BIOD21H
Molecular Biology Laboratory I
Host, Vectors and Cloning
Course outline Summer 2019

Instructor:

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PHILOSOPHY: **Molecular techniques have evolved rapidly over the last 20 years**, however, the **fundamental principles remain unchanged**. This course combines the opportunity to perform commonly used basic techniques that provide the foundation for all the newly developed techniques while also introducing the newer/more advanced techniques. **Significant attention** is paid to the **principles behind the techniques** which will allow you to gain familiarity with the **techniques and most importantly gain insight into the theory behind the techniques to aid in troubleshooting**.

The goal of this course is to provide you with the tools and the background to go forward to find a technical position in a research , biotechnology or pharmaceutical laboratory or go forward into graduate school in any research field that requires the use of molecular biology techniques. In addition you will develop skills in data interpretation, experimental design and data presentation both oral and written,Moreover this course will provide the background necessary to understand the techniques used in primary research papers you discuss fourth year courses. Molecular tools are a mainstay in all biology fields. **Therefore the emphasis is on the laboratory component of this course. We attempt to mimic the experience of a fourth year research project**.

Learning outcomes:

- **Compare and** contrast types of molecular biology techniques involved in gene identification, gene expression and cell biology
- Evaluate sequencing data using a variety of bioinformatics tools
- Evaluate data sets and create appropriate hypothesis to explain experimental outcomes
- Apply molecular biology techniques to a unique student driven project
- Evaluate data in the context of primary literature
- Demonstrate strong communication skills in oral presentation and written work
- Defend hypotheses

Important:

We will be doing significant problem solving in class and therefore you will need to come prepared having read the lecture notes/textbook prior to class

Communication

I encourage you to ask questions during lecture/classwork. If you have a question about the material, whether it is lecture or laboratory material I encourage you to talk to me during the laboratories as I will be around for much of the lab period or to visit me in my office. It is not feasible to give detailed answers to questions regarding material covered in the lecture or laboratory via email. Therefore **I have an open door policy** and in addition I hold scheduled office hours.

Please use E-mail (UTORONTO ACCOUNT ONLY) when it is critical you get in touch with me, and you are unable to see me in person

Office hours:

Tuesday 10 to 11 and 2:30 to 3:15 pm
Wed 11 to noon
Thursday 1 to 2 pm
or email me and we can arrange an alternative time

AccessAbility statement:

"*Students with diverse learning styles and needs are welcome in this course. In particular, if you have a disability/health consideration that may require accommodations, please feel free to approach me and/or the AccessAbility Services Office as soon as possible.*
AccessAbility Services staff (located in Rm SW302, Science Wing) are available by appointment to assess specific needs, provide referrals and arrange appropriate accommodations 416-287-7560 or email ability@utsc.utoronto.ca. The sooner you let us know your needs the quicker we can assist you in achieving your learning goals in this course."

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Academic integrity/plagiarism

The University treats cases of cheating and plagiarism very seriously. The University of Toronto's Code of Behaviour on Academic Matters (http://www.governingcouncil.utoronto.ca/policies/behaveac.htm) outlines the behaviours that constitute academic dishonesty and the processes for addressing academic offences.
Potential offences in papers and assignments include using someone else's ideas or words without appropriate acknowledgement, submitting your own work in more than one course

without the permission of the instructor, making up sources or facts, obtaining or providing unauthorized assistance on any assignment.
On tests and exams cheating includes using or possessing unauthorized aids, looking at someone else's answers during an exam or test, misrepresenting your identity, or falsifying or altering any documentation required by the University, including (but not limited to) doctor's notes."

http://academicintegrity.utoronto.ca/ (http://www.governingcouncil.utoronto.ca/policies/behaveac.htm) outlines the behaviours that constitute academic dishonesty and the processes for addressing academic offences. Potential offences include, but are not limited to:

(source http://www.utsc.utoronto.ca/~vpdean/academic_integrity.html)

Good tutorial

http://library.acadiau.ca/sites/default/files/library/tutorials/plagiarism/

In papers and assignments:

- Using someone else's ideas or words without appropriate acknowledgement.
- Submitting your own work in more than one course without the permission of the instructor.
- Making up sources or facts.
- Obtaining or providing unauthorized assistance on any assignment.

On tests and exams:

- Using or possessing unauthorized aids
- Looking at someone else's answers during an exam or test.
- Misrepresenting your identity.

In academic work:

- Falsifying institutional documents or grades.
- Falsifying or altering any documentation required by the University, including (but not limited to) doctor's notes.

