

## BIOL 09 – Researching Cellular Mysteries

Spring 2024

### **Course Description:**

This course focuses on understanding and applying the scientific method to answer questions in Biology through discovery-based, hands-on laboratory experiences. This course is structured in a ‘flipped’ format, lectures will be delivered virtually with time for questions and discussion during class. Class time will also be devoted to designing and performing experiments. Lecture topics will cover an introduction to the structure and function of DNA, RNA, and proteins. Class meetings will occur in a laboratory setting. We will design and carry out experiments to address a novel question in Biology. This course is designed to create new knowledge through laboratory research activities (hence “discovery-based”), meaning that we will be addressing novel questions – not even the instructors know what the outcome will be! Throughout the course, students will design and carry out experiments, record the experimental procedures and results, analyze and interpret data, and present their discoveries in written and oral formats.

### **Course Goals and Learning Objectives:**

By the end of this course, students will be able to use the scientific method to design and execute an experiment to answer a question in Biology.

The learning objectives are to:

- Describe background information on the research topic
- Design an experiment to test a hypothesis with proper controls and methods
- Conduct experiments and record results in a laboratory notebook
- Interpret the results of experiments
- Communicate your findