

**MBG\*3350**  
**Laboratory Methods in Molecular Biology**  
**Summer 2017**

**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  
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Ext. 53833  
Email: [djosephy@uoguelph.ca](mailto:djosephy@uoguelph.ca)

**LAB  
COORDINATOR:** Amanda van der Vinne  
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Ext: 56189  
Email: [avander@uoguelph.ca](mailto:avander@uoguelph.ca)

## **COURSE SCHEDULE**

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

**Lecture:** Tuesday  
11:30am – 12:50pm  
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 4481** 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 11:30am – 12:50pm

### 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*)

MBG\*3350 Summer 2017; Lectures: Tuesday, 11:30-12:50

Class	Date	Topic
1	2017-05-16	intro. to nucleic acid structure, properties; plasmids; restriction enzymes; PCR
2	2017-05-23	DNA analysis: spectrophotometry; electrophoresis
3	2017-05-30	plasmid "mini-preps"; transformation; molecular cloning
4	2017-06-06	restriction enzymes; DNA polymerase
5	2017-06-13	plasmid vectors
6	2017-06-20	Class test 1; presentation about 4th-year projects
7	2017-06-27	protein expression in E. coli; presentation about graduate studies
8	2017-07-04	protein purification; electrophoresis
9	2017-07-11	SDS-PAGE; Western blotting
10	2017-07-18	PCR methods (in more detail)
11	2017-07-25	qPCR; RT-PCR
12	2017-08-01	Class test 2

## Laboratory Schedule

### Week 1 (May 16-17)

#### 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 23-24)

#### 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 30-31)

#### Day 1

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

#### Day 2 – Lit Review Due

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

### Week 4 (June 6-7)

#### Day 1 – PR#1 Due

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

#### Day 2

- Transformation of Ligation Reactions into *E. coli* DH5 $\alpha$
- PCR Primer Design

### Week 5 (June 13-14)

#### Day 1 – PR #2 Due

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

#### Day 2

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

**Week 6 (June 20-21)**

Day 1 – PR#3 Due

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 27-28)**

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 4-5)**

Day 1

No Lab Scheduled

Day 2

No Lab Scheduled

**Week 9 (Jul 11-12)**

Day 1 – PR#4 Due

SDS-PAGE and Western Immunoblotting

Day 2

SDS-PAGE and Western Immunoblotting

**Week 10 (Jul 18-19)**

Day 1 – PR#5 Due

qPCR – Isolation of DNA from a Soy sample

Day 2 – PCR Assign Due

qPCR – GMO detection

**Week 11 (Jul 25-26)**

Day 1

qPCR – RNA Isolation from *Arabidopsis*

Day 2 – Lab Report Due

qPCR – *Arabidopsis* Gene Expression Assay

**Week 12 (Aug 1-2)**

Day 1 No Lab Scheduled

Day 2 No Lab Scheduled – GMO Assign Due; Arabidopsis Expression Assign Due

## Key Dates

May 17: Web exercise assignment  
May 31: Literature Review  
June 6: Progress Report #1  
June 13: Progress Report #2  
June 20: Test #1  
June 20: Progress Report #3  
July 7: 40th class day--Last day to drop the course  
July 11: Progress Report #4  
July 18: Progress Report #5  
July 19: PCR Report  
July 26: Lab Report  
Aug 1: Test #2  
August 2: qPCR GMO Assignment  
August 2: 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 17	Lab Weeks 1-11	3 and 5
Literature Review*	10%	May 31	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 26	Lab Weeks 1-9	1, 2, 3, 4 and 5
PCR Report*	4%	July 19	Lab Weeks 4-11	1, 2, 3 and 4
GMO Assign.*	4%	August 2	Lab Week 10	1, 2, 3, 4 and 5
<i>Arabidopsis</i> Assign.*	4%	August 2	Lab Week 11	1, 2, 3, 4 and 5
Lab Performance	15%	May 23 – July 26	Lab Weeks 2-11	2, 3 and 4
Test 1	15%	June 20	Lectures and Labs weeks 1-5	1, 2, 3 and 4
Test 2	15%	Aug 1	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.

All assignments and reports (with the exception of the web-based assignment) will be submitted electronically to the Dropbox on Courselink and are due by 1:30pm on the assigned dates (listed above).

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 other Assignments and Reports 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.

**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](mailto: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 40<sup>th</sup> 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 (with the exception of the web-based assignment) are due at 1:30pm and must be submitted electronically to the Dropbox on Courselink.

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.

## **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.uoquelp.ca/~ksomers/>

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

The Student Accessibility Services (SAS) 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://wellness.uoquelp.ca/accessibility/>