Neural Defects: BIOSC 0067 Foundations of Research

Department of Biological Sciences, University of Pittsburgh

Instructor: Dr. Nancy Kaufmann

Location: Langley A148. Lab runs synchronously, in person. There is no hybrid option.

Class time: Mondays 9:00- 11:50am We work with biological organisms (*Drosophila melanogaster*) that have their own time schedules (life cycles!). **Never schedule conflicts during our course time.**

Office hours: Tuesdays 1-2PM or by appointment (nkaufman@pitt.edu). Zoom link in Canvas.

Communication: Email me directly (nkaufman@pitt.edu). Please practice professional communication skills. Enter the topic in the "subject" and use a professional opening and include the purpose of the meeting in the email body. Include the day and time your Neural Defects lab meets. If the email contains information beneficial to your teammates, cc them in the email. *Do not send messages via Canvas.* I will post class announcements in Canvas. Please enable Canvas announcements to be sent to your email.

This lab is a true research project developed with Dr. Jeff Hildebrand.
Research objective: Identify genes which interact with the Shroom gene in the cellular process of apical constriction to form the mammalian neural tube, tubules forming in kidney and lung, and other tissue morphogenetic processes. Determine the role of Shroom and candidate proteins in development of the fruit fly wing to better understand their function in mammalian development.

Research approach: Use *Drosophila melanogaster* (fruit flies) genetically engineered to over-express ShroomB to search for genes that interact with ShroomB that may distinguish its function from ShroomA. Over-production of ShroomB protein in the developing wing makes the wing crumple. We will make genetic crosses between ShroomB over-expressing female flies and male flies with loss of function mutations in genes we want to test and then examine the F1 progeny wings for changes in the wing phenotype.

Learning objectives: To design and carryout research experiments through all parts of the scientific process including modeling hypotheses, performing careful experiments with precision and accuracy, record keeping, analyzing data, making ethical decisions, problem solving, and reporting using different modalities of science communication.

Course Materials: A PC/Mac laptop or surface computer with webcam, WiFi, Zoom App and Microsoft Office Pro365 products PowerPoint and Excel *free from Pitt*. Pitt's configuration recommendations:https://www.technology.pitt.edu/services/computer-

purchasing-students, **We have some Laptops available in an office hours room to borrow in the room- let me know if you need this.**
Canvas, Lab Archives and Fiji will be used extensively. Download the image processing program Fiji if you do not already have it https://imagej.net/Fiji.
Pitt Computer Help Desk 24/7 412-624-HELP (4357). Call if urgent.

- Face masks that fit well- over both your mouth and nose. Always carry extras.
- Paper and pen for notes, colored pencils or markers for drawing genetic and cellular models

Safety: Keep your mask on at all times, even when you talk, cough, or sneeze. During this pandemic, it is extremely important that you abide by the public health regulations , the University of Pittsburgh's health standards and guidelines, and Pitt's Health Rules. These rules have been developed to protect the health and safety of all of us. Universal face covering is required in all classrooms and in every building on campus, without exceptions, regardless of vaccination status. This means you must wear a face covering that properly covers your nose and mouth when you are in the classroom. If you do not comply, you will be asked to leave class. It is your responsibility to have the required face covering when entering a university building or classroom. For the most up-to-date information and guidance, please visit coronavirus.pitt.edu and check your Pitt email for updates before each class. **Always swipe into the building with your Pitt ID to confirm your health status to keep our community safe.**

We are scientists. This is a research lab course. Data shows the vaccine is safe. Do your part to protect our community. https://www.coronavirus.pitt.edu/healthy-community/why-get-vaccinated. Choices we all make, in and out of lab, throughout the semester will determine the success of this term. Keep masking up! KF94/K/N95 best supported by data.

- No food or drink is allowed in the lab or hallway. Step outside the building if needed.
- Pull back long hair. Do not wear clothing and accessories with dangling parts.
- Come to lab prepared, awake, and focused. Stay focused during lab.

Lapses in safety will result in you being asked to leave. Your absence will then be marked "un-excused."

If you test positive for the coronavirus, notify Pitt contact tracers that everyone in our lab section, including UTAs and instructors, is a close contact as the lab room doesn't allow physical distancing. This isn't shaming or punishment for getting sick; reporting helps everyone to prevent further spread of the virus. Reporting allows us all access to University testing and resources to help keep everyone as safe as possible. *Knowing exposures empowers everyone to make scientifically informed decisions of personal health and infection risk.*

Course work: This lab course emulates the structure of authentic research work in a Pitt faculty lab as much as possible. Just like a student in a faculty lab, you will set up experiments, collect, record and analyze data. You will present your findings in lab meetings and more formal presentations and participate in journal clubs to analyze and discuss relevant research articles.

