

BIOD21H3
Advanced Molecular Biology Laboratory
Course outline Fall 2014

DESIGN OF THE COURSE

BIOD21 is a laboratory and lecture course. The lectures provide essential background knowledge for molecular biologists, while the laboratory provides hands-on experience in many essential molecular biology techniques.

The protocols used in this course are those commonly used in a research lab. You will: construct, isolate and characterize cDNA clones that encode a specific protein; isolate RNA and characterize gene expression patterns; and isolate genomic DNA to identify mutations in a gene of interest.

- This course is offered by:
Professor Sonia Gazzarrini
Office hours: by appointment.
Office room: SY222 (SRB-Science Research Building)
e-mail: gazzarrini@utsc.utoronto.ca
Note: email only for important matters; questions involving detailed answers about lectures and lecture notes will be addressed during office hours.

- Laboratory TA coordinator (contact for lab related material)
Dr. Shelley Brunt
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- Laboratory TA
Rex Chiu
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- Laboratory TA
Aaron Chan
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COURSE REQUIREMENTS

A Background in microbiology, cell and molecular biology is absolutely required for this course

Exclusion: ([BGYD21H](#))

Prerequisite: [BIOB12H](#) & [BIOC15H](#) & [[BIOC17H](#) or [[IMCB01H](#) & [IMCB02H](#) (for Applied Microbiology students only)]]

Corequisite: [BIOC12H](#) (Note: Although listed as a corequisite, it is recommended that [BIOC12H](#) be taken in advance of [BIOD21H](#).)

TEXT AND REQUIRED MATERIALS

- Text: “Laboratory DNA Science”
Authors: Mark V. Bloom, Greg A. Freyer, David A. Micklos
The Benjamin/Cummings Publishing Company Inc.
- Lab handouts:
 - Data files for the laboratories will be provided on Blackboard. Please check Blackboard for any postings of laboratory material prior to each lab.
- Lecture notes:
 - Lecture notes will be provided on Blackboard prior to each lab.

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. Alternatively, a Staedtler lumocolor permanent marker (fine point). The Sanford markers are usually cheaper and can be bought at any business supply store. 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.

REFERENCE BOOKS

Copies of the books below are in the UTSC library, certain of them e.g. Alberts, *et al.* and also Lodish *et al.*, are also available at PubMed Bookshelf. These contain information relevant to the lecture material and/or laboratory procedures.

Lecture

Genes. Lewin, B. Oxford University Press (any recent edition).

Molecular Biology of the Cell. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and J.D. Watson. Garland Publishing, Inc. (any recent edition)

Molecular Cell Biology. Lodish, H., Baltimore, A. Berg, S. L. Zipursky, D., Matsudaira, P., and J. Darnell. Scientific American Books (any recent edition)

Many Cell and Molecular or Biochemistry or upper level Biology textbooks e.g. *Cell and Molecular Biology*. Karp, G. J. Wiley & Sons, Inc.

Laboratory

Molecular Cloning : A Laboratory Manual, Vol. I, II and III,
by Sambrook, J. and D.W. Russell,. Cold Spring Harbor Laboratory Press. 2001.
(highly recommended; copies are on reserve in the library)

Short Protocols in Molecular Biology. by Ausubel, F., Brent, B., Kingston, R.E., Moore, D.D. Seidman, J.G. Smith, J.A. and Struhl, K.E. J. Wiley & Sons, Inc. 1999 (or later)

Genome Analysis: A laboratory Manual, Vol. I, II and III,
by Birren, B., Green, E.D., Klapholtz, S., Myers, R.M., and Roskams, J.
Cold Spring Harbor Laboratory Press, 1999 (or later)

GRADE BREAKDOWN

Midterm Exam	25%
October 8, 2014 (in the labs); covers lecture (15%) and lab (10%) material	
Final Exam	35%
During exam period - TBA; covers lecture (20%) and lab (15%) material	
Laboratory	40%

Laboratory Grade Breakdown

A) Lab performance	11%
preparation: 3%	
technical performance: 3%	
laboratory log: 5%	
B) Quizzes (3 x 1%)	3%
C) Assignments (3)	26%
bioinformatics (5%)	
formal lab report 1 (9%)	
formal lab report 2 (8%)	
primer design (4%)	

