

BIOD21H
Molecular Biology Laboratory I
Host, Vectors and Cloning
Course outline Summer 2014

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 older techniques that provide the foundation for all the newly developed techniques while also introducing the newer 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 today's research that involves 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.**

I encourage you to ask questions during lecture. 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: Remember I have an open door policy in addition to set office hours when I will definitely be in my office

Tuesday 10 to 11 and 2:15 to 3:15 pm
Thursday 11am to noon
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 *AccessAbility* Services Office as soon as possible. I will work with you and *AccessAbility* Services to ensure you can achieve your learning goals in this course. Enquiries are confidential. The UTSC *AccessAbility* 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

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 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 include, but are not limited to:

(source: <http://ctl.utsc.utoronto.ca/home/integrity>)

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.

All suspected cases of academic dishonesty will be investigated following procedures outlined in the Code of Behaviour on Academic Matters. If you have questions or concerns about what constitutes appropriate academic behaviour or appropriate research and citation methods, you are expected to seek out additional information on academic integrity from myself as your instructor or from other institutional resources (see <http://www.utoronto.ca/academicintegrity/resourcesforstudents.html>)

Text and required materials:

Text: Introduction to Biotechnology
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/ data files will be provided for the lecture. Please check blackboard for any postings of lecture material

Lecture material will come in two forms. A more detailed PDF file and a more bare bones powerpoint. The word file will help you supplement your lecture material. You are responsible for what I cover in lecture and the related material in the posted the PDF files. Use the material I presented in lecture as a guide to what I emphasize.

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 (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 this seven hours.. On average there is two hours of lecture per week. Some

weeks will have only one hour if the lab schedule is very busy

Attendance is mandatory. You will be carrying out a laboratory exercise every Wednesday and Thursday, starting May . 7, 2014. 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 will **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.

- 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 required
- **More than two unexcused absences and you will forfeit all grades associated with the laboratory, which means you will not pas the course**

Lectures:

BIOD21H is a laboratory course. The lecture material covered 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.**

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. I will record whether the preparation was done and to what level (unacceptable/acceptable/good/excellent).

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 DATA analysis**
- **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 your book up to date. Your book will be graded on a regular basis.

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

Each pair will be provided with in :

- 1) Sterile pipette tips for micropipettors
 - a box (blue) for a large volume pipettor (100 µl-1000 µl)
 - a box for small to mid range micropipettors (1µl to 200 µl)
- 2) a can containing sterile 1.5 ml microcentrifuge 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 microcentrifuge 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 micropipettors. 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 micropipettors.

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 10% is directly lab/applied)

includes (In class Wed June 11, 2014, 1: to 3 pm

19%

Final Exam (minimum of 20% is lab or applied)

32%

(cumulative, for applied): lab and lecture (theory based on material since midterm)

Lab performance

11%

preparation 3%

technical performance 3%

laboratory log including data analysis and summaries 5%

Small assignments/presentations/data analysis/summaries/reflective practice/concept map. These may take place in both the lab and lecture

9%

Quizzes (4 x 1%)

4%

Assignments (3)

25%

1) bioinformatics (6.5%)

2) formal lab report #1 (9%)

3) formal lab report #2 (9.5%)

*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 lab 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 Dr. 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.**

"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".

If you wish to opt out of turnitin you must state this to Dr. Brunt in writing and submit an electron copy to Dr. Brunt

BIOD21 2014 Lab assignments and quizzes

Assignments (3) (24.5%)

1. Computational analyses of amino acid sequences provided to you (6.5%)

-introduced week 3 and finished outside class. You will be asked to analyze and compare two protein sequences provided to you.

Due Thursday June 5, 2014

2. Construction of cDNA library cloning characterization and expresion of cDNA clones

-week 1 to 7 (9%)

due Thursday July 17 2014

3. Construction of a genomic library/genomic southern and cloning and characterization of genomic clones (9.5%)

-weeks 5-12

due Tuesday August 5, 2014

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

Your log books will be looked at throughout the year. If you are not keeping up you mark will reflect this. The book will be collected on the day you hand in your final lab report just to look at the analysis of the last week of data. You can not fix missing material from previous checks

Quizzes (4x1%)

1. Thursday May 15, 2014 (Growth, plasmid isolation restriction digests and gel electrophoresis)
2. Wednesday June 4, 2014 (, cDNA library construction, probe preparation, screening of library)
3. Wednesday June 25, 2014 (Southern blot, PCR, yeast genomic DNA isolation)
4. Wednesday July 16, 2014
(Construction and screening of genomic libraries, characterization of genomic clones, RNA, RTPCR if done)

Lecture and Laboratory Schedule for BIOD21H

Date	Quiz	Lecture: Schedule is tentative	Laboratory exercise	assignment
May7	-----	Lecture 12 to 2:30 pm Brief outline of course: first lecture Topic 1 and topic 2 on bacterial growth:	Appendix week 1A/1B <u>Exercise 2</u> -inoculation of Individual colonies into LB/amp broth	-----