You must participate in the first lab period in order to take this class -our research project begins Day 1. You may not add the class after missing Day 1.

Attendance and participation: Both are mandatory. ***Any unexcused absence will cause your grade to drop. If you miss two lab periods for any reason (excused or unexcused), your grade drops a full letter grade. If you miss three or more lab periods for any reason, you cannot pass the class. Do not plan absences.***

Communicate with me, and your teammates, any unforeseen emergencies before the lab period. Any missed lab for which you did not provide a written explanation before lab will cause your grade to drop except in extreme, documented circumstances. Documentations must be official to be accepted (ex. record of visitation to Student Health or hospital on day of class, quarantine request, etc.). I must receive the official documentation within one week of the missed lab period or it results in a full letter grade drop. If you miss more than two lab periods for any reason, your grade drops a full letter grade.

Punctuality: Lab time is packed. Arrive on time; do not leave early. Do NOT schedule appointments during lab. If you arrive late or leave early or are not participating fully more than twice for any reason, your grade will drop by half a letter grade.

Focus: Do NOT use any devices for non-lab work during this time. Participate the whole time.

Accountability: *You are independently responsible for understanding the research work and making up any possible missed work, regardless of whether it will be graded, as the research project builds continuously throughout the semester.*

Assignments: come directly from your lab work. You must have done the research work in order to complete and get credit for the assignment- **do not miss class**.

- **In-class work must be immediately turned in** when requested to receive any credit. **You cannot receive credit for in-class work if you were not on-time , leave early, or were absent.** (for example notebook updates, discussions, pre-lab questions)

- **Homework assignments** are due 1 hour before the start of class (unless specifically stated by the instructor, ex. final presentation slides). Lab notebook and Canvas submissions are time-stamped. Homework assignments due for the next class are listed in Canvas modules.

- **Late homework** is first docked 10%. It is docked 20% when turned in more than 12 hours, but less than 48 hours late. **Assignments more than 48 hours late will not receive credit.** When answers to questions (ex. Journal club figures, course data, etc) are discussed in class, late submissions will not be accepted.

Working individually and with peers: We encourage you to talk about our research and other science with your peers, instructors, and consult outside sources. *However*, the work you turn in must be your own, written in your own words, designed by *yourself*. **You may not copy or quote from peers, instructors, online or printed sources.** Ask me for help when you are uncertain about following these guidelines. **Plagiarism and violations of research ethics cannot be tolerated in science and in universities.**

Lab notebooks: Lab notebooks are a real-time recording of your research and essential aspects of all real research. We use Lab Archives, an electronic research notebook program. You will have assignments to set up your lab notebook, record your findings, and analyze your results. Each entry is date stamped. In recording data- Always record what YOU see, not what you hope you see. Add commentary about your analysis and how it relates to your hypothesis and information that will help other people understand your finding. You can add notes about what other people see or think about your results, but make clear who the observer is, by name. Changes to protocols must be recorded. ***You cannot start an experiment before your notebook is set up.*** **Notebooks can be graded at any time-keep them up to date, in real time.**

Research ethics: As a scientist on this research project, you must observe carefully, be skeptical and questioning, and accurately report what you actually see in your experiments. It can be tempting to report what you *hoped* to see instead of what you *actually* see, especially if your data does not support your hypothesis. Often data will NOT support your hypothesis. This is real science. Ask your peers and instructors to help you analyze your data-second opinions are important.

Lab work quality includes (but is not limited to): Doing focused work independently *and* with your team. Paying attention and asking questions during group discussions to get clarification. Getting help when you need it, not when you weren't paying attention or could have thought through the answer with your teammates. Labeling lab materials clearly with expected detail. *If your notebook is not set up, you cannot do the lab work- whether online or in person.* If you are participating online, you will direct the hands of the instructor or teaching assistant- make sure you come online to class prepared. It's ok to ask questions, but you must read the protocol, watch any required videos, and prepare you notebook as if you were going to be physically present in lab. Make use of office hours!

Grading: Final grades are calculated from work scored for points, work scored for completeness/effort, participation in discussions and lab work. Whether you join online or in the lab room, daily participation is mandatory.

Re-grade requests must be submitted in writing and provide detailed and tangible reasoning for the request. The request must be received by me within a week of the graded assignment being returned.

