BIOD21H3
Advanced Molecular Biology Laboratory
Fall 2021

DESIGN OF THE COURSE

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

The protocols described in this course are those commonly used in modern research laboratories. You will learn how to: i) isolate genomic DNA, generate and characterize knock-out mutants; ii) isolate RNA and characterize gene expression patterns; iii) clone genes and DNA sequencing and iv) conduct bioinformatic analysis of DNA and protein sequences. Most importantly, you will learn how to plan and conduct experiments independently, how to interpret and troubleshoot results, and how to present and discuss scientific data.

Instructor:

Professor Sonia Gazzarrini, sonia.gazzarrini@utoronto.ca

Office hours: Online only and by appointment. Include “BIOD21” in the title and use only @utoronto.ca email. Note: email only for important matters; questions involving detailed answers about lectures and laboratories will be addressed during office hours.

Laboratory TAs:

Deka Mohamed, deka.mohamed@mail.utoronto.ca

Jasmin Patel, jas.patel@mail.utoronto.ca

Manisha Kabi, manisha.kabi@mail.utoronto.ca

Note: TAs will only reply to emails related to the lab material and only during designated office hours. Include “BIOD21” in the subject and use only @utoronto.ca account. For all other inquiries, please contact Prof. Gazzarrini.

COURSE REQUIREMENTS

A background in microbiology, genetics, 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

There is no text or lab manual for this course.
Laboratory protocols/handouts, **lecture notes** and other **course material** (data, including pictures of gels, spectrophotometer absorbances, bioinformatics files, etc.) will be posted on **Quercus**. Please check Quercus for any postings of laboratory/lecture material prior to each lab.

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

Lecture

- *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). **Highly recommended, available on PubMed bookshelf (https://www.ncbi.nlm.nih.gov/books/).**
- *Molecular Cell Biology*. Lodish, H., Baltimore, A. Berg, S. L. Zipursky, D., Matsudaira, P., and J. Darnell. Scientific American Books (any recent edition). **Highly recommended, available on PubMed bookshelf.**
- *Cell and Molecular Biology*. Karp, G. J. Wiley & Sons, Inc.

Laboratory

- *Molecular Cloning: A Laboratory Manual* (Vol. I, II and III), by Sambrook and Russell. Cold Spring Harbor Laboratory Press. 2001. **Highly recommended, copies are on reserve at the library**
- *Laboratory DNA Science*, by Bloom, Freyer, Micklos. The Benjamin/Cummings Publishing Company Inc. **Highly recommended, copies on reserve at the library**
- *Short Protocols in Molecular Biology* by Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl. Wiley & Sons, Inc. 1999 (or later)
- *Genome Analysis: A laboratory Manual* (Vol. I, II, III), by Birren, Green, Klapholtz, Myers and Roskams. Cold Spring Harbor Laboratory Press, 1999 (or later)

COURSE WORK AND GRADING

Final Exam or Take-Home Assignment (covers lecture and lab material)	**30%**
Laboratories	**70%**

Labs - Grade Breakdown:

A) Electronic Laboratory Notebooks (10 x 2%)	**20%**
B) Quizzes (4 x 2%)	**8%**
C) Assignments	**42%**

Bioinformatics, 5x2% (10%)

Oral Presentation (12%)

Primer Design (5%)

Formal Lab Report (15%)

A) Electronic Laboratory Notebook
The electronic Laboratory Notebook (eLN) will include weekly reports of the labs. They must be submitted weekly and will include:

Part A (pre-lab)
i) Introduction & Rationale. Describe the experiment and the rationale for doing it.
ii) Flow Chart. Describe how the laboratory procedures is carried out.
This will ensure you are prepared for the laboratory and will help you formulate any questions regarding the exercises. Section i) and ii) must be completed and uploaded every week before the start of the labs, and will be checked by the TA.

Part B (post-lab)
iii) Data recording. Organize the data in figures and/or tables (complete with title and figure legend). Data should be properly organized, labeled and have sufficient information that would allow an external reader to understand your experiment.
iv) Conclusion. Summarize and interpret your results. Section iii) and iv) will be started during the lab and completed at home. eLN Part B (post-lab) must be completed and uploaded every week after the labs.

B) Quizzes
Multiple choice questions and short answers. They will cover: genomic DNA isolation, T-DNA and PCR, RNA isolation and qPCR, cloning, genome editing.

