University of Pittsburgh Department of Biological Sciences

DNA Regulation & Disease (BIOSC 0067)

Research-based Introductory Biology Laboratory Course

Spring 2018

Course objectives:

The main goal of this course is for students to gain practical knowledge of the fundamentals of laboratory research through participation in a current authentic research project. Students will gain experience with many standard molecular biology techniques and be expected to learn the basics of experimental design, execution, and data interpretation. Participants in this course will also gain exposure to primary scientific literature and present their research findings at a scientific poster session.

Experimental overview:

The research project performed by students in this course aims to explore the mechanisms that underlie the organization of eukaryotic DNA. The genomes of eukaryotic cells are compacted and organized into chromatin through association with the histone proteins. Chromatin serves organizational and regulatory functions that are critical to proper gene expression; defects in these processes are linked to human cancers and developmental disorders. We will study the Chd1 protein. This protein is a "chromatin remodeler" that utilizes the energy derived from ATP hydrolysis to modify chromatin structure during transcription; it both opens the promoter to allow access by transcription factors and restores normal chromatin structure within genes after RNA polymerase II passes. We will study Chd1 in the yeast *Saccharomyces cerevisiae* (baker's yeast). However, this protein has nine known human homologs in which mutations have been linked to disorders ranging from prostate cancer to autism. We will utilize PCR and cloning techniques to randomly mutagenize the Chd1 protein in order to identify the amino acids that are critical for its function. The resulting discoveries will be sent to research labs, including that of Karen Arndt in our own department, to integrate into their research programs. Identification of functional residues in proteins is critical to understanding how they function and in creating drugs that alter or correct their activity. A detailed schedule of experiments can be found at the end of this syllabus.

Learning objectives:

At the conclusion of this course, students should:

1) have an understanding of the role of three dimensional structure and functional domains in protein activity and how mutation can impact protein function.
2) understand the role of chromatin structure in regulation of gene expression and how errors in this process can lead to disease.
3) comprehend the value and applications of a simple model system in the scientific process.
4) understand the mechanism of action and proper application of the experimental techniques utilized in random mutagenesis, mutant selection, propagation, isolation, and sequence determination and analysis.
5) Keep an accurate record of experiments that can be easily interpreted by other scientists
6) Read and present primary scientific data.

Prerequisites: Satisfactory completion of BIOSC0050 (Foundations of Biology Laboratory I) or BIOSC0058 (SEA-PHAGES I) or appropriate advanced placement credits

Textbooks and materials: There is no textbook required for this class. We will utilize LabArchives, an online electronic notebook software accessible through my.pitt.edu. Protocols and readings will be uploaded to the "Course Documents" folder on the Courseweb page for this course.

Instructor:

Marcie H. Warner, Ph.D.
A252 Langley Hall
mwarner1@pitt.edu
412-624-2230

Office hours:

A252 Langley Hall
Thursday: 12:00-2:00 pm
Or email to schedule an appointment

Undergraduate Teaching Assistants:

Section	Name	Email
Wednesday	Charu Arora	CHA33@pitt.edu
12:00-2:50 pm	Caitlin Viele Haggerty	caitlinvielehaggerty@pitt.edu
	Madeline Gerwig	mag267@pitt.edu
Wednesday	Lihn Pham	LNP27@pitt.edu
3:30-6:20 pm	Austin Souryavong	asouryavong@pitt.edu

Course meeting time:

Section 1090: Wednesday 12:00-2:50 pm
Section 1095: Wednesday 3:30-6:20 pm

Location: G4 Clapp Hall

Grading:

Final grades will be based on your performance on assignments, attendance, participation in class discussions and laboratory procedures, keeping a laboratory notebook, and a final presentation of your experimental findings. **While we strongly encourage scientific discussions with your peers, you must do your own work.** Plagiarism will not be tolerated (see Academic Integrity Policy below).

See grading summary below for detailed point allotments. Due to the unpredictable nature of a scientific research project, assignments may be added or removed (which may change the overall point total for the course), at the instructor's discretion.

