BIOLOGY 3050 LAB and Bio 6090 (section 3)

COURSE: Biology 3050L and Bio 6090L (section 3), Advanced Cell Biology lab, Sp2021 (1 unit)
Wed. 6:00 – 9:30 or Thurs. 6:00 – 9:00 @ Sater Hall 120

INSTRUCTORS: Dr. Mike Dorrell (Bio3050L); Office: Rohr Science 158,
619-849-2962, mdorrell@pointloma.edu
Office Hours (most days): MW 10:00 – 12:40 or by appointment. Just email to find a time to set up a zoom meeting anytime you want help or to talk.

Dr. Helen Goodluck (Bio6090);

PLNU mission: *To Teach ~ To Shape ~ To Send*. Point Loma Nazarene University exists to provide higher education in a vital Christian community where minds are engaged and challenged, character is modeled and formed, and service becomes an expression of faith. Being of Wesleyan heritage, we aspire to be a learning community where grace is foundational, truth is pursued, and holiness is a way of life.

Course Description:
A laboratory course normally associated with Advanced Cell Biology lab. Students will learn and perform many techniques commonly used in cell biology laboratories.

Learning outcomes;

1) Students will maintain cells in culture for multiple weeks. Students will grow, passage, and prepare cells for several experimental and analysis procedures.
2) Students will perform immunocytochemistry, viability assays, and gene and protein expression analyses. Students will also know how tissue slides are prepared and stained for analysis.
3) Students will understand the difference between cell lines and primary cells.
4) Students will maintain a lab eNotebook and perform common data analysis associated with cell biology experimentation.

REQUIRED TEXTS: There are no texts required for this laboratory course. All lab handouts have been prepared by the professors and are available on canvas.

FINAL EXAMINATION POLICY – There is no final examination for this laboratory course. Major projects include maintaining a lab notebook, lab reports, and data analysis. Further information can be found below.

Lab Schedule

Date	Lab activity	Text	Due / reminders
3-3	**Tissue culture of L-cells / Introduction to chamber slides and cell counting.**	**Read: Tissue culture of L-cells and chamber slides protocol;** Quiz on handout	
3-10	**Staining L-cells: parts 1 and 2 (split groups)** *discuss cell viability exp.*	**Read: Staining L-cells handouts 1 and 2;** Quiz on handout / previous work	
3-17	**Fluorescence and bright light microscopy – *imaging slides from week 2.***	*Sign up for a time slot (in pairs)*	
3-24	**Cell culture viability assay** *Calculations (preparation) for TGF-B1 activity assay -demonstration of fluorescence microscope*	**Read: cell viability assay handout;** Quiz on handout / previous work	***Make sure you are keeping up on the E-notebooks***
3-31 (week 5) ***(day off for undergraduates)***	**Cryosectioning mouse tissue (grad students only)**	**Read: Cryosectioning handout;** -Quiz on handout	***Everyone: set up TGF-B1 assay during the week***
4-7	**Isolating DNA and protein from the cells, including Bradford assay**	**Read: TGF-B1 handout and isolating DNA and protein handout** -Quiz on handouts	
4-14	**Start western blot analysis through transfer.**	**Western blot analysis handout** -quiz on handout	**Check cultures and then stop growing**
4-21	**Western blot analysis** **-Staining and analysis**	**Continued (Western blot analysis handout) - no quiz**	***Make sure you are keeping up on the E-notebooks***
4-28	**qPCR analysis – one-step RT-qPCR SYBR green analysis**	**qPCR lab handout** -quiz on handout	
5-5 (week 10) ***(day off for undergraduates)***	**Staining cryosectioned tissue (Wed section accomplishes through primary antibody, Thurs section does secondary ab) (grad students only)**	**Cryosectioning handout continued (part 2);** ***- due to covid, we will have to image slides using confocal and send to students for lab report***	**E-notebooks for tissue culture project due**
5-12 ***(the rest of this is optional for graduate students)***	**Hematopoietic stem cell culturing, day 1 (undergrads and interested graduate students)**	**Read: Mouse BMCs handout-1 Quiz on handout / previous work**	***Lab report for Cryosectioning and Immunohistochemistry due (graduate students)***
5-19	**HSC culturing, day 2 (undergrads and interested graduate students)**	**Read: Mouse BMCs handout-2 Quiz on handout / previous work**	
5-26	**Two –way ANOVA analysis for BMCs experiment (undergrads and interested graduate students)**	**Slides on two-way ANOVA** ***Data analysis for the BMCs lab is due 6-2-2021***	***Counting of HSC colonies must be performed and uploaded by lab today***
6-2	**No lab**		**Data analysis for BMCs lab is due**

Attendance:
Attendance at lab sessions is required and role will be taken. Absence from a lab, results in a zero for any work done on that day. Extenuating circumstances will be considered as long as the instructor is notified up front.

Laboratory Work – Brief Introduction

There will be three main laboratory projects for this course. For each of them, you will be working as part of a group of four people. Your group may split the efforts equally, but you are always expected to perform the work at least in pairs to minimize errors. **Handouts with pertinent background information and procedural details for each lab can be found on canvas.** There will be a quiz at the beginning of the lab period, which will cover the material in this introductory handout. The goal is to insure that you have put some effort into reading and digesting the handout material, so that a high quality lab experience results. At the conclusion of a body of laboratory work, you will be responsible for turning in a "summary product" for me to evaluate. ***There will be an opportunity for each student to evaluate the quality of the group experience (by evaluating yourself and other group members) as we reach major assignment "milestones" for the semester. The intent is to ensure that there is accountability within each group.***

(1) Artificial Cell Culture/Fluorescence Microscopy (40% of lab grade)

The laboratory student teams as constituted above will conduct the work involving the artificial culture of cells, and their use as subjects for microscopy. This work will stretch over several weeks. You will also be responsible for preparation of cells for use in the microscopy labs listed in this syllabus. As microscopic data accumulate (e.g. digital images) you will also be responsible for selecting a set of these and including them, with your analytical comments. I will give you a handout later on with more details on this. Grades will be based on successful maintenance of cells over the course of several weeks, success during the projects using these cells, and your electronic lab notebook that you keep throughout the project.

(2) Cryosectioning and staining of mouse heart tissue (20% of lab grade) ***(graduate students only; undergraduate students can participate in the technique if interested without having to submit the assignment)***

We will be practicing an important technique used by cell biologists to study cells within a tissue system. I will provide some mouse hearts in a cryomold and groups will prepare sections of this tissue so that we can stain them for various markers of cardiac muscle and myocardial precursor cells. Your grade will be based on the success of the cryosectioning, the success of the staining, and your individual lab report.

(3) Mouse Bone Marrow Clonal Progenitor Cell assay (Hematopoietic Stem Cells) (20% of lab grade) ***(undergraduate students only; graduate students can participate in the technique if interested without having to submit the assignment)***

You and your lab group will prepare a set of special culture dishes which will support the growth of primitive bone marrow "progenitor" cells, which are responsible for populating the marrow and peripheral blood. This is the same culture technology which is used in research to study the control of progenitor cell physiology, and to assess the growth of marrow cells from patients with hematopoietic abnormalities. As a class we will collect data from the growth of bone marrow cells under control, and an experimental treatment condition. Your grade will be slightly based on successful growth of the BMCs, quantification of the bone marrow colonies, and your electronic lab notebook (details further below).

Lab Quizes: (20% of lab grade)

There will be several short quizzes that will be given at the beginning of lab. These quizzes will cover material from the lab handout for that day to ensure that you have read the handout thoroughly and come prepared to perform the lab activities. You should come to lab prepared and knowledgeable of the experiment you are about to begin. Some of the questions will also cover activities and material from previous laboratories to ensure that you are understanding and maintaining the desired material.