Submitted work may be requested to be submitted turnitin

- *"Normally, students will be required to submit their course essays to Turnitin.com for a review of textual similarity and detection of possible plagiarism. In doing so, students will allow their essays to be included as source documents in the Turnitin.com reference database, where they will be used solely for the purpose of detecting plagiarism. The terms that apply to the University's use of the Turnitin.com service are described on the Turnitin.com web site".*

Intellectual Property (CTSI) - http://teaching.utoronto.ca/teaching-support/course-design/developing-a-syllabus/ - *"Recording or photographing any aspect of a university course - lecture, tutorial, seminar, lab, studio, practice session, field trip etc. – without prior approval of all involved and with written approval from the instructor is not permitted. For further information on University policies, please refer to the following links for details*

Text and required materials:

Text: Introduction to Biotechnology (background information and questions are excellent)
Thieman and Palladino fourth edition 2013 Pearson

Lab manual/handouts:

- The lab manual will **be posted on-line each lab will be posted at least a week in advance on Quercus.**
- Any additional laboratory material including reference material will be posted on Querucus

Lecture material will be provided prior to the lecture. Please check Quercusfor any postings of lecture material
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students also require:

a)a lab coat (no exceptions)

b) at least one permanent marker with a fine tip (Black). The best choice is a Sanford "Sharpie" fine point or extra fine point. (I would suggest you buy yourself two markers (a fine point and extra fine point)
c) a book for recording your work (your laboratory log book). This book can be hard covered and bound, or a binder with paper added.

Course schedule:
class meets 2 days a week: Wednesday 1 to 5 pm (1-2 pm or 4-5 pm is generally used for lecture) and Thursday 2 to 5 pm. I lecture at different times during the seven hours.

On average there is 1.5 hours of lecture/class work and 5 to 6 hours of lab per week.

Attendance is mandatory. You will be carrying out a laboratory exercise every Wednesday and Thursday, starting May 8, 2019. Missing a laboratory will be equivalent **to missing a midterm**. Therefore, the **procedure for missed laboratories/ quizzes/term tests is as follows:**
A UTSC medical certificate filled in by a Medical Doctor will be required.. **Lab work cannot be made up**, but should you provide a proper medical note I will provide a makeup assignment for the missed laboratory. **If you miss the term test** contact me within 48 Hr. To write a makeup a UTSC medical note is required. However, it may **not** be the same exam
Self-declaration can be used for assignments only. No assignment will be accepted more than 5 days late

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A reminder, if you miss the final exam, I cannot give a makeup exam. You must deal with the registrar, fill out the appropriate forms to defer the exam.

What happens if you miss laboratories

- If you have **one unexcused absence** you will forfeit all of your participation grade and grades associated with that lab (5%)
- **Two unexcused absences** leads to a loss of all laboratory grades associated with those days including the lab report and all participation grades (10%)
- **Three absences regardless of the reason and you will forfeit all grades associated with the laboratory, which means you will not pass the course and you will be asked to leave the course.**

Lectures/ in class work:

BIOD21H is a laboratory course. The lecture material covered/material discussed/ problems solved will relate to the laboratory techniques carried out throughout the course. It will include in depth explanations of methodologies, the theory behind the methodology, and discussions on how to apply the methodology to studies in molecular genetics. **The time and length of lecture will vary** week to week. **The course outline gives you a general idea of the length of each lecture, but exact times may vary** . On a weekly basis I will give you an update as to any changes in the schedule for the following week. In this way you will be able to keep ahead in your reading, and will be prepared for the laboratory/lecture in the upcoming week. **Lecture is an ideal time to ask questions if you have a questions likely another student has the same question. We will be using a modification of a reverse classroom at times in the lecture period**

Laboratories:

As I have stated above the emphasis of this course is on the laboratory, and the lecture complements the laboratory. Think of this course as a supervised study course. It is your responsibility to carry out the experiments correctly and within the time frame of the laboratory schedule. **You will be graded on how you work in the laboratory, whether you are prepared, and how well you keep a log of your experiments, detailing exactly what you did and what you observed** (diagrams/tables are excellent additions). In research **you MUST have excellent notes on your daily work**, as REPRODUCIBLE DATA is an absolute must. Your mental mistakes and oversights will be reflective of how well you prepared and will be considered when you are graded. **Therefore, simply showing up to the lab will not ensure you any success in this course.** You must arrive well informed and prepared to carry out the laboratory exercises. Since each week builds on the previous week of work, you will often be preparing the materials you need for subsequent experiments. **The intent of this course is to introduce you to how you would work within a research, industry or government laboratory, where you are producing materials you need for subsequent experiments.**

