BioS368 Cell Bio Lab – Tentative Experiment Schedule, Fall 2014

Week	Date	Topic	Lab Report Due (~1-2 weeks later)
		Section 1: Cell Culture	
Week 1	Tue 8-26	Introduction / Aliquot and Prepare Cell Medium	---
	Thr 8-28	Passaging Cells by Trypsinization (Seed cells in 10cm dishes for Cryopre.)	Passage 9-9
Week 2	Tue 9-2	Cell Count Using Hemocytometer / Trypan Blue Live-Dead Staining	Count 9-16
	Thr 9-4	Cryopreservation of Cells (Start)	
Week 3	Tue 9-9	Growth Curve (Start) / ECM & Cell Attachment (Start) (need MC3T3s!)	ECM 9-23
	Thr 9-11	ECM & Cell Attachment (End) / Cont. Growth Curve	
Week 4	Tue 9-16	Cont. Growth Curve / Thaw and plate frozen cells incl. Live-Dead stain	Cryop. 9-25
	Thr 9-18	Growth Curve (End) / Evaluate plated frozen cells (End)	Growth C. 10-2
		Section 2: Live-Cell Stains	
Week 5	Tue 9-23	Hoechst, DNA stain	Hoechst 10-9
	Thr 9-25	Acridine Orange, DNA, RNA stain	AO 10-14
Week 6	Tue 9-30	MitoTracker Green, Mitochondria stain	Mito 10-16
	Thr 10-2	LysoTracker Red, Lysosome stain	Lyso 10-21
Week 7	Tue 10-7	PACING BREAK	
	Thr 10-9	ER-Tracker blue/white, ER-stain; BODIPY-TR-ceramide, Golgi stain	ER 10-23
Week 8	Tue 10-14	Triple live cell stains (Mito/Lyso/Hoechst; Mito/Golgi/Hoechst)	---
		Section 3: Indirect Immunofluorescence	
	Thr 10-16	Phalloidin/DAPI, actin/DNA stain	Actin 10-30
Week 9	Tue 10-21	Vinculin/actin/DNA	Vinculin 11-6
	Thr 10-23	-"-	
Week 10	Tue 10-28	Microtubules, α-/γ-tubulin/DNA	MTs 11-13
	Thr 10-30	-"-	
		Section 4: Expression of fluorescent protein-tagged proteins	
Week 11	Tue 11-4	GFP/DsRed; Cx43-GFP/Cx43-DsRed	GFP 11-20
	Thr 11-6	-"-	
Week 12	Tue 11-11	Scrape-loading dye transfer (or similar)	Dye trans. 11-20
	Thr 11-13	-"-	
Week 13	Tue 11-18	Clathrin/Dynamin drugs (or similar)	Clathrin 11-27
	Thr 11-20	-"-	
Week 14	Tue 11-25	Anti-cancer drugs (or similar)	Cancer D. 12-2
	Thr 11-27	THANKSGIVING BREAK	
Week 15	Tue 12-2	Wrap-up, Q&A, oral presentation preparation, etc.	
	Thr 12-4	FINAL ORAL PRESENTATIONS	

SYLLABUS

BioS 368 | **Classroom: C108**
Cell Biology Laboratory | **Office: D-218**
Spring 2014 | **Office Phone: 610-758-5896**
Tuesday/Thursday 1:10 – 4:00 | **Office Hours: By appointment**
Instructor: Prof. Matthias M. Falk | **email: MFalk@Lehigh.edu**
Teaching Assistant: | **Charles (Chuck) Fisher (cgf211@lehigh.edu)**
Course Site location: | **https://coursesite.lehigh.edu/course/view.php?id=52805**

COURSE OBJECTIVE:

Cell Biology is an integrative field that overlaps with many other research areas such as Molecular Biology, Biochemistry, Physiology, Neuroscience, Biophysics, Mathematics, and Bioengineering. Lehigh University offers specialized laboratory courses in all of these areas.

The Cell Biology Lab will accompany the Cell Biology Lecture (BioS367 & 411) that is taught in parallel in the fall semester. This course is a pre-requisite for this lab and needs to be taken before, or at least in parallel. The lab has been designed to clearly illustrate the theory taught in the lecture ("Structure and Function of Cells") by **visualizing** cells, sub-cellular organelles/structures, proteins, and cellular processes. In the course, state-of-the-art fluorescence microscopy techniques, including imaging proteins in *living* cells, will be applied. The course will have four main sections: (1) We will learn thoroughly how to culture immortalized cell lines, (2) to stain sub-cellular structures in fixed and *living* cells using specific probes and antibodies (including double and triple color labeling), (3) to express and observe proteins tagged with fluorescent protein probes (GFP and derivatives, RFPs) in *living* cells, and (4) to interfere with cellular processes using specific drugs. The Biology Department has acquired a new cell-incubator based fluorescence microscope (Nikon BioStation TM) specifically designed to observe living cells for extended periods (days). We plan to use this new microscope system in this class as well.

