

Biology 480L – Cell Physiology lab

Spring 2011

Tue 2:30-6:30 ST 327

Instructor: Dr. Natalie Prigozhina

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Office hours: by appointment. I'm always available by e-mail and will respond ASAP, usually within a couple of hours.

Expected Learning Objectives:

After completing Bio 480 Laboratory, a student will:

- Acquire good laboratory skills for a cell culture lab (sterile technique, use of basic lab equipment, accurate dilutions, etc.).
- Be able to maintain cell lines in culture and evaluate the health of the cells.
- Learn how to use brightfield and fluorescent microscopes to visualize and image the cells.
- Be able to design, perform and analyze cell-based experiments.
- Be able to effectively communicate scientific results in both oral and written form (presentation and lab reports), specifically learning to:
 - perform critical review of primary scientific literature to propose new experiments in a format appropriate for a short grant application;
 - articulate own observations;
 - organize and interpret own results in the context of previously published research;
 - write a clear, logical and concise manuscript in a format of primary scientific paper;
 - deliver a 40 minute PowerPoint seminar to describe the results of own research.

Materials:

- 1) Lab coat.
- 2) 1.5" or 2" binder or lab notebook.
- 3) The lab Manual for each week that will be provided weekly and posted on WebCT prior to each class.

4) WebCT: go to <http://pope.sandiego.edu> and sign in. You are expected to log in often (at least 2-3 times a week); otherwise you might miss important deadlines, announcements and discussions. The chatroom and discussion forums will be set for your convenience.

*****To make sure you don't miss any e-mails sent through WebCT, please set up e-mail forwarding!*****

Books: Required: *Essential Cell Biology, 3rd Edition*, by Alberts *et al.* 2004. Garland Science.

Strongly Recommended: *A Student Handbook for Writing in Biology, 2nd edition*, by K. Knisely (Sinauer Associates, Inc., Sunderland, MA, 2005)

Grading: Your final grade will be based on the total points achieved on all assignments and the final presentation. Grading will essentially be straight scale. There will be no exams.

** Course schedule, assignments and point totals may be changed at any time if deemed necessary by the instructor.

A	93
A-	90
B+	87
B	83
B-	80
C+	77
C	73
C-	70
D	60

Total points for the exams and assignments:

Lab Worksheets (20 points each x8)	= 160points
Lab Reports (100 points each x2)	= 200 points
Grant Proposal	= 100 points
Final Presentation	= 100 points
Lab participation	= 40 points
Total	= 600 points

Attendance and participation: Attendance is required for ALL laboratory sessions. You are expected to arrive on time and be prepared for class. This means you should have read that day's assignment in the lab manual. Please respect others by being on time for class with your cell phone turned off and stored prior to class. Computers should only be used for class purposes and not for checking your email or facebook.

Participation is expected in all facets of the course: laboratory work, assignments, class discussions, performing calculations, conducting experiments, recording data AND cleaning up at the end of the lab period. It is critical that you understand each calculation and exercise and take an active part in your group. You will also be required to take care of your cells between labs – usually on Fridays, and occasionally on Wednesdays (see lab schedule).

Team work: I generally give the same grade to all group members for their collective work. However, occasionally, I might have to assign individual grades in cases when one or two people clearly do most of the work, or when one or two people clearly contribute less than their group mates. *****Please talk to me if you have any concerns regarding your group dynamics.*****

Assignments and lab reports: a guide to writing lab reports can be found on WebCT. Read it and refer to it often throughout the semester! You will work in groups during the lab exercise and you are welcome to discuss your results with your classmates, but you must TURN IN YOUR OWN ASSIGNMENTS. This means that you must write your own text, create your own graphs, tables and figure legends. If assignments are turned in with identical text, tables or figures or only slightly altered text, you will receive a score of ZERO and additional steps may be taken according to the University's Academic Integrity Policy. The only writing assignment where team work is allowed and expected is the grant proposal.

Penalties: while you will submit the lab report and grant proposal drafts on WebCT, the final versions will be accepted only in printed form in class. All late assignments will be penalized 10% for each day overdue and will not be accepted 1 week after the due date (1 week late = ZERO). *I will not accept any assignments by e-mail => please plan your work to make sure that you have enough time to resolve all computer issues and find a working printer!*

The lab participation points may be taken off if you don't keep your work space clean, do not participate in lab activities and/or if your teammates are unhappy with your performance as a group member.