Lab Participation and attitude (20% of grade)

Most of this course is based on learning from experience. Thus, it is critical that you attend each lab session, are on time, and come with a "can-do" attitude ready to learn. Mistakes are a part of science and this is the way we learn. However, you must be willing to put yourself out there by participating in all aspects of this lab

(don't just let your partners carry you), and you must be willing to ask questions when you have them. Much of your participation will also determine how well you score on other parts of lab grading, but this allows us to really give credit (and deductions, if necessary) for participation and attitude. If you put your all into the labs, this course should be an easy 'A', but you will need to put in the work. Points for this will also be derived from completing the pre-lab video EdPuzzles (watch the videos and answer the questions).

Project information

Electronic Lab Notebook (Cell culture project)

Keeping a detailed notebook procedures performed, data accumulated, and analyses of the data and subsequent next steps is critical for any researcher. Be sure to keep this updated as we go, and **make sure that you write exactly what was done and how (don't just copy and paste the protocol from the lab handout**). Things do not always go perfectly according to the protocol so you need to keep good detailed notes on exactly what was done (concentrations, timing of incubations, etc.) for both the parts that corresponded perfectly with the protocol as well as mistakes or necessary alterations. Sometimes, changes to the protocol are the reason that the lab does not work, and sometimes they turn out to be what helps a particular procedure work. You need to note exactly what was done so that you can either replicate it at some later date, or determine areas that need improvement for a protocol to work. I suggest keeping a written lab book to take notes on, followed by completing an electronic lab book. Your lab notebook should contain:

1) Introduction and purpose: A section describing the purpose (what are we trying to do, and why). Be sure to relate the lab procedure to its relevance and importance in broader cell biology experimentation and/or clinical techniques. This includes all the sub-projects such as staining, viability, western blots, qPCR, etc.

2) A link to the technique protocol (can be a formalized document with procedures) with pertinent notes about any deviations from the main protocol, hints to help you know how to do key steps, in the eNotebook. This should be one main protocol for culturing cells and then notes about the health of cells at each feeding or splitting and key deviations of the protocol (for example if you split at a higher or lower dilution, etc.)

3) Results of the lab including any images obtained pertinent to the lab (such as images of cells, images obtained from fluorescent staining, analyzed results of the viability assays, images of western blots, analysis of the qPCR data, etc.). Be sure to directly reference the images along with a thorough description of what is being observed and the relevance of this. This should include:

- images of your cells right after splitting, at mid confluency (feeding days) and at high confluency (just before splitting).
- The results from each sub-project (data graphs, images, etc. as appropriate)
    - Images from IHC and H& E stainig
    - Viability assay graphs
    - Western blot images
    - qPCR data graphs or table
- along with a brief analysis of the results and conclusions. Ex: Is the staining real? Were the results what you would expect? Why or why not? Conclusions about the IC50 of methotrexate (dose at which 50% of the cells died, analysis of western blot and qPCR data and what they show, Etc.

4) Finally, discuss possible next steps based on that data.

I would strongly suggest that your group set up a Googledocs file online so that each member can access and add to the same file as appropriate. Further information on the electronic lab notebook will be given throughout the course. **If you have any questions at all, please ask.**

Lab Report for cryosectioning.

- The **introduction** should start broad and become more specific as you go along. The analogy I like to use is that of an hourglass. Start with general information about the techniques or lab and its purpose and get

more specific as you go. For example, in the cryosectioning lab report, you should start by introducing cryosectioning and its purpose / advantage, followed by immunofluorescence, and then get more specific by discussing the tissue we are using and the markers we are staining for, and what you expect to observe.

- The **methods** section describes the procedures by which this was accomplished (***in past tense; this should always be formatted in a "blank was done" manner***). This should not be in the form of a bulleted protocol, but rather a paragraph. Avoid the first or third person (don't say "we did this", say "this was done)
- The brief **results** section should include the **labeled** images from your slides, complete with figure legends. **This section should also have a short 1-2 paragraph description of what the results show** within the body. Describe your observations thoroughly in the main paragraph and reference the figures appropriately. **The separate figure legends should briefly describe the image being shown**.
- The **discussion** should relate what you see in your images to what was expected, along with some modifications that could be made to improve the results, and future directions for similar experiments (be creative and show some understanding of the utility of this method). This is the bottom of the hourglass. You start specific describing and analyzing the meaning of the observations / results. Then put them into broader context and discuss issues / future directions, etc.

