

Biology 3326G (Cell Biology Laboratory) Course Outline

1. General Course Information

Course Information Biology 3326G (Cell Biology Laboratory), Winter 2024 Lectures/Tutorials Lab sections:

List of Prerequisites

The prerequisites for this course are Biology 2290F/G, Biochemistry 2280A; a minimum mark of 70% in Biology 2382A/B.

Unless you have either the prerequisites for this course or written special permission from your Dean to enroll in it, you may be removed from this course and it will be deleted from your record. This decision may not be appealed. You will receive no adjustment to your fees in the event that you are dropped from a course for failing to have the necessary prerequisites.

2. Instructor and Contact Information

Instructor: Office hours:

Teaching Assistants: Laboratory Technician:

Students must use their Western (@uwo.ca) email addresses and include - PAGE, fluorescence staining of cellular organelles, and bioimaging. A discovery-based component of this course focuses on application of the mentioned methods to study changes in protein expression and cytoskeleton organization in cells exposed to microenvironmental stress stimuli.

Course Content and Learning Outcomes: The work in this course is divided into two modules, outlined below. **The first module** is intended to give students an understanding of and experience with the basic animal cell culture techniques and cell line characterization and authentication. **The second module** is based on application of SDS-PAGE and fluorescence microscopy methods to study protein expression and remodeling of cytoskeleton systems in cultured cells in response to microenvironmental stress stimuli. The major training objectives are:

<u>Module I. Basics of Animal Cell Culture:</u> • Examining the microscope potential (upright vs inverted; magnification; numerical aperture; resolution; field of view; working distance) • Proper alignment of a bright-field light microscope (Köhler illumination) and a phase contrast microscope • Identification of cells and cellular components on slides and in cell culture flasks • Harvesting methods for adherent cell lines • Cell counts using hemacytometer • Cell density and confluency • Test for cell viability • Monitoring morphology of animal cells in culture • Bioimaging using an inverted microscope • Chromosome spreads from monolayer cultures • Staining procedure for chromosome spreads with Giemsa and DAPI • Karyotype analysis of a cell line • Modal number of chromosomes and heteroploidy • Genetic instability in cell culture • Genomic DNA isolation • PCR assay for identifying cell lines • Agarose gel electrophoresis.

<u>Module II. Cell Stress Biology and Bioimaging:</u> • Preparation of cell lysates using RIPA buffer • Spectrophotometrical determination of protein concentration using Bradford assay • SDS-PAGE • Staining proteins in gels using Coomassie blue • Gel analysis using ImageLab software (Bio-Rad) • Fluorescence microscopy • Fluorochromes • Fluorescence microscope operation • Image capture • Direct fluorescence staining of actin microfilaments with phalloidin • Preparation of slides for fluorescence microscopy • Fixation procedure • Monoclonal and polyclonal antibody • Primary and secondary antibody • Indirect immunofluorescence staining • Blocking solution • Observation of microfilament, microtubules and intermediate filament modifications in response to stress stimuli • Bioimaging using fluorescence microscope • Northern Eclipse and ImageJ software.

- 6 Demonstrate a knowledge of animal cell culture models in cell biology.
- 6 Perform animal cell line characterization using regular light, inverted and fluorescence microscopy, karyotyping, SDS-PAGE, and PCR assay.
- 6 Isolate and quantify genomic DNA and proteins in animal cells.
- 6 Run horizontal and vertical electrophoresis to analyze biological molecules.
- 6 Prepare chromosome spreads, stain chromosomes, and analyze cell karyotype.
- 6 Stain the cytoskeleton and cell nucleus using fluorescent probes.
- 6 Operate with inverted and fluorescence microscopes and perform bioimaging of live and stained animal cellseletleuorescee mnisces in animal cene, successfuin(ni)-d[P)3(r-0j0.001 T(s)2(.)] J0 Tc 0 T