Pursued experiments are not standard Cell Biology experiments available commercially in kit form, but are based on actual, unique research projects pursued in the instructor's laboratory that have been adapted to the classroom. **You will culture your own cells during the entire course and grow cells in dishes and on cover slips for experimental manipulation and microscopic observation.** All course topics are designed to give you hands-on experience in cell biological experimentation.

No exams will be given in this course. Most important is that you are highly motivated! It is imperative that you actively participate in all classes and that you write thorough lab reports of all experiments! The lab reports should allow you to do these experiments in any future research environment with consulting only minimal additional help. To learn to formulate/communicate scientific experiments/results, all experiments and results will be communicated in the format of an oral presentation (PowerPoint; one quarter of the experiments per group, one oral presentation per group).

Laboratory Sessions:

Lab sessions will be divided into two main units. In the first unit you will learn the experimental techniques mentioned above (see below for details). In the second unit we will use the learned techniques to observe and interfere with cellular behavior. Again, your motivation is what counts most; unexpected results are tolerable as long as you try to explain the outcome of your experiments in your lab reports.

EXPERIMENTAL PROCEDURES AND TENTATIVE SCHEDULE:

First course day: Tuesday, August 26, 2014

(1) Cell Culture: (about 4 weeks, Aug. 26 – Sept. 18)
Intro into cell culture: Sterile techniques, medium (components and pH indicators), cell culture flasks and dishes, incubators, measuring CO_2 concentration using a Fyrite
To start a culture from frozen stocks, counting, splitting, and seeding cells
Viability testing
Cryo-preserving cells
Potential contamination with bacteria/yeast/fungi; testing for *Mycoplasma* contamination
Coating glass cover-slips with different cell-adhesion substrates (e.g. uncoated, BSA, collagen, fibronectin, poly-L-lysine, gelatin) and its influence on cell attachment, morphology and growth
Growing cells on glass coverslips for microscopic observation

Cell types that will be grown in the course:
HeLa (Human cervix carcinoma, not contact inhibited, not polarized, negative for gap junctions)
PAECs, primary endothelial cells (positive for tight-, gap- and adherens junctions)
MC3T3, mouse osteoblast precursor cells
MDCK, polarized epithelial dog kidney cells
Others if required for certain experiments

Introduction into fluorescence microscopy techniques using high-end upright and inverted fluorescence microscopes

(2) Specific sub-cellular compartment stains for fixed and living cells (single, double, and triple stains): (about 3 weeks, Sept. 23 – Oct. 14)
Specific for nucleic acids (DNA, RNA, cell nucleus), endoplasmic reticulum (ER), Golgi apparatus, Mitochondria, acidic compartments (Lysosomes/Autophagosomes), actin filaments

Pacing Break: Oct. 6-7 (no class)

(3) Indirect immuno-fluorescence techniques using specific monoclonal and polyclonal antibodies (single, double, and triple stains): (about 2 weeks, Oct. 16 - 30)
Antibodies specific for microtubules, intermediate filaments, actin binding proteins (myosins, vinculin), tight-, adherens- and gap junctions, secretory and endocytic machinery components

(4) Transfection and expression of auto-fluorescent protein tagged proteins: (about 1 week, Nov. 4-6)
Examples include:
Connexin43-GFP/connexin43-DsRed (gap junctions), N-cadherin-GFP (adherens junctions), Tubulin-YFP (microtubules), α-actinin-GFP (actin cytoskeleton), rab9-YFP (Late Endosomes), Myosin-VI-GFP (endocytic vesicles), LC3-GFP (autophagosomes)

Selected experiments that analyze, and interfere with, specific cellular functions using the learned techniques. Will also include to image cellular processes over extended periods (days) (about 3 weeks, Nov. 11 - 25):

-- Direct cell-to-cell communication via gap junctions measured by scrape-loading dye transfer
-- Extracellular calcium and N-cadherin mutants and their influence on adherense junctions
-- Specific drugs and their influence on sub-cellular components and cellular processes (mitosis, secretion, endocytosis, etc.):
Actin – Cytochalasin D, Latrunculin A, etc.
Microtubules – Taxol, Nocodazole, etc.
Golgi – Brefeldin A
-- Clathrin-mediated endocytosis – high levels of extra-cellular sucrose, ikarugamycin treatment
-- Mitochondria morphology and function, etc.

Thanksgiving Break, Nov. 26 – 28 (no class)

Last Day, Thursday, Dec. 4: Oral Presentations of all experiments

Laboratory attire: Please wear appropriate laboratory clothing; **sandals and "Flip-Flops" are not appropriate and will NOT be tolerated** (see attached EH&S Memo). Laboratory coats will be supplied and have to be worn at all times. Gloves and protective eyeglasses are also provided and are recommended when performing sterile techniques, or handling corrosives.

Attendance: **Missed Laboratories:** As you will be working within a lab group, and individual laboratories require considerable set-up and preparation, you will be expected to attend your normal laboratory session**. Please make all efforts to attend class! If you have to miss a laboratory, please notify the instructor prior to the lab and of the reason for absence. No make-ups will be offered due to the nature of the course!**

Academic Honesty**:** Issues of academic dishonesty will be handled according to the guidelines put forth by the Lehigh Academic Honesty Committee.