Hematopoietic Stem Cells project

We will be analyzing the results of this project. You will submit your data analysis, including statistical analyses and a brief discussion of the results and what they mean.

Points Breakdown for lab work

Pre-lab Quizzes on handouts		**20%**	
Artificial cell culture (groups of 3)		**40%**	
Quality of work	10%		
Health of cells			
Quality of staining			
Viability assay			
Western blot and qPCR			
Electronic lab book	20%		
Group participation / effort	10%		
Cryosectioning and staining		**20%**	**(Graduate students)**
Quality of work	5%		
Lab report (not a lab notebook)	15%		
Bone marrow cells in culture		**20%**	**(undergraduates)**
Quality of work	5%		
Data analysis	15%		
Lab participation and attitude		**20%**	

Includes some points from EdPuzzle pre-lab activities, assigned videos introducing techniques, etc.

Total **100%**

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PLNU ACADEMIC HONESTY POLICY

Students should demonstrate academic honesty by doing original work and by giving appropriate credit to the ideas of others. Academic dishonesty is the act of presenting information, ideas, and/or concepts as one's own when in reality they are the results of another person's creativity and effort. A faculty member who believes a situation involving academic dishonesty has been detected may assign a failing grade for that assignment or examination, or, depending on the seriousness of the offense, for the course. Faculty should follow and students may appeal using the procedure in the university Catalog. See Academic Policies for definitions of kinds of academic dishonesty and for further policy information.

PLNU ACADEMIC ACCOMMODATIONS POLICY

While all students are expected to meet the minimum standards for completion of this course as established by the instructor, students with disabilities may require academic adjustments, modifications or auxiliary aids/services. At Point Loma Nazarene University (PLNU), these students are requested to register with the Disability Resource Center (DRC), located in the Bond Academic Center. (DRC@pointloma.edu or 619-849-2486). The DRC's policies and procedures for assisting such students in the development of an appropriate academic adjustment plan (AP) allows PLNU to comply with Section 504 of the Rehabilitation Act and the Americans with Disabilities Act. Section 504 (a) prohibits discrimination against students with special needs and guarantees all qualified students equal access to and benefits of PLNU programs and activities. After the student files the required documentation, the DRC, in conjunction with the student, will develop an AP to meet that student's specific learning needs. The DRC will thereafter email the student's AP to all faculty who teach courses in which the student is enrolled each semester. The AP must be implemented in all such courses.
If students do not wish to avail themselves of some or all of the elements of their AP in a particular course, it is the responsibility of those students to notify their professor in that course. PLNU highly recommends that DRC students speak with their professors during the first two weeks of each semester about the applicability of their AP in that particular course and/or if they do not desire to take advantage of some or all of the elements of their AP in that course.

PLNU ATTENDANCE AND PARTICIPATION POLICY

Regular and punctual attendance at all classes is considered essential to optimum academic achievement. If the student is absent from more than 10 percent of class meetings, the faculty member can file a written report which may result in de-enrollment. If the absences exceed 20 percent, the student may be de-enrolled without notice until the university drop date or, after that date, receive the appropriate grade for their work and participation. See Academic Policies in the Undergraduate Academic Catalog.

FERPA POLICY

In compliance with federal law, neither PLNU student ID nor social security number should be used in publicly posted grades or returned sets of assignments without student written permission. This class will meet the federal requirements by (Note: each faculty member should choose one strategy to use: distributing all grades and papers individually; requesting and filing written student permission; or assigning each student a unique class ID number not identifiable on the alphabetic roster.). Also in compliance with FERPA, you will be the only person given information about your progress in this class unless you have designated others to receive it in the "Information Release" section of the student portal. See Policy Statements in the (undergrad/ graduate as appropriate) academic catalog.