BIOLOGY 3050 Lab

Lab Schedule

Date	Lab activity	Text	Due / reminders
8-31 **Week 1**	**Tissue culture of L-cells / Introduction to chamber slides and cell counting.**	**Read: Tissue culture of L-cells and chamber slides protocol;**	
9-7 **Week 2**	**Staining L-cells: parts 1 and 2** *Discuss TGF-B1 activity assay*	**Read: Staining L-cells handouts 1 and 2;**	
9-14 **Week 3**	**Fluorescence and bright light microscopy – *imaging slides from week 2.***	*Assigned times (in groups)* *45 mins per group* **Read: "fibrosis handout" (TGF-beta activity assay**	***Implement TGF-B1 activity assay***
9-21 **Week 4**	**Isolating protein from the cells --- BCA analysis of protein conc.** **Group 4 primary literature presentation (membranes)**	**Isolating protein handout**	***HeLa (Immortal Life of Henrietta Lacks) discussion – Part 1, 'Life'***
9-28 ***Week 5***	**Exam 1; Units 1 – 2 ; Chapter 1,3, 8-11**		
10-5 **Week 6**	**Cryosectioning mouse brains**	**Immunohistochemistry handout**	***Final check on L-cells in culture***
10-12 **Week 7**	**No lab _ Dr. D. at a conference** **Group 3 primary literature presentation (cell transport)**	***Students attend with TA and zoom the presentation with Dr. D. Potential option to pre-record the presentation instead***	***Make sure you are keeping up on the E-notebooks***
10-19 Week 8	**No lab (Fall break)**		
10-26 Week 9	**Exam II ; Unit 3; Chapters 12-13**		
11-2 **Week 10**	**Western blot analysis – electrophoresis, transfer & block** ***-during electrophoresis – block → primaries for IHC***	**Western blot analysis handout**	***Work on E-notebooks***
11-9 **Week 11**	**Completion of Western blot and immunohistochemistry -washes, secondary, and analysis** **Group 2 primary literature presentation**	**Continued (Western blot analysis and Immunohistochemistry handout)**	***Work on E-notebooks***
11-16 Week 12	**Exam III; Unit 5: Chapters 16,19**		
11-23 Week 13	**No lab --- Thanksgiving**		
11-30 **Week 14**	**Hematopoietic stem cells, day 1**	**Mouse BMCs handout-1**	**Henrietta Lacks parts 2-3 discussion**
12-7 **Week 15**	**HSC culturing, day 2** **Group 1 primary literature presentation (TRAIL)**	**Read: Mouse BMCs handout-2** **Quiz on handout / previous work**	**E-notebooks for tissue culture and IHC 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 and Grading: Your laboratory grade makes up 20% of the course grade

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 to insure that you have put some effort into reading and digesting the handout material, so that a high quality lab experience results. ***There will be an opportunity for each student to evaluate the quality of the group experience (by evaluating yourself and other group members to ensure that there is accountability within each group.***

(1) Artificial Cell Culture and associated experimets (~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 cell staining, western blot analysis of protein expression, qPCR analysis of gene expression, and cell viability. This work will stretch over several weeks. Grades will be based on successful maintenance of cells over the course of several weeks, success during the projects using these cells, your effort towards the group work (including doing your part during the labwork required outside of regular lab time and maintaining the eNotebook), your electronic lab notebook that you keep throughout the project. See detailed information for the eNotebook further below and on canvas.

(2) Mouse Bone Marrow Clonal Progenitor Cell assay (Hematopoietic Stem Cells) (~10% of lab grade)

You and your lab group will prepare a set of special culture dishes which will support the growth of primitive bone marrow derived stem cells. This is the same culture technology which is used in research to study the control of stem cell differentiation, 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 based on successful growth of the BMCs, and the quantification and analysis of the bone marrow colonies.

Lab Quizes: (~15% 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 (~15% 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. Participation by working with your group whenever required to come in outside of regular lab time will also be a HUGE part of this grade.

Henrietta Lacks Discussions (~20% of lab grade)

We will be discussing the book "The Immortal Life of Henrietta Lacks" which is a great story of the history of tissue culture and social and cultural justice topics affecting research and healthcare. Much of this will be

answering prompts on the discussion board, but we will use some lab time (usually during incubations) to discuss these key issues together as a class.

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. You may create a link to the protocols for each experiment, but **ensure that the protocols have important hints and comments that you feel would allow you to remember how to accomplish it if you were to return to the eNotebook at a later date**, and be sure to make a note where deviations from the protocol occurred. **You must note exactly what you did so that troubleshooting or repeating the experiment is possible at a later time.** 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. This should be there for the overall lab and the various sub-labs that we do (western blots, 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 **LABELED image**s 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, graphical 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 staining
    - Viability assay graphs
    - Western blot images
    - qPCR data graphs or table
- A brief analysis of the results / conclusions. Ex: Is the staining real? Did the results fit expectations? 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 the results show / mean, 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 , including the grading rubric and an example eNotebook, can be found on canvas. **Please ask if you have any questions.**

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.

PLNU COPYRIGHT POLICY

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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.