Peer Collaboration**:** Students will work in groups of two to three students. However, ALL students should participate in performing the laboratory procedures. For example, everybody should get ample chance to feed, harvest and seed cells during cell culture experiments. Although ideas should be shared, each student should write her/his own lab reports.

COURSE EVALUATION:
Written or oral exams will not be conducted in this laboratory course. Performance will be evaluated from your motivation and lab conduct/participation, and the quality of your lab reports, and your group's final oral presentation. Students are expected to maintain their own cell cultures throughout the course. Each student should write her/his own lab reports. The final oral presentation should be prepared by the group and each group member should present a portion of the presentation. Please have a copy for me to share with all course students. Lab reports are due at the specified time (about 1 – 2 weeks after the lab).

Your final grade will be based on the following:

Lab Conduct/Participation	50 %
Lab reports	25 %
Oral group presentation	25 %
	100 % total

Each category will be graded on a scale from 0 to 10. Points will be added and weighted according to the percentages above. The final grade is the summary of the three categories above. Grade weights will be given according to Lehigh University's Faculty Resource Guide as follows: A (100-92), A- (91-90), B+ (89-87), B (86-82), B- (81-80), C+ (79-77), C (76-72), C- (71-70), D+ (69-67), D (66-64), D- (63-61), F (60 and less).

Laboratory Notebooks and Lab Conduct:

You should write and maintain a Lab notebook. A notebook is a diary. It is to be used to recount what happens from day to day during your project. The notebook will help you to remember how you performed the experiments and to write the lab reports. You should include dates, experimental goals, alterations made to procedures, calculations, results and analysis of results, manufacturers and concentrations of chemicals/solutions used, etc. Lab notebooks will not be evaluated, however, thorough notes are the key to good lab reports.

Lab reports:

Lab reports are due one to two weeks from the completion of the lab. You will lose 10% of your earned grade for every day that a report is late.

The goal of the lab reports is to give you experience in writing a detailed protocol of an experiment you performed. This is important to track why experiments may not have worked, to document to a co-worker what you have done, and to summarize materials and methods in a publication. For each lab report, you should prepare a **Materials** list (including manufacturers and concentrations), a **Methods** section (with a detailed step by step protocol of the procedure), a **Results** section, and a short **Discussion** section. In the Results section, you should present the data you obtained in the experiment performed to test your hypothesis. If you add figures, include figure titles and figure legends that summarize the figure/experiment, give proper units (when necessary), identify symbols, etc. In the Discussion section, evaluate your experiment. Include a brief discussion of possible reasons for any discrepancies in your data, as well as any suggestions for better ways to design or execute the experiment.

Oral Presentations:

On the last day of the course the groups will present their section of labs to the other groups in a short talk. The presentation will be about 20-30 minutes long plus time for discussion. The talk should give background information, the hypothesis, experimental results, and some discussion of the results. The oral presentations are a great way to refresh the learned material and to critically rethink the individual experiments.

Accommodations for Students with Disabilities: *If you have a disability for which you are or may be requesting accommodations, please contact both your instructor and the Office of Academic Support Services, University Center 212 (610-758-4152) as early as possible in the semester. You must have documentation from the Academic Support Services office before accommodations can be granted.*

Lehigh University endorses The Principles of Our Equitable Community (http://www4.lehigh.edu/diversity/principles). We expect each member of this class to acknowledge and practice these Principles. Respect for each other and for differing viewpoints is a vital component of the learning environment inside and outside the classroom.

Lehigh University
Environmental Health & Safety

MEMORANDUM

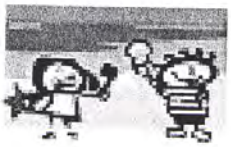
TO: Mailing List Recipients

FROM: Dr. Barbra A. Plohocki
Director, Environmental Health and Safety

RE: Proper Laboratory Attire and Children and Pets in University Buildings

Please keep in mind it is a Pennsylvania state law that eye protection must be worn in ***all*** laboratories. The laboratory supervisor has flexibility in determining the type of eyewear appropriate for the task. The issue of personal protective equipment must be addressed by all laboratory supervisors. In addition, laboratory supervisors should set an example by always wearing safety glasses in the laboratory.

There is also a concern regarding ***"proper laboratory attire"***. In many cases, graduate students wear shorts and sandals when using chemicals and other hazardous materials. This is not appropriate laboratory clothing and should not be allowed by the laboratory supervisor. I strongly encourage all laboratory supervisors to enforce the Chemical Hygiene Plan in regards to appropriate laboratory attire.



It has come to my attention that children and animals have accompanied graduate students and employees to work and have been seen in University laboratories. Under no circumstances, are children or animals permitted in research as well as undergraduate teaching laboratories.

Please call me at X83643 or e-mail me at bap2@lehigh.edu, if you have questions.

BAP:dd

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