Grading Scale:

	93-100% A	90-92% A-
87-89% B+	83-86% B	80-82% B-
77-79% C+	73-76% C	70-72% C-
67-69% D+	63-66% D	60-62% D-
		59% and below F

Percentages are determined at the end of the semester based on earned points of graded and ungraded work throughout the semester. Assignment point breakdown as anticipated is available in Canvas Syllabus. It is subjected to change based on class needs for educational purposes and occasionally due to changes in research work or organization. Expect homework for each class and in lab assignments each lab period.

University policies:

Academic Integrity Policy: *Students in this course will be expected to comply with the University of Pittsburgh's Policy on Academic Integrity. Any student suspected of violating this obligation for any reason during the semester will be required to participate in the procedural process, initiated at the instructor level, as outlined in the University Guidelines on Academic Integrity. This may include, but is not limited to, the confiscation of the examination of any individual suspected of violating University Policy. Furthermore, no student may bring any unauthorized materials to an exam, including dictionaries and programmable calculators. To learn more about Academic Integrity, visit the Academic Integrity Guide for an overview of the topic. For hands- on practice, complete the Understanding and Avoiding Plagiarism tutorial.*

Disability Services: *If you have a disability for which you are or may be requesting an accommodation, you are encouraged to contact both your instructor and Disability Resources and Services (DRS), 140 William Pitt Union, (412) 648-7890, drsrecep@pitt.edu, (412) 228-5347 for P3 ASL users, as early as possible in the term. DRS will verify your disability and determine reasonable accommodations for this course.*

Equity, Diversity, and Inclusion: *The University of Pittsburgh does not tolerate any form of discrimination, harassment, or retaliation based on disability, race, color, religion, national origin, ancestry, genetic information, marital status, familial status, sex, age, sexual orientation, veteran status or gender identity or other factors as stated in the University's Title IX policy. The University is committed to taking prompt action to end a hostile environment that interferes with the University's mission. For more information about policies, procedures, and practices, visit the Civil Rights & Title IX Compliance web page. I ask that everyone in the class strive to help ensure that other members of this class can learn in a supportive and respectful environment. If there are instances of the aforementioned issues, please contact the Title IX Coordinator, by calling 412-648-7860, or e-mailing titleixcoordinator@pitt.edu. Reports can also be filed online. You may also choose to report this to a faculty/staff member; they are required to communicate this to the University's Office of Diversity and Inclusion. If you wish to maintain complete confidentiality, you may also contact the University Counseling Center (412-648-7930).*

Weekly plan fall 2021 8_27_21
Note: Research work and thus assignments are subject to change based on research findings and student learning needs.

Week	**Lab work overview**	**Assignments to complete 1 hours or more before next class**
1 (Aug 30-Sept 3)	Before class: Complete "Getting to Know You" Survey; Read and sign "How to Keep a Lab Notebook" assignment in Canvas which should create your lab notebook in Lab Archives. Introduction to our Neural Defects research project and *Drosophila melanogaster* as a model system. Observe live flies in vials. Syllabus overview and expectations. Expt 1: Determining the over-expression ShroomB phenotype. Step 1- Sexing and sorting of flies	Read course information and weekly plan in syllabus section of Canvas. Assignment in Canvas of research and reading to make PowerPoint slides on neural tube defects with focus on genes and environment with socioeconomic factors, current treatment options and hope for the future. Read: Sadava Life (Intro bio text book) Chapter 12.1 "Inheritance of genes follows Mendel's Laws" and 12.2 "Alleles can produce multiple phenotypes" (if you no longer have your Biology textbook, there is a PDF in the Lab Archives reading folder).
2 (Sept 6-10) Labor Week	Work on the journal club now! *Lab will not meet this week, but the homework assignment will take lots of time.*	Read: Sadava Life Chapter 12.4 "Genes are carried on chromosomes" Journal club 1 preparation- reading and assertion/evidence slides Hildebrand and Soriano, 1999: "Shroom, a PDZ Domain–Containing Actin-Binding Protein, Is Required for Neural Tube Morphogenesis in Mice" Review Expt 1 progress and write procedure for how to dissect wings and make slide in Lab Archives to prepare for Step 2.
3 (Sept 13-17)	Expt 1: Determining the over-expression ShroomB phenotype. Step 2- Dissecting wings and making microscopy slides. Fly genetics introduction. Journal club #1	Submit Expt 1. Practice wing measurement with Fiji (Expt 2). Submit. Introduction PowerPoint slides - edits and addition of Shroom background information.
4 (Sept 20-24)	Fly genetics continued: loss and gain of function mutations and over-expression. Expt 3: Photography and measurement of fly wings. Submit.	Journal club 2 preparation- reading and assertion/evidence slides Hildebrand, et al., 2021: "A modifier screen identifies regulators of cytoskeletal architecture as mediators of Shroom-dependent changes in tissue morphology" Part 1 Fig 1-4.