Log books for laboratory

Every class you should arrive with a introduction (paragraph) written in your log book (pages should be numbered) that describes in general what your goals are for the day. This introduction will be followed by **a flow chart/outline that will diagrammatically describe how you will carry out the procedures** within the exercises. Include all relevant information (for example incubation times, volumes to use). If two experiments are ongoing then indicate within this flow chart when you might be carrying out certain steps of the various exercises. **You should be able to use at the flow chart/outline to carry out the experiment without constant referral to your manual**. This will ensure you are prepared for the laboratory and will help you formulate any questions before starting your work. **This preparation is required and** will be checked each day. We will record whether the preparation was done and to what level (unacceptable/acceptable/good/excellent).

Students that use the laboratory exercises rather than their flow chart are not functioning as a researcher and will receive a reduction in their performance grade.

- **During the course of the experiment you will record a detailed log of what you did**. Each step you carry out will be written down (use past tense). Indicate volumes used, time of incubation (write the actual times). Describe exactly what you did and what you observed. If a step was carried out by your partner and not you indicate that in your log book.

For example:
Thursday Sept 23:
1) An isolated white colony from plate number 1, containing *E. coli* strain D21-1, was

aseptically transferred to a 5 ml aliquot of sterile LB containing 100 µg/ml of ampicillin. This tube was assigned the number 1.

2) The culture was incubated at 37°C with constant shaking in a water bath shaker. The cultures were grown overnight (if you know the exact time indicate it) and placed at 4°C the next morning by the teaching technician the .

Wed Sept 29:

1)Culture tube #1 was removed from the 4°C fridge. The cells had sedimented to the bottom of the tube. The pellet was resuspended by gently tapping the tube. The cell pellet dispersed and the culture was turbid.

2) One ml of *E.coli* strain D21-1 (XL1B cells containing the plasmid) was aseptically removed from the 5 ml overnight culture and placed in a 1.5 ml microfuge tube. The remaining culture was put back into the fridge to keep as a source of culture if needed.

3) The sample was centrifuged at 1000xg for 5 min at RT(room temperature). A small cream coloured pellet was observed at the bottom of the tube. The supernatant was clear.

4) The spent media was poured off and excess media drained from the tube by inverting the tube on a paper towel for 1 min.

5) 100 µl of solution 1 was added to the cell pellet and the pellet was resuspended by agitation using a vortex at speed 6. The sample was left on ice for 5 min. 2:20 pm-2:25 pm.

6) While I waited for the sample to resuspend I labelled my tubes for subsequent steps in the procedure.

another example

1) John prepared the DNA samples for Eco RI digestion, for specific details see John's log book. I prepared the samples for Hind III digestion. See table below.

- **At the end of the experiment** there should be a summary of what you did, what you observed, and how this relates to the next experiment and most importantly DATA analysis which must be present the day following your receipt of the date
- **an inventory table** will also be kept at the back of your log book (explanation in class)

Your log book will be checked at some point during the day, usually in the first hour of lab. I or the TA will initial various pages. At the end of the year you will be assigned a final grade for your preparation, lab performance and record keeping (see mark breakdown). You will be required to keep you book up to date. You book will be graded on a regular basis therefore you can't repair the book at the end of term.

The exercises will be carried out in pairs. Remember the success you have will depend on each of you being will prepared.

per pair of students you will be provided with a set of micropipettes. These will be numbered with your group number and placed in zip lock bag or plastic container. These are your responsibility for the term. You are responsible for properly using, and storing your micropipettes.

You will be responsible for returning the empty pipet tip boxes and picking up a new box.
Therefore it is up to you to properly look after your laboratory tools. Responsible use of your supplies and equipment is critical to obtaining good results in a research setting. **Misuse of equipment is very costly and will not be tolerated.**

Grade Breakdown

Midterm (includes lecture and lab (a minimum of 15% is directly lab/applied) this is a written exam (In class Thur June 13, 2019, 2 to 4 pm-in lab) **14%**

Final Exam (minimum of 30% is lab or applied) **31%**
(cumulative): lab and lecture –written exam

Lab performance **12%**
- Includes preparation 3%
- Technical performance 4%
- laboratory log including data analysis and summaries 5%

Small assignments/presentations/data analysis/summaries/reflective practice/concept map/class participations and group work including problem solving. These may take place in both the lab and lecture **10%**

Quizzes (4 x 0.5%) **2%**

Quiz 1:	May 16	PCR and Restriction Digests
Quiz 2:	May 22	Genomic DNA isolation
Quiz 3:	June 26	RNA isolation and RT-PCR / RT-qPCR
Quiz 4:	July 4	Yeast transformation and protein expression

