BIOD21H
Molecular Biology Laboratory I
Host, Vectors and Cloning
Course outline Summer 2017

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. 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**.

Important:

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

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

Accessibility:

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 Access*Ability* Services Office as soon as possible. I will work with you and Access*Ability* Services to ensure you can achieve your learning goals in this course. Enquiries are confidential. The UTSC Access*Ability* Services staff (located in S302) are available by appointment to assess specific needs, provide referrals and arrange appropriate accommodations (416) 287-7560 or ability@utsc.utoronto.ca.

Academic integrity/plagiarism (taken from code behaviour)

Academic integrity is essential to the pursuit of learning and scholarship in a university, and to ensuring that a degree from the University of Toronto is a strong signal of each student's individual academic achievement. As a result, the University treats cases of cheating and plagiarism very seriously. The University of Toronto's Code of Behaviour and academic integrity links are below:

http://academicintegrity.utoronto.ca/
(http://www.governingcouncil.utoronto.ca/policies/behaveac.htm)

behaviours that constitute academic dishonesty and the processes for addressing academic offences. Potential offences include, but are not limited to:

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,

For the laboratory:
Students require:

1. **lab coat** (no exceptions) and closed toed shoes. You will be asked to leave if you come with inappropriate attire and no lab coat: **this will also lead to a loss in associated marks**
2. safety glasses for most labs
3. a permanent black marker (Sanford :sharpie fine point)
4. a book for recording your work (your log book). This book can be hard or soft cover, or a binder.

Text and required materials:

Text: Introduction to Biotechnology (background information and questions are excellent)
Thieman and Palladino third 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.**
- Any additional laboratory material including reference material will be posted on blackboard

Lecture material will be provided prior to the lecture. Please check blackboard for any postings of lecture material
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Lecture material will come in two forms. A more detailed PDF file and a more bare bones powerpoint (when I am lecturing. The word file will help you supplement you lecture material and should be read prior to lecture. **You are responsible for what we cover in lecture and the related material in the posted the PDF files**.

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 noon to 4 pm (noon to 1 pm or 3 to 4 pm is used when I can't complete lecture during lab periods) and Thursday 2 to 5 pm. I lecture at different times during the seven hours.. On average there is two 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 4 , 2016. 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.

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
- **Two unexcused absences** leads to a loss of all laboratory grades associated with those days including the lab report and all participation grades
- **More than two unexcused absences 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.

Each pair will be provided with in :

1) Sterile pipette tips for micropipettes
   - a box (blue) for a large volume pipette (100 µl-1000 µl)
   -a box for small to mid range micropipettes (1µl to 200 µl)
2) a can containing sterile 1.5 ml micro centrifuge tubes
3) a bag of disposable gloves for each student (the size given to you will be determined in the first week). There will be enough gloves to last you the entire course. If you however use more than three pairs a day, you will run out. If this happens you will be required to buy any extra gloves you require. Going without gloves when they are required is not an option. This will introduce you to the cost of research. Extra gloves will cost 50¢ a pair.

4) racks for tubes
5) sectioned box for micro centrifuge tube storage (2 boxes each pair).
5) you will be given a variety of solutions that you will keep in your locker , fridge or freezer over the course of the labs.

- It is your responsibility to keep track of your supplies and tools

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) includes (In class Wed June 7, 2017, 1: to 3 pm) **14%**

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

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%**

Assignments (4) **31%**

1) bioinformatics (6%)

2) formal lab report #1 (8.5%)

3) formal lab report #2 (8.5%)

4) research proposal (8%)

*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 two 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 Turnitin and to the TA** as well as in hard copy. Each page must have your name as a header and you must initial each page. All assignments will be submitted to 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".

BIOD21 2017 Lab assignments and quizzes
Assignments (3) (31%)
1. **Computational analyses of amino acid sequences** provided to you (6%)
-introduced week 3 and finished outside class. You will be asked to analyze and compare two protein sequences provided to you.
Due Thursday June 1, 2017

2. Research proposal: no more than 4 pages double spaced based on NSERC application
Due: by the last day of classes (July 31th). I suggest you work towards handing it in on July 19th, 2017

2. **Construction of genomic library cloning and characterization of clones** -week 1 to 8 (8.5%)
due Wed July 12 2017

3. Expression study and sequence analysis on hsp90 (8.5%)
-weeks 6-11
due Friday July 28, 2017 with you lab books

You may be asked to hand in calculations or graphs for certain labs which will be graded as Pass/Fail and will contribute to laboratory /log data analysis grade

<u>Quizzes (4x0.5%)</u>

1. Thursday May 11, 2017 (Growth, plasmid isolation restriction digests and gel electrophoresis)

2. Wednesday May 31, 2017 (transformation library construction, probe preparation, screening of library)

3. Wednesday June 21, 2017 (Southern blot, PCR, yeast and Drosophila genomic DNA isolation)

4. Wednesday June 28, 2076 (RNA isolation, RTPCR)

Lecture and Laboratory Schedule for BIOD21H subject to change

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Date	**Quiz**	**Lecture:** **Schedule is tentative**	**Laboratory exercise**	**assignment**
May 3 May 4	-----	Lecture 12 to 2:30 pm Brief outline of course: Discuss research proposal first lecture Topic 1 and topic 2 on bacterial growth: We will do this as a class discussion. Please have read all course information and lecture material	**Appendix week 1A/1B** <u>Exercise 2</u>-inoculation of Individual colonies into LB/amp broth **Introductory laboratory** **-evaluate the results from yesterday** **Appendix week 1B:** - <u>completion of exercise 2.</u> -the completion of exercise 2 provides the cultures for Week 2 plasmid prep Appendix week 1A	-----

May10		**Start Topic 3:** vectors used/class discussion of vector characteristics ;read the material before class as well as relevant text material	**Appendix week 2A:** exercise 3- isolation of plasmid DNA (vector [pB] only and plasmid containing *Achlya* insert cDNA using rapid alkaline lysis exercise 4- quantification of plasmid DNA using gel electrophoresis: digest set up (part 1)	
May 11	Quiz 1	**Topic 3: vectors used in molecular cloning** **Topic 4:** DNA analysis: restriction endonucleases, gel electrophoresis in class discussions read topic 3 and 4	**Appendix week 2B:** exercise 4 (part 2)- load gel exercise 5 (part 1)- restriction analysis of pB and cDNA clone PCR on plasmid DNA (part 1) Exercise 5A isolation of Genomic DNA from *Achlya*	
May 17			**Appendix week 3A**: exercise 5 (part 2)-load gels for restriction digest and Exercise 5(i) PCR reactions gel Exercise 7 Part 1: EcoRI Digest of genomic DNA and pB with EcoRI for genomic library (noon to 1:30 pm) **Appendix week 3A**: exercise 6 (1:30 to 4) - programs for computer analysis of nucleotide and amino acid sequences **(bring your**	

			computers)	
May 18		Topic 5: basic cloning Brief discussion if time	- **Appendix week 3B** <u>Exercise 7 part 2</u> check digest of Achlya genomic DNA and plasmid DNA <u>Exercise 7 part 3:</u> Set up ligation data interpretation for week 2 and Week 3 class discussion come with your data, -figure legend example/group discussion QPCR software discussion	
May 24	.	**Topic 5: basic cloning/cDNA and genomic theory and practice** **Hand in a concept map of your bioinformatics assignment**	**Appendix week 4A**: <u>Exercise 7;</u> Genomic library construction: transformation of XL1B and plating	
May 25			**Appendix week 4B**: <u>exercise 8 part 1</u>-Screening of a plasmid library: colony lifts	
May 31	Quiz 2	**topic 6:** **cloning: limitations discussion**	**Appendix week 5A:** <u>exercise 9</u>-random primer labelling using DIG system <u>exercise 10 part 1</u>-colony hybridization <u>exercise A</u>:part 1Yeast	

			DNA isolation Exercise B: part 1 *Drosophila* DNA isolation	
June 2		Discuss how to write an abstract during incubations	**Appendix week 5B**: <u>exercise 10</u>- part 2 completion of colony hybridization <u>exercise 11</u>- enhanced chemiluminescence detection of positive genomic clones <u>exercise A</u>: part 2 Yeast DNA isolation exercise <u>exercise B</u>: Drosophila DNA part 2	Bioinformatic assignment due
June 7			**Appendix week 6A**: <u>exercise 12 part 1</u>- isolation of plasmid DNA from putative genomic clones : inoculation into LB/amp	Midterm after picking you positives and digests
June 8			**Appendix week 6B**: <u>Exercise 12 part 2</u> plasmid isolation and digests Exercise D: primer analysis for amplifying the ORF of yeast and Drosophila and order primers: three primer sets needed	
June 21	Quiz 3	**Topic 7:** Southern and PCR Discuss the writing of a materials and methods	**Appendix week 7A**: <u>exercise 13 part 3</u>- agarose gel analysis of restricted genomic plasmids (Southern gel) <u>Exercise C:</u> RNA	

			isolation from Achya Spores and yeast	
June 22			**Appendix week 7B**: <u>exercise 13 part 4:</u> Southern transfer <u>exercise 14 part 1</u> PCR using primers based on A.a. hsp 90 sequences to confirm cloning <u>Exercise D</u> PCR on Yeast and Drosophila to amplify the ORF Exercise 15: finish RNA	
June 28	Quiz 4	Brief presentation of your research proposal (2-3 mins each)	**Appendix week 8A**: <u>exercise 13, part 5:</u> Southern blot continued: prehybridization and hybridization <u>exercise 14 part 2 and Exercise C :</u> agarose gel analysis of PCR products from Ex 14 part 1 and QPCR on Achlya genomic DNA <u>Exercise 16 end point RT PCR</u> Discussion of data for genomic PCR	
June 29		Finish research proposal presentations	**Appendix week 8B**: <u>exercise 16 Run RT PCR and set of Q PCR</u>	

			for RNA Exercise 13 Southern hybridization: washes and ECL	

July 5			**Appendix Week 9A** Class presentations for lab report 1	
July 6		Data analysis question	**Appendix week 9B** Exercise E: Design primers to amplify Achlya and Yeast promoter region QPCR or RT PCR if needed and go through the data	
July 12		**Topic 8 : overview of advanced techniques**	**Appendix week 10A** Exercise E: PCR on Achlya and Yeast Promoter regions	Formal lab 1 (week 1 through 9)

			Go through all RNA data and sequence info	
July 13			**Appendix week 10B** Ecercise E: Run PCR on Promoter regions Class discussion of RNA data, promoter and brief discussion of bioinformatics	
July 19			**Appendix 11A** Group presentation of RNA data/class discussion	
July 20 And Jul26		Finish any lecture material Brief overview of research	Wed July 28 Clean up	
Jul 28				Lab report and books due and your research proposal Monday July 31 (your choice of handing it in earlier)