Tentative Plan --- Spring 2011

Lab	Date	Lab Topics	Due
1	Jan 25	Introduction to Cell Culture <ul style="list-style-type: none"> • Introduction • lab safety • Review dilution calculations and pipette usage • Sterile method demonstration • Learn how to use a hemacytometer • Stain epidermal cells with Giemsa-Wright • Review basic microscopy 	
2	Feb 1	Cell Culture Basics and Maintenance <ul style="list-style-type: none"> • Discussion: optimal growth conditions and possible sources of contamination • Discussion: anatomy of a primary research paper. • Practice sterile technique (make media, split/passage cells) • Set-up Experiment: Optimal Growth Conditions for Swiss 3T3 Cells 	Worksheet 1
	Friday Feb 4	Take 3 pictures of each flask Change media on (-)serum and low density pass master flask 1:5	
3	Feb 8, 2011	Cell Culture monitoring <ul style="list-style-type: none"> • Gain experience passaging and counting cells • Continue the Growth Conditions experiment • Monitor cell culture and collect data • Analyze the data to assess the health of your cultures • Introduction to Image J software for measuring cell and nuclear size 	Worksheet 2
	Friday Feb 11	Take 3 pictures of each flask Change media on (-)serum and low density pass master flask 1:5	
	Mon Feb 14	Plate cells on 2 coverslips for each group: 1 @ low density, 1 @ high density	Done by Instructor or TA or for extra credit
4	Feb 15, 2011	Monitoring Cell Culture and Intro to Fluorescent Microscopy <ul style="list-style-type: none"> • Continue the Growth Conditions experiment • Monitor cell culture and collect data • Analyze the data to assess the health of your cultures • Fix the cells on coverslips and mount in DAPI-contained media. • Learn to use a fluorescent microscope • Analyze mitotic index of DAPI-stained cells 	Worksheet 3
	Friday Feb 18	Take 3 pictures of each flask Change media on (-)serum and low density pass master flask 1:5	
	Mon Feb 22	Prepare control (2x), serum-free (2x) coverslips per group to use for LPA-treated TRITC-phalloidin-stain experiment	Done by Instructor or TA or for extra credit
5	Feb 22, 2011	Monitoring Cell Culture & Effects of LPA on actin <ul style="list-style-type: none"> • Complete the Growth Conditions experiment – final data collection and analysis • Discuss essential components of a scientific lab report • Discuss data presentation, analysis and interpretation • Treat pre-plated cells on coverslips with LPA and label with TRITC-phalloidin 	Worksheet 4
	Friday Feb 25	Pass all cells from the master flask to T75	

	Sunday Feb 27	Submit your drafts by 12pm using WebCT	Lab Report #1 draft
6	March 1, 2011	Freezing Cells & Effects of LPA on actin (cont.) <ul style="list-style-type: none"> • Gain experience freezing cell lines • Take pictures and analyze +/-LPA actin-stained slides • Discuss the lab report drafts 	Worksheet 5
	Wed March 2	Transfer frozen cells to LN	
	Mon March 7	Plate cells on cover-slips a day before (7 per group, in 3cm dishes) Scratch the the 6h +/-LPA for them at 9am	Done by Instructor or TA or for extra credit
7	March 8, 2011	Wound Healing Experiment <ul style="list-style-type: none"> • Perform wound healing experiment to study migration of cells • Fix the coverslips at different time points • Continue analyzing +/-LPA actin-stained slides • Brainstorm with your group about what you would like to investigate and why (student designed experiments) 	Lab Report #1
	March 14-18	Spring Break (no lab)	
8	March 22, 2011	Wound Healing Experiment Continued <ul style="list-style-type: none"> • Practice indirect immunolabeling of 2 structures (GA and MTs) • Utilize microscopy and image analysis to determine wound width from DAPI stained slides • Analyze data • Thaw and plate frozen cells • Keep thinking about your experiments, start writing detailed protocols! 	Worksheet 6
	Wed March 23	Assess the viability of defrosted cells Change the media or pass 1:5 Decide whether you'll need to pass them again on Fri – avoid confluency!!!	
	Sunday March 27	Submit your drafts by 12pm using WebCT	“Grant” proposal draft
9	March 29, 2011	Wound Healing Experiment Continued & Thawing of Frozen Cells <ul style="list-style-type: none"> • Monitor and maintain cell culture • Image slides immunolabeled with multiple fluorophores • Analyze data • Discuss student projects/experiments proposals 	Worksheet 7
	Friday April 1	Pass the master flask 1:5	
10	April 5, 2011	Finish Wound Healing Data Analysis & Prepare for Student Designed Experiments <ul style="list-style-type: none"> • Monitor and maintain cell culture • Finish gathering and analyzing wound healing data • Discussion: Wound healing results and presentation of data for Lab Report #2 • Begin student design experiments 	“Grant” proposal Worksheet 8
	Friday April 8	Pass the master flask 1:5	
	Sunday April 10	Submit your drafts by 12pm using WebCT	Lab Report #2 draft
11	April 12, 2011	Student designed experiments – set up and gather data Discuss Lab Report #2	
12	April 19, 2011	Student designed experiments – gather data and begin analysis	Lab Report #2
13	April 26, 2011	Student designed experiments – complete data analysis	outline of the presentation
14	May 3, 2011	Final Presentations (40 min each)	
15	May 10, 2011	No class	