	Shared data entry for use in Expt 4; discussion of research integrity and ethics. Loss of function candidate gene research introduction.	Reading: Genetics on the Fly: A Primer on the Drosophila Model System Hales, et al. pp.815-824 (focus on parts most relevant for your research ex. can skip sex determination) Fly genetics worksheet. Suggested reading: Sadava Life Ch5.3 p.98-103. “The cytoskeleton is important in cell structure and movement”
5 (Sept 27-Oct 1)	Expt 5: looking for genetic interactors with Shroom B via a candidate genetic cross: Collect Nubbin>ShroomB wing over-expression/CTG virgin females. Journal club #2 part 1 Course-wide control data graphing and summary statistics. Expt 4.	Suggested reading: Sadava Life Ch47.1 “Interactions of actin and myosin cause muscles to contract” Part 2 Journal club #2 Fig 5-8 with assertion/evidence slides. Expt 4: submit Presentation slides: wing qualitative and quantitative data slides.
6 (Oct 4-8)	Expt 5: Collect loss-of-function candidate fly males; Set cross with Nubbin>ShroomB wing over-expression/CTG virgin females and candidate males. (Cross day 0) Write out genetic crosses with expected F1 progeny genotypes and marker phenotypes. Journal Club #2, part 2 Candidate research for JC#3 and hypothesis models. From structural data to cartoon icons. Drawstring bags. Wing predictions.	Drosophila introduction slides: genetics, timeline of cross with life cycle icons, Shroom in drosophila. Candidate research for JC#3 and background slides **Register to vote!!** **10/18/2021 is the last day to register before the 11/02/2021 election.** https://www.pavoterservices.pa.gov/pages/VoterRegistrationApplication.aspx
7 (Oct 11-Oct 15) Fall Break Week	**Remove parents from vial; GFP larvae analysis-cross day 7** (Friday section comes on Thursday (Day 6)).	Sadava Life Ch47.1 “Interactions of actin and myosin cause muscles to contract” Sketching hypothesis models.

		Friday section clears flies on Wednesday or Thursday.
8 (Oct 18-22)	(Cross day 14) Sorts F1s M/F and dominant markers, briefly score wings. Complete Expt 5. Collect F1 progeny into 70% ethanol. Expt 6. Team work: Choosing journal club #3- candidate primary research and review articles. Team work: Hypothesis modeling, adding in your candidate. Organizing a research presentation	Expt 6: Fixation and dissection solutions math. Pipetting video. Team work: Candidate journal club #3 preparation. Slides should also be useable in Final Presentation Team work: Final presentation slide compilation and editing
9 (Oct 25-29)	Solution math. Pipetting practice. Expt 6. Solution changes from 70% ethanol into 15% glycerol 35% ethanol and 20% glycerol. Candidate journal club #3 Hypothesis/conclusion model presentation	Plan to vote! Model slides (individual assignment).
10 (Nov 1-5)	Expt 6 Dissect and mount wings. Submit. Expt 7 Photograph and measure. Tuesday November 2 is Election Day. Please vote!	Team work: slide compilation and editing Edit models for conclusion. Presentation preparation reading- specific for teams' needs
11 (Nov 8-12)	Expt 7: Complete wing measurements. Course-wide data entry for Expt 8.	Qualitative candidate cross wing slides for presentation

	Share wing pictures.	Future directions research slides
12 (Nov 15-19)	Course-wide data analysis and lab meeting. Expt 8. CV writing and looking for research workshop.	Complete and edit data and conclusion and future directions slides. Make final edits. Practice presentation. Anticipate questions.
13 (Nov 22-26) Thanksgiving week	Thanksgiving	Thanksgiving Write memo to Dr. Hildebrand. Practice presentation to family/friends with reflection.
14 (Nov 29-Dec 3)	Semester lab mtg: genes to proteins to cells to tissues Neural tube defects review. Professional presentations: asking and anticipating questions Assemble presentation. Practice and review.	Practice and review with team.
15 (Dec 6-10)	Team presentations with Q+A	Post course surveys, reflections on team work and presentation, OMETS