C) Assignments
Details about each assignment will be explained during class. There is a 10% penalty per day for late assignments. If you can't complete the lab report on time, please speak to the

instructor BEFORE the due date. *Note: plagiarism can have serious academic consequences.*

1. **Bioinformatics.** Computational analyses of nucleotide and amino acid sequences; primer design and *in-silico* PCR; gene expression analysis and qPCR; Gateway cloning and guide RNA design. Introduced in Bioinformatics lab and finished outside class.

3. **Oral presentation (after reading week, on October 20th).** Covers weeks 1 to 5.

2. **Primer Design (after reading week, TBA).** Design of gene-specific primers and/or guide RNAs.

4. **Formal lab report.** Covers weeks 6 to 11. Due Monday, December 6th 2021.

All written assignments in this course must be submitted electronically by the deadline. Normally, students will be required to submit their course essays to the University's plagiarism detection tool 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 tool's reference database, where they will be used solely for the purpose of detecting plagiarism. The terms that apply to the University's use of this tool are described on the Centre for Teaching Support & Innovation web site https://uoft.me/pdt-faq. If you choose to opt out, let you instructor know well in advance of submitting your paper. Ideally, during the first class.

Missing a laboratory or a test

If you miss a laboratory or term work (including term tests) due to illness you must **self-declare within 48 hours via Acorn**. **Please note it is mandatory for you to fill in the notes field within the self-declaration tool on Acorn to specify what term work you are missing and applicable due dates to be considered**. For some additional instructions on how to declare illness please review the following resource https://help.acorn.utoronto.ca/blog/ufaqs/how-do-i-declare-an-absence/. If you are missing term work for another reason including: short-term illness under the care of a Physician or someone affiliated with Health and Wellness, disability reasons, a family death, vehicle accident, essential travel that is not vacation related, or varsity activities must e-mail the course instructor **and** Jennifer Campbell (jac.campbell@utoronto.ca) in advance or within 48 hours of the term work due date. All documentation will be verified for authenticity by Jennifer Campbell and any accommodations (if applicable) will be determined by the course instructor. Please note that we understand that life happens and you may miss term work for valid reasons and we will help you navigate through those situations. Please remain in communication with our departmental admin office as well as your course's teaching team. Mental health resources are available for UTSC students, please visit the Health and Wellness Centre.

You will lose 4% of your grade for each missed laboratory (including the first week) without a valid reason. If you miss more than three laboratories without a valid documentation you may be dropped from the course. Students missing laboratories will be required to fill in eLN part A (prel-ab) and will be provided with data to be analysed and included in eLN

part B (post-lab).

Religious accommodation
The University has a commitment concerning accommodation for religious observances. I will make every reasonable effort to avoid scheduling tests, examinations, or other compulsory activities on religious holy days not captured by statutory holidays. According to University Policy, if you anticipate being absent from class or missing a major course activity (like a test, or in-class assignment) due to a religious observance, please let me know as early in the course as possible, and with sufficient notice (at least two to three weeks), so that we can work together to make alternate arrangements.

LECTURE TOPICS

Lectures will be presented once per week (online synchronous) on Zoom (Link will be announced on Quercus). Lecture slides will be posted on Quercus prior to each lecture. Lecture topics are listed below and may change.

Lecture Topics:

1. Isolation of genomic DNA and genotype analysis (PCR and/or PCR-RFLP)
2. Isolation of RNA and gene expression analysis (RT-PCR and/or RT-qPCR)
3. Promoter analysis by histochemical assays (transcriptional reporters)
4. Overview of cloning (recombinant DNA technology, Gateway and Golden Gate; hosts and vectors and *E. coli* transformation)
5. DNA sequencing (sequencing techniques and chromatogram analysis)
6. Generation of knockout mutants by genome editing (CRISPR-Cas9 and its applications).
7. Generation of fluorescent fusion proteins (translational reporters) for subcellular localization of proteins.

COURSE MATERIALS AND RECORDING

Notice of video recording and sharing (download and re-use prohibited)
This course uses the University's learning management system, Quercus, to post information about the course (https://q.utoronto.ca). This includes posting readings and other materials required to complete class activities and course assignments, as well as sharing important announcements and updates. Please make it a habit to log in to the site on a regular basis. This course, including your participation, will be recorded on video and will be available to students in the course for viewing remotely and after each session. Course videos and materials belong to your instructor, the University, and/or other sources depending on the specific facts of each situation, and are protected by copyright. Do not download, copy, or share any course or student materials or videos without the explicit permission of the instructor. For questions about recording and use of videos in

which you appear please contact your instructor.

LABORATORIES

A) Covid-19 self-assessment & protocol.
Beginning September 13, in order to attend University premises you must:

1. Provide proof of vaccination.
2. Complete daily health screenings via UCheck.

Those awaiting their final vaccine dose (required by October 15) or who have a University-approved exemption must submit negative rapid screening results through UCheck.

Individuals who are neither fully vaccinated nor enrolled in the rapid screening program by September 13 cannot come to any University premises, including BIOD21 labs. Covid protocols, including wearing PPE, hand sanitizing and physical distancing, will be discussed prior to and during the labs. Covid-19 protocol, checklist and videos will be posted on Quercus.

B) Laboratory schedule and attendance.
Laboratories run twice a week: Wednesdays and Thursdays 2-5 pm (SW 133, SW135 or SW 248) for a total of 6h per week. You will be carrying out a laboratory exercise (in person) or bioinformatics work (online) every Wednesday and Thursday. The first two weeks of labs will be online synchronous. A laboratory schedule will be posted on Quercus.

Attendance in laboratories is mandatory. 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. 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. Please 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.

C) Log books will be organized with the following format:

Introduction & flow chart. **Prior to the lab, every student will write an Introduction (paragraph)** in the log book that describes in general the goals/purpose for the day (include date; pages should be numbered). This introduction will be **followed by a flow chart/outline that will diagrammatically describe how the laboratory procedures** within the exercises will be carried out. Include all relevant information (for example incubation times, volumes to use). You should be able to use the flow chart/outline to carry out the experiment without constant referral to your handouts. 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 by the TA**. It will be

recorded whether the preparation was done and to what level (unacceptable / acceptable / good / excellent). At the end of the year, you will be assigned a final grade for your preparation, lab performance and record keeping (see mark breakdown). Although lab books will not be collected at the end of the course due to COVID protocols, they are required for experimental recording, writing assignments and the final exam.

Methods/procedure. **Follow the handouts, and during the course of the experiment write down on your log book each step in-detail** (use past tense). Indicate volumes used, time of incubation (write the actual times). Describe exactly what you did and what you observed.

Results. Your data/results should be properly labeled and included in Figures (eLN part B – post-lab). Label each lane of a gel and have sufficient information so that an external reader can understand your results. Each Figure will have a title and figure legend.

Discussion. Summarize and interpret your results (eLN part B – post-lab).

Examples:

Thursday Sept 23:
1) Aseptically transferred an isolated white colony from plate # 1 (*E. coli* strain D21-1) into a culture tube of LB amp (100 µg/ml).
2) Culture incubated at 37°C with constant shaking in a water bath shaker, grown overnight, and placed at 4°C the next morning by the TA.

Wed Sept 29:
1) Cells from culture tube #1 were resuspended by gently tapping.
2) 1 ml of *E. coli* strain D21-3 was aseptically transferred from the 5 ml overnight (O/N) culture in a 1.5 ml microfuge tube.
3) Sample was centrifuged at 1000xg for 5 min at RT (room temperature). A small pellet was observed at the bottom of the tube. The supernatant was clear.
4) 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. Sample was left on ice for 5 min.

D) Laboratory Material.
Each student will be provided with:
1) Sterile pipet tips for micropipettes; a box (blue) for a large volume pipettor (100 µl-1000 µl) and a box for small to mid-range micropipettes (1µl to 200 µl).
2) A container with sterile 1.5 ml microcentrifuge tubes
3) A box of disposable gloves for each student.
4) Racks for tubes.
5) Sectioned box for microcentrifuge tube storage (2 boxes each pair).
6) A variety of solutions that you will keep in your locker, fridge or freezer over the course of the labs.
7) A set of micropipettes. These will be numbered with your group number and placed in

zip lock bag. You are responsible for properly using and storing your micropipettes. 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.

EQUITY DIVERSITY AND INCLUSION

The University of Toronto is committed to equity, human rights and respect for diversity. All members of the learning environment in this course should strive to create an atmosphere of mutual respect where all members of our community can express themselves, engage with each other, and respect one another's differences. U of T does not condone discrimination or harassment against any persons or communities.

ACCESSABILITY

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 AA142, Arts and Administration Building) 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.

ACADEMIC INTEGRITY

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.