May 8			<p>-Introductory laboratory -evaluate the results from yesterday</p> <p>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</p>	
May14		<p>Start Topic 3: vectors used In molecular cloning Lecture time 3 to 4 pm</p>	<p>Appendix week 2A: <u>exercise 3-</u> isolation of plasmid DNA (vector [pB] only and plasmid containing <i>Achlya</i> insert cDNA using rapid alkaline lysis <u>exercise 4-</u> quantification of plasmid DNA using gel electrophoresis: digest set up (part 1)</p>	
May 15	Quiz 1	<p>Topic 3: vectors used in molecular cloning Topic 4: DNA analysis: restriction endonucleases, gel electrophoresis 3:30 to 4:30 pm</p>	<p>Appendix week 2B: <u>exercise 4 (part 2)-</u> load gel as soon as possible- no later the 12:45 pm <u>exercise 5 (part 1)-</u> restriction analysis of pB and cDNA clone (part 1) REFLECTIVE PRACTICE</p>	
May 21		<p>topic 4 Bioinformatics lecture followed by</p>	<p>Appendix week 3A: <u>exercise 5 (part 2)-</u>load gels -data interpretation for</p>	

		data analysis and figure legend exercise and introduction of concept maps using sequence analysis	week2 class discussion come with your data -figure legend example/group discussion	
May 22			Appendix week 3B: <u>exercise 6</u> - programs for computer analysis of nucleotide and amino acid sequences (computer lab) TBA	
May 28		Topic 5: basic cloning Approx 2:30 to 3:30 pm Hand in a concept map of your Bioinformatics assignment	Appendix week 4A: <u>exercise 7</u> - transformation of XL1B cells with cDNA clone using rapid method (construction of cDNA plasmid library using <i>Achlya</i> cDNA)	
May 29			Appendix week 4B: <u>exercise 8 part 1</u> - Screening of a plasmid library: colony lifts	
June 4	Quiz 2	topic 6: cDNA cloning 3 to 4 pm	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 <i>Drosophila</i> DNA isolation	
June 5		Discuss how to write an abstract	Appendix week 5B: <u>exercise 10</u> - part 2 completion of colony hybridization <u>exercise 11</u> - enhanced	Bioinformatic assignment due

			chemiluminescence detection of positive cDNA clones <u>exercise A</u> : part 2 Yeast DNA isolation exercise exercise B: Drosophila DNA part 2	
June11			Appendix week 6A: <u>exercise 12 part 1</u> - isolation of plasmid DNA from putative cDNA: inoculation into LB/amp exercise 13 part 1: Southern analysis: set up analytical digests of genomic DNA	Midterm after picking you positives
June12			Appendix week 6B: <u>Exercise 12 part 2</u> plasmid isolation and digests Exercise 13, part 2 precipitation of genomic DNA	
June 25	Quiz 3	Topic 7: Southern and PCR Approx 2 pm to 3:30 Discuss the writing of a materials and methods	Appendix week 7A: <u>exercise 13 part 3</u> - agarose gel analysis of restricted cDNA plasmids and genomic DNA (Southern gel) <u>exercise 14 part 1</u> - PCR using primers based on A.a. hsp90 cDNA exercise 15 part 1- digestion of <i>A. ambisexualis</i> genomic DNA for genomic mini-library construction	
June			Appendix week 7B:	

26			<u>exercise 13 part 4:</u> Southern transfer <u>exercise 14 part 2:</u> agarose gel analysis of PCR products from Ex 14 part 1 <u>exercise 15</u> <u>part 2</u> analysis of genomic DNA digests carried out in Exercise 15 part 1 for genomic library construction <u>exercise 15, part 3:</u> ligation of genomic DNA insert and pB vector for library construction	
July 2		Topic 7 complete 2:30 to 3:30 pm	Appendix week 8A: <u>exercise 15, part 4:</u> construction of genomic library continued: transformation of <i>E. coli</i> XL-1 Blue with recombinant vectors containing genomic DNA <u>exercise 13, part 5:</u> Southern blot continued: prehybridization and hybridization	
July 3			Appendix week 8B: <u>exercise 16 part 1:</u> Screening of a genomic library: colony lifts <u>exercise 13 part 5</u> <u>continued and part 6:</u> Southern hybridization: washes and ECL <u>exercise C RNA</u> <u>isolation from spores</u> <u>using qiagen plant kit</u>	

July 9		Topic 8 genomic cloning 3 to 4pm (tentative based on progress, may dedicate it to presentations)	Appendix week 9A: <u>exercise 16 part 2:</u> prehybridization and hybridization of colony lifts, <u>data analysis</u> <u>presentations from cDNA cloning</u>	
July 10			Appendix week 9B: <u>exercise 16, part 2</u> <u>continued and part 3:</u> colony hybridization: washes and ECL -complete RNA isolation if needed	
July 16	Quiz 4	Topic 9 : overview of advanced techniques 1 pm- 3 pm	Appendix week 10A <u>exercise 17 part 1:</u> isolation and characterization of putative positive genomic clones	
July 17			Appendix week 10B <u>exercise 17 part 2:</u> plasmid prep on putative positives <u>exercise 17 part 3:</u> restriction digests of plasmids <u>exercise 17, part 4:</u> RT-PCR analysis	Formal lab 1 (week 1 through 7)
July 23		Topic 9	Appendix week 11A <u>exercise 17 part 5-</u> agarose gel analysis of restriction digests of putative clones <u>exercise 17 part 6</u> agarose gel analysis of, Exercise C RT-PCR products <u>exercise 17, part 4:</u> PCR on genomic clones based on	

			digestion patterns	
July 24			Appendix week 11B: data analysis and PCR gel analysis: full class discussion, summarize your data	
July 30 and July 31		Topic 9	Data analysis and completion of experiments class presentations	
Aug 5				Lab report and books due