments.

Additionally you should record the variables of the experiment (reaction conditions), insert the actual results you obtained, in table format or gel image (labelled) and a statement of whether or not the experiment was successful. Your lab notebook will be graded for the PCR assignment.

When You Cannot Meet a Course Requirement

When you find yourself unable to meet an in-course requirement because of illness or compassionate reasons, please advise the course instructor (or designated person, such as a teaching assistant) in writing, with your name, id#, and e-mail contact. See the undergraduate calendar for information on regulations and procedures for Academic Consideration:

<http://www.uoguelph.ca/registrar/calendars/undergraduate/current/c08/c08-ac.shtml>

Accessibility

The University of Guelph is committed to creating a barrier-free environment. Providing services for students is a shared responsibility among students, faculty and administrators. This relationship is based on respect of individual rights, the dignity of the individual and the University community's shared commitment to an open and supportive learning environment. Students requiring service or accommodation, whether due to an identified, ongoing disability or a short-term disability should contact the Centre for Students with Disabilities as soon as possible.

For more information, contact CSD at 519-824-4120 ext. 56208 or email csd@uoguelph.ca or see the website: <http://www.uoguelph.ca/csd/>

Academic Misconduct

It is the nature of undergraduate labs to complete experiments with a partner. Your results should be discussed with your partner as this is expected in all scientific research. However, **ALL** assignments must be completed **INDEPENDENTLY**.

The University of Guelph is committed to upholding the highest standards of academic integrity and it is the responsibility of all members of the University community – faculty, staff, and students – to be aware of what constitutes academic misconduct and to do as much as possible to prevent academic offences from occurring. University of Guelph students have the responsibility of abiding by the University's policy on academic misconduct regardless of their location of study; faculty, staff and students have the responsibility of supporting an environment that discourages misconduct. Students need to remain aware that instructors have access to and the right to use electronic and other means of detection.

Please note: Whether or not a student intended to commit academic misconduct is not relevant for a finding of guilt. Hurried or careless submission of assignments does not excuse students from responsibility for verifying the academic integrity of their work before

submitting it. Students who are in any doubt as to whether an action on their part could be construed as an academic offence should consult with a faculty member or faculty advisor.

The Academic Misconduct Policy is detailed in the Undergraduate Calendar: <http://www.uoguelph.ca/registrar/calendars/undergraduate/current/c08/c08-amisconduct.shtml>

E-mail Communication

As per university regulations, all students are required to check their <uoguelph.ca> e-mail account regularly. E-mail is the official route of communication between the University and its students.

Drop Date

The last date to drop one-semester courses, without academic penalty, is the 40th class day. To confirm the actual date, please see the schedule of dates in the Undergraduate Calendar. For regulations and procedures for Dropping Courses, see the Undergraduate Calendar:

<http://www.uoguelph.ca/registrar/calendars/undergraduate/current/c08/c08-drop.shtml>

Copies of out-of-class assignments

Keep paper and/or other reliable back-up copies of all out-of-class assignments: you may be asked to resubmit work at any time.

Recording of Materials

Presentations which are made in relation to course work—including lectures—cannot be recorded or copied without the permission of the presenter, whether the instructor, a classmate or guest lecturer. Material recorded with permission is restricted to use for that course unless further permission is granted.

Resources

The Academic Calendars are the source of information about the University of Guelph's procedures, policies and regulations which apply to undergraduate, graduate and diploma programs:

<http://www.uoguelph.ca/registrar/calendars/index.cfm?index>

Grading

All assignments are due at 1:30pm and a hard copy is to be submitted in lab.

Students who wish to have their assignments re-graded must submit them to the Lab Demonstrator within 5 class days of their return. The entire assignment will be re-graded so the mark may go up, down or remain unchanged.

Turnitin

In this course, your instructor will be using Turnitin, integrated with the CourseLink Dropbox tool, to detect possible plagiarism, unauthorized collaboration or copying as part of the ongoing efforts to maintain academic integrity at the University of Guelph.

All submitted assignments will be included as source documents in the Turnitin.com reference database solely for the purpose of detecting plagiarism of such papers. Use of the Turnitin.com service is subject to the Usage Policy posted on the Turnitin.com site.

A major benefit of using Turnitin is that students will be able to educate and empower themselves in preventing academic misconduct. In this course, you may screen your own assignments through Turnitin as many times as you wish before the due date. You will be able to see and print reports that show you exactly where you have properly and improperly referenced the outside sources and materials in your assignment.

Note: The final copy has to be a hard copy.

CAMPUS RESOURCES

If you are concerned about any aspect of your academic program:

- make an appointment with a program counsellor in your degree program.
<http://www.bsc.uoguelph.ca/index.shtml> or
<https://www.uoguelph.ca/uaic/programcounsellors>

If you are struggling to succeed academically:

- There are numerous academic resources offered by the Learning Commons including, Supported Learning Groups for a variety of courses, workshops related to time management, taking multiple choice exams, and general study skills. You can also set up individualized appointments with a learning specialist.
<http://www.learningcommons.uoguelph.ca/>

If you are struggling with personal or health issues:

- Counselling services offers individualized appointments to help students work through personal struggles that may be impacting their academic performance.
<https://www.uoguelph.ca/counselling/>
- Student Health Services is located on campus and is available to provide medical attention.
<https://www.uoguelph.ca/studenthealthservices/clinic>

- For support related to stress and anxiety, besides Health Services and Counselling Services, Kathy Somers runs training workshops and one-on-one sessions related to stress management and high performance situations. <http://www.uoquelfh.ca/~ksomers/>

If you have a documented disability or think you may have a disability:

The Centre for Students with Disabilities (CSD) can provide services and support for students with a documented learning or physical disability. They can also provide information about how to be tested for a learning disability. For more information, including how to register with the centre please see: <https://www.uoquelfh.ca/csd/>