LECTURES

The lectures are not designed to be explanations of specific laboratory exercises. Lecture notes will be posted on Blackboard prior to each lecture. Lecture topics include but are not limited to:

1. **Recombinant DNA technology.** Overview of cloning: hosts, vectors, restriction and modification enzymes.
2. **Recombinant DNA analysis.** Isolation of plasmid DNA. Restriction digest and restriction mapping. Gel electrophoresis.
3. **Cloning strategies and vectors**
4. **Construction and screening of cDNA libraries.**
5. **PCR**
6. **Isolation of genomic DNA and genotype analysis**
7. **Isolation of RNA and gene expression analysis by RT-PCR**
8. **Sequencing**

LAB ASSIGNMENTS

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. If you have an issue getting your lab report completed on time you will have to speak to your TA before the lab is due.

1. Bioinformatics: Computational analyses of amino acid sequences provided to you. Introduced week 3 and finished outside class. You will be asked to analyze and compare two protein sequences provided to you.

2. Formal lab report 1: Construction of cDNA library, cloning and characterization of cDNA clones. Weeks 2 to 7.

3. Formal lab report 2: Molecular genotyping: isolation of genomic DNA, PCR, RFLP-PCR; characterization of gene expression pattern: isolation of RNA, RT-PCR. Weeks 8 to 12.

NB: LAB reports will be electronically submitted as well as submitted as a hard copy.

4. Primer Design: Design of gene-specific and RT-PCR primers

LOG 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 Your log books will be looked at throughout the year. If you are not keeping up your mark will reflect this. The log books will be collected **the last day of class.**

QUIZZES

1. Growth, plasmid isolation and gel electrophoresis
2. cDNA library construction, probe preparation, screening of library
3. genomic DNA isolation, PCR

IMPORTANT NOTE: All written assignments in this course must be submitted electronically via Turnitin.com by the deadline for the submission. If you are unwilling to participate in the system, you must tell your instructor.

"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".

Please check Turnitin.com - Getting Started: A Guide for Students

<http://www.teaching.utoronto.ca/teaching/academicintegrity/turnitin/conditions-use.htm>

LABORATORIES

Laboratory schedule:

Laboratories meet 2 days a week: Wednesday and Thursday, 2 to 5 pm.

You will be carrying out a laboratory exercise every Wednesday and Thursday.

Attendance in laboratories is mandatory. 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. **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 or industry laboratory, where you are producing materials you need for subsequent experiments.

Missing a laboratory will be equivalent to missing a midterm. Therefore, the procedure for missed laboratories / quizzes is as follows: A UTSC medical certificate filled in by a Medical Doctor will be required. Vague notes that say “you were seen at this office” will not be sufficient. Lab work cannot be made up, but should you provide a proper medical note a makeup assignment for the missed laboratory will be provided.

Log books:

Every class you should arrive with an 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 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. It will be recorded 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 assistant.

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 D21-3 (XL1B cells containing the actin 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 - 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. 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.

Exercises

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:

The locker will contain the following items:

- 1) Sterile pipet tips for micropipettors- a box (blue) for a large volume pipettor (100 μ l- 1000 μ l)- a box for small to mid range micropipettors (1 μ l to 200 μ l)
- 2) a can (lined with tinfoil) 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. A box of 50 pairs of gloves (you have been given 36 pairs) costs between \$8.50 and \$12.00 depending on the supplier and the size of the order. Therefore you can see that in gloves alone this course spends over \$200 dollars (approximately 1/15 of the course budget). Extra gloves will cost 50¢ a pair.
- 4) racks for tubes.
- 5) sectioned box for microcentrifuge tube storage (2 boxes each pair).
- 6) you will be given a variety of solutions that you will keep in your locker, fridge or freezer over the course of the labs.

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. 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.**

The University of Toronto is dedicated to fostering an academic community in which the learning and scholarship of every member may flourish, with vigilant protection for individual human rights, and a resolute commitment to the principles of equal opportunity, equity and justice.

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. 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 STATEMENT

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: 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 your instructor or from other institutional resources (see <http://www.utoronto.ca/academicintegrity/resourcesforstudents.html>).