Weeks	Dates	Tutorial topics
Week 1		Orientation and the first lab overview
Week 2		Animal cell culture methods
Week 3		Authentication of animal cell lines. Karyotype analysis
Week 4		Basics of PCR
Week 5		Midterm paper discussion
Week 6		In-class Test #1
Week 7	Spring Reading Week	
Week 8		SDS-PAGE and protein analysis
Week 9		Methods of fluorescence staining in cell biology
Week 10		Data analysis; ImageJ
Week 11		Poster project requirements
Week 12		In-class Test #2
Week 13		Student's questions

Laboratory Schedule: In-person lab classes (4 h):

Weeks	Dates	Laboratory classes
Week 1		Light microscopy in cell biology
Week 2		Animal cell culture methods (trypsinization, cell counts, viability assay)
Week 3		Cell line characterization by chromosomal staining
Week 4		Genomic DNA isolation and PCR
Week 5		Agarose gel electrophoresis and gel imaging (ImageLab)
Week 6		

5. Methods of Evaluation

Click <u>here</u> for a detailed and comprehensive set of policies and regulations concerning examinations and grading. The table below outlines the University-wide grade descriptors.

A+	90-100	One could scarcely expect better from a student at this level
А	80-89	Superior work which is clearly above average
В	70-79	Good work,

with the guidelines and instructions for the preparation and peer evaluation of BIO3326F/G Poster. The poster project and presentation must be completed to pass the course.

6. Student Absences

Attendance in the lab and completion of all lab exercises is mandatory. If you miss a lab class due to a valid reason confirmed by the Dean's office, your average grade will be applied to that class an(6i1g)2(rad)1(e)-

writing at least two weeks prior to the holiday to the course instructor and/or the Academic Counselling office of their Faculty of Registration. Please consult University's list of recognized religious holidays (updated annually) at https://multiculturalcalendar.com/ecal/index.php?s=c-univwo.

Accommodation Policies

Students with disabilities are encouraged to contact Accessible Education, which provides recommendations for ac

9. Support Services

Please visit the Science & Basic Medical Sciences Academic Counselling webpage for information on adding/dropping courses, academic considerations for absences, appeals, exam conflicts, and many other academic related matters: https://www.uwo.ca/sci/counselling/

- 1. Invest in a planner or application to keep track of your courses. Populate all deadlines at the start of the term and schedule time at the start of each week to get organized and manage your time.
- 2. Make it a daily habit to log onto OWL to ensure you have seen everything posted to help you succeed in this class.
- 3. Take notes as you go through the lecture/tutorial material. Keeping handwritten notes or even notes on a regular Word document will help you learn effectively.
- 4. Connect with others. Try forming an online study group and try meeting on a weekly basis for study and peer support.
- 5. Do not be afraid to ask questions. If you are struggling with a topic, check the online discussion Forums or contact your instructor and or teaching assistant.
- 6. Reward yourself for successes. It seems easier to motivate ourselves knowing that there is something waiting for us at the end of the task.
- 7. Take advantage of office hours with your instructor.

11. Equal Opportunity and Evaluation Policy

All individuals involved in the offering of Biology 3326F/G were, at one time, undergraduate students themselves. Accordingly, your professor and your teaching assistants thoroughly understand the importance of course grades and the hard work that you will invest into this course. They are there to help you achieve your goals. We want you to do well in the course, but we also have to be fair. The university is committed to academic integrity and has high ethical and moral standards. All students will be treated equally and evaluated using the criteria presented in this course outline and their respective weights. The evaluation criteria are based strictly on actual achievement, not on effort or how hard the student tried. Claims of an excellent academic history, of attendance in the course components, or of personal issues (family, relationship, financial, etc.) cannot be used to justify a higher grade in the course because they are not criteria for evaluation. There is no extra work available for extra credit or to "make up" another grade. We do not offer any extra assignments, essays, or other work of any kind to any student. The requirement for a higher grade in order to, for example, maintain