Assignments (4) **31%**

1) bioinformatics-Gene of interest (6%) –electronically Jun 7 11:59pm

2) formal lab report #1 (8.5%)-electronically July 5 11:59 pm

3) formal lab report #2 (8.5%)- electronically August 3 11:59 pm

4) research proposal (8%)- electronically July 19, 11:59 pm-
no more than 4 pages double spaced based on NSERC application

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*The content required for each assignment will be explained during the appropriate class. Assignments will be considered late if they are not handed in at the beginning of the class on the due date. **Late lab reports will not be accepted more than five days late with a 10% penalty a day. If you have an issue getting your lab report completed on time you will have to speak to Professor Brunt, preferably before the lab is due. All labs must be submitted electronically to Quercus**

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The general lab and lecture schedule is given below. **However, this course is designed as a research course and therefore, the order / type of experiment and the topics covered may need to be modified as the semester progresses.** Therefore, this lab schedule should be taken as a general guideline and required modifications will be communicated to the students as necessary.

Dates	Lecture Topics	Laboratory Exercises
May 8 /9	• Brief outline of course • Discuss research proposal • Bacterial growth • Introduction to Bioinformatics and PCR • Outline requirements for picking gene of interest (GOI)	**Wednesday** • Introduction to Bioinformatics • Primer design exercise **Thursday** • Practice pipetting, plating, dilutions • Inoculation of individual colonies into LB/Amp liquid media to get plasmid samples (pB, p90)
May15/16	• Continue bioinformatics and PCR • Vectors characteristics • DNA analysis • Restriction endonucleases	**Wednesday** • Plasmid purification • Set up restriction digests • PCR of known gene sequence on yeast genomic DNA (yeast hsp90, hsc90) **Thursday** • Run digests and PCR products on gels • **Quiz 1 – PCR and restriction digests** • **Discussion of GOI and submit GOI primers for ordering**

May22/23	• Basic cloning • Discussion of GOI (if necessary)	**Wednesday** • Yeast genomic DNA isolation • **Quiz 2 – Genomic DNA isolation** **Thursday** • Gel to quantify DNA and check integrity • Evaluate gel results from last week's labs and data discussion • **Due: Concept map of GOI assignment**
May29/30	• Continue basic cloning • cDNA and genomic library theory and practice • Advanced PCR techniques	**Wednesday** • PCR using student primer sequences and genomic DNA **Thursday** • Run PCR products on gel • Purification of PCR products and set up of diagnostic digests • Set up digest for vector
June5/6	• Continue Advanced PCR techniques	**Wednesday** • Test vector and PCR product digests on gel and set up ligation • **Brief oral presentation of your research proposal** (2-3 minutes each) **Thursday** • qPCR on yeast genomic DNA to test GOI • *E.coli* transformation with ligated product (may be done later) • **Due: Bioinformatics analysis of GOI-Fri June 7**

June12/13	• DNA sequencing techniques	**Wednesday** • Prepare clones for sequencing • Discuss data for lab report • Go over lab report requirements **Thursday** • **Midterm in class**
June18/19	• Reading week	
June26/27	• Finish Sequencing techniques (if necessary)	**Wednesday** • RNA isolation from Yeast under control and experimental conditions • Quantify RNA • **Quiz 3 – RNA isolation and RT-PCR / RT-qPCR** **Thursday** • RT-PCR and RT-qPCR on isolated RNA • **Class presentations for lab report 1**
July 3/4	• Protein expression and function	**Wednesday** • Run gels with PCR products • Bioinformatics analysis on sequence data • **Due: Formal lab report 1- Friday July 5 11:59 pm** **Thursday** • Finish data analysis and if necessary, transform yeast with expression vector • **Quiz 4 – Yeast transformation and protein expression**

July10/11	• Fluorescence microscopy • In-class discussion of GOI protein and its function	**Wednesday** • Set up qPCR / RT-PCR if needed • Go through RNA data analysis **Thursday** • Protein localization using fluorescence microscopy • Go over requirements for lab report 2
July 17/18	• Overview of advanced molecular biology techniques • Finish analyzing RNA and expression data • Data analysis question in-class • **Due: Research proposal –Friday July 19 11:59 pm**	
July 24/25	• Finish any lecture material • **Class presentations for lab report 2**	• Lab cleanup
July 31/August 1	• **Class discussions if necessary of material** • **Due: Notebooks and lab report 2** • **Report 2-August 3 11:59 pm**	