

MBG*3350
Laboratory Methods in Molecular Biology
Winter 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. A. Nassuth
Office: SSC 4459
Ext. 58787
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**LAB
DEMONSTRATOR:** Amanda van der Vinne
Office: SSC 3519
Ext: 56189
Email: avander@uoguelph.ca

COURSE SCHEDULE

Laboratory: Either Monday & Wednesday **or** Tuesday & Thursday
1:30pm – 5:20pm
SCIE 4108/4109

Lecture: Friday 1:30pm – 2:20pm
ANNU 156

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 2302** 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: Friday 1:30pm – 2:20pm ANNU 156. Note that in addition some lectures will be presented on Mo/Tue from 1:30 - 2:20pm in SSC1511 (i.e. during the first hour of the first lab periods) in weeks 1 to 3, to ensure that you receive relevant information before you do a lab.

Laboratory

Two lab sessions per week: Monday/Wednesday or Tuesday/Thursday 1:30pm – 5:20pm
SCIE 4108/4109

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.

Tentative Lecture Topics and Schedule

Week 1

Lecture 1, Jan 11/12: Course introduction; DNA, plasmids and E. coli

Lecture 2, Jan 15: Plasmid isolation, restriction enzyme digestion and gel electrophoresis

Week 2

Lecture 3, Jan 18/19: Polymerase chain reaction (PCR) principles, primer design

Lecture 4, Jan 22: PCR fragment cloning and other applications

Week 3

Lecture 5, Jan 25/26: DNA transformation into bacteria

Lecture 6, Jan 29: cloning GFP fusions and other constructs

Week 4

Lecture 7, Feb 5: Recombinant protein expression and purification

Week 5

Quiz 1 in class Feb 12, covering lectures & labs of weeks 1-4 (15%)

Combination of short answer, multiple choice and problem solving questions

Week 6 Feb 15-19 READING WEEK

Week 7

Lecture 8, Feb 26: Protein Analysis by SDS-PAGE

Week 8

Lecture 9, Mar 4: Western, Northern and Southern blotting

Week 9

Lecture 10, Mar 11: DNA isolation, quantitative PCR (qPCR) RNA isolation, Reverse Transcription Polymerase Chain Reaction (RT-PCR) and qRT-PCR, measuring and comparing gene expression levels

Week 10

Lecture 11, Mar 18: RNA isolation, Reverse Transcription Polymerase Chain Reaction (RT-PCR) and qRT-PCR, measuring and comparing gene expression levels
Trouble-Shooting PCR and qPCR; Lecture Review,

Week 11

No lecture

Week 12

Quiz # 2 in class covering lectures 1-10 & Labs 1-10 (20%)

Combination of short answer, multiple choice and problem solving questions

Week 13

No Lecture

Laboratory Schedule

Week 1 (Jan 11-15)

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 (Jan 18-22)

- Micropipetting
- Plating Cultures
- Inoculating Cultures

Day 2

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

Week 3 (Jan 25-29)

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 (Feb 1-5)

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 (Feb 8-12)

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 (Feb 15-19)

Day 1 No Lab Scheduled – READING WEEK

Day 2 No Lab Scheduled

Week 7 (Feb 22-26)

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 8 (Feb 29-Mar 4)

Day 1

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

Day 2

SDS-PAGE and Coomassie Stain

Day 1

SDS-PAGE and Western Immunoblotting

Day 2

SDS-PAGE and Western Immunoblotting

Week 10 (Mar 14-18)

Day 1

qPCR – Isolation of DNA from a Soy sample

Day 2
qPCR – GMO detection

Week 11 (Mar 21-25)

Day 1
qPCR – RNA Isolation from *Arabidopsis*
Day 2
qPCR – *Arabidopsis* Gene Expression Assay

Week 12 &13 (Mar 28-Apr 8)

Day 1 No Lab Scheduled
Day 2 No Lab Scheduled

Key Dates (where two dates are listed: the first applies to sections 1 and 2, the second applies for sections 3 and 4)

Jan 13/14: Web exercise assignment
Jan 27/28: Literature Review
Feb 1/2: Progress Report #1
Feb 8/9: Progress Report #2
Feb 12: Quiz #1
Feb 24/25: Progress Report #3
Mar 7/8: Progress Report #4
Mar 11: 40th class day--Last day to drop the course
Mar 14/15: Progress Report #5
Mar 23/24: PCR Report
Mar 30/31: Lab Report
Apr 1: Quiz #2
Apr 4/5: qPCR GMO Assignment
Apr 6/7: qPCR *Arabidopsis* Assignment

METHODS OF ASSESSMENT

Assessment				
Form of Assessment	Weight of Assessment (% of final)	Due Date of Assessment	Course Content /Activity	Learning Outcome (see above)
Web-based Assignment	3%	Jan 13/14	Lab Weeks 1-11	3 and 5
Literature Review*	10%	Jan 27/28	Lab Weeks 1-11	1 and 5
Progress Reports (5)	15% (3% each)	Various (see key dates)	Lab Weeks 1-9	2, 3 and 4
Lab Report*	10%	Mar 30/31	Lab Weeks 1-9	1, 2, 3, 4 and 5
PCR Report*	4%	Mar 23/24	Lab Weeks 4-11	1, 2, 3 and 4
GMO Assign.*	4%	Apr 4/5	Lab Week 10	1, 2, 3, 4 and 5
<i>Arabidopsis</i> Assign.*	4%	Apr 6/7	Lab Week 11	1, 2, 3, 4 and 5
Lab Performance	15%	Jan 18-Mar 25	Lab Weeks 2-11	2, 3 and 4
Quiz 1	15%	Feb 12	Lectures and Labs weeks 1-4	1, 2, 3 and 4
Quiz 2	20%	Apr 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.

The three later Assignments are due at 1:30pm on your scheduled lab day. 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.

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.

Quiz 1 and 2 will be held during regular lecture time; if you fail to write the quiz 1 a grade of 0% will be assigned unless an acceptable cause such as sickness or family emergency is documented. In the situation where academic consideration is given the Quiz 2 will be adjusted to 35%. For missed Quiz 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 nature of the lab exercises also 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.uoguelph.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.uoguelph.ca/csd/>