Grading summary:

Assignments	Group or individual	Due date	Points
Lab notebook (includes scientific background, experimental design, and data analyses)	Individual	Weekly; (11 total at 10 points each; drop lowest); MUST BE TIMESTAMPED IN YOUR NOTEBOOK BEFORE CLASS! More may be added if additional experiments must be completed.	100
Safety contract	Individual	Jan 17	2
Chd1 structure paper	Individual	Jan 24	5
Poster observations	Individual	Jan 31	5
Poster introduction	Group	Feb 14	5
Gel practice figure	Group	Feb 21	5
Research paper #2	Individual	Mar 14	5
Poster draft	Group	March 30	10
Final poster	Group	April 13	5
Poster presentation and summary	Individual	April 18	10
Analysis of other students' posters	Individual	April 23	3
TOTAL			**155**

Grading scale:

97 - 100%:	A+	77 - < 80%:	C+
93 - < 97%:	A	73 - < 77%:	C
90 - < 93%:	A-	70 - < 73%:	C-
87 - < 90%:	B+	70 - < 73%:	C-
83 - < 87%:	B	67 - < 70%:	D+
80 - < 83%:	B-	60 - < 63%:	D-
		< 60%:	F

Assignments:

Assignments are due in lab at the start of class on the specified due date. No credit will be given for late assignments.

Courseweb:

Protocols, course materials, and announcements will be made on the Blackboard Courseweb system. You are required to regularly check the page for this class.

Attendance and Participation:

You must be present to perform the labs during class time in order to perform well in this course. If you miss a lab, you should make arrangements to meet with the instructor or a TA to discuss the material that you missed so you do not fall behind. If you miss lab due to an illness (for which you have a medical excuse) or severe personal trauma, you will not receive points for the assignments from the day, but instead your grade will be based on a prorated scale that includes only the points possible from the dates that you attended class. If you miss more than 2 class meetings (regardless of the cause of your absences), you are strongly encouraged to drop the course. **If you have more**

than 1 unexcused class absence, you will lose a letter grade for each occurrence. Routinely showing up late or not participating in group assignments will result in a loss of points on the final poster project.

University policies

Students with disabilities

If you have a disability for which you are or may be requesting an accommodation, you are encouraged to contact both your instructor and the Office of Disability Resources and Services, 216 William Pitt Union, 412-648-7890/412-383-7355 (TTY), as early as possible in the term. Disability Resources and Services will verify your disability and determine reasonable accommodations for this course.

Academic Integrity

Academic Integrity Policy: Cheating/plagiarism will not be tolerated. Students suspected of violating the University of Pittsburgh Policy on Academic Integrity will be required to participate in the outlined procedural process as initiated by the instructor. A minimum sanction of a zero score for the quiz, exam or paper will be imposed.

The integrity of the academic process requires fair and impartial evaluation on the part of faculty and honest academic conduct on the part of students. To this end, students are expected to conduct themselves at a high level of responsibility in the fulfillment of the course of their study. It is the corresponding responsibility of faculty to make clear to students those standards by which students will be evaluated, and the resources permissible for use by students during the course of their study and evaluation. The educational process is perceived as a joint faculty-student enterprise which will perforce involve professional judgment by faculty and may involve—without penalty—reasoned exception by students to the data or views offered by faculty. *Senate Committee on Tenure and Academic Freedom, February 1974*.

Students agree that by taking this course all required papers may be subject to submission for textual similarity review to Turnitin.com for the detection of plagiarism. All submitted papers will be included as source documents in the Turnitin.com reference database solely for the purpose of detecting plagiarism of such papers. Use of Turnitin.com page service is subject to the Usage Policy and Privacy Pledge posted on the Turnitin.com site.

E-mail Communication Policy

Each student is issued a University e-mail address (username@pitt.edu) upon admittance. This e-mail address may be used by the University for official communication with students. Students are expected to read e-mail sent to this account on a regular basis. Failure to read and react to University communications in a timely manner does not absolve the student from knowing and complying with the content of the communications. The University provides

an e-mail forwarding service that allows students to read their e-mail via other service providers (e.g., Hotmail, AOL, Yahoo). Students that choose to forward their e-mail from their pitt.edu address to another address do so at their own risk. If e-mail is lost as a result of forwarding, it does not absolve the student from responding to official communications sent to their University e-mail address. To forward e-mail sent to your University account, go to http://accounts.pitt.edu, log into your account, click on **Edit Forwarding Addresses**, and follow the instructions on the page. Be sure to log out of your account when you have finished.

Copyright Notice

Course materials may be protected by copyright. United States copyright law, 17 USC section 101, et seq., in addition to University policy and procedures, prohibit unauthorized duplication or retransmission of course materials.

Classroom Recordings

You may record lectures for this course, but any recordings are for your own personal use and may not be further disseminated.

Student health and wellness:

If at any time throughout the semester or your time at Pitt you are in need of assistance to evaluate or improve your physical or mental health, please visit the Student Health Center (https://www.studentaffairs.pitt.edu/shs/) or Counseling services (https://www.studentaffairs.pitt.edu/cc/services/).

Student opinion of teaching surveys

Students in this class will be asked to complete a *Student Opinion of Teaching Survey*. Surveys will be sent via Pitt email and appear on your CourseWeb landing page during the last three weeks of class meeting days. Your responses are anonymous. Please take time to thoughtfully respond, your feedback is important to me.

Operational agreement:

Students are expected to demonstrate respect toward each other and their instructors while in the classroom. During class and class-related activities, students, instructors, and teaching assistants are expected to abide by the following rules:

Students will follow the University policy on nondiscrimination in group work, class discussions, all other classroom activities, and communications relevant to class that occur outside of class time:

> "The University of Pittsburgh, as an educational institution and as an employer, does not discriminate on the basis of disability, race, color, religion, national origin, ancestry, genetic information, marital status, familial status, sex, age, sexual orientation, veteran status or gender identity and expression in its programs and activities."

Students should arrive promptly. Important announcements are frequently made at the beginning of class and students are expected to be present to receive this information. Additionally, arriving late is disruptive and disrespectful.

When you have completed your lab work for the day, you may leave in a quiet and nondisruptive manner provided you have satisfactorily communicated with your instructor that you have completed all necessary work.

Talking and other disruptive behavior during announcements and while other students are providing information relevant to the class is not permitted.

Failure to comply with this agreement may result in the deduction of points or other interventions by the University.

Tentative schedule

In class (discussions/activities/labs)	Assignments (due next class)
Week of Jan 10/11	
Discuss chromatin and chromatin remodeling	"Confirming strain identities" lab notebook entry
Discuss Chd1	Read summary document up to "Experimental details" heading
Look up Chd1's connections to disease	Safety contract
Discuss media	
Pour rich media (YPD; Yeast-Peptone-Dextrose media)	
Discuss model organisms and *Saccharomyces cerevisiae*	
Streak yeast strains for single colonies	
Week of Jan 17/18	
Pour drop out (synthetic) media	PCR notebook entry
Streak yeast to drop out media	Read Chd1 structure paper (Farnung et al.) and complete worksheet
Discuss Chd1 domains and segments	Read summary document beginning at "Experimental details" to beginning of last paragraph on page 8.
Discuss PCR	
Week of Jan 24/25	
Gather and interpret marker screening results	Restriction digests and gel electrophoresis lab notebook entry
Set up PCR reaction	Read summary document through *FLO8* reporter
Discuss plasmids	Poster observations
Discuss restriction digests	Read summary document beginning with last paragraph of p. 8 up to "Step 3" heading"
Discuss gel electrophoresis	
Week of Jan 31/Feb1	
Set up restriction digest	Prepare yeast transformation notebook entry
Run PCR and restriction digests on gel	Read summary document from "Step 3" through the end
Discuss yeast transformation	
Discuss *FLO8* reporter	
Week of Feb 7/8	
Yeast transformation	Prepare mutant candidate confirmation notebook entry
Discuss mutant candidate confirmation	Poster introduction

Week of Feb 14/15	
Streak candidates to SC-L	Prepare notebook to rescreen candidates
	Gel figure practice
Week of Feb 21/22	
Streak candidates to SC-H-L	Prepare notebook for colony PCR
Discuss colony PCR	
Week of Feb 28/Mar 1	
Colony PCR	Prepare notebook for gel electrophoresis and DNA sequencing
Discuss DNA sequencing	Select a paper of interest and write a short summary
Week of Mar 7/8	
SPRING BREAK	**NO CLASS**
Week of Mar 14/15	
Run gel to check candidate plasmid digests	Prepare notebook for DNA sequencing
Send candidates for sequencing	
Week of Mar 21/22	
Interpret DNA sequencing	Enter results of sequencing into lab notebook
Week of Mar 28/29	
Discuss sequencing results; make class/course map	Prepare "exotic" phenotype notebook entry
Discuss "exotic" phenotypes	POSTER DRAFT DUE BY Friday March 30 @ 9 AM
Work on posters	Submit posters in "Drafts" folder in course PittBox
Week of April 4/5	
Test "exotic" phenotypes	Finish notebook
Work on poster	
Week of April 11/12	
Catch up	FINAL POSTER DUE BY FRI April 13th @ 9 AM!
Work on poster	Submit posters in "Final poster" folder in course PittBox as PDFs!
Clean up lab	
Lab course survey	
Week of April 18/19	
Poster session	Written presentation summary due before class the day of your poster presentation

Week of April 25/26	
This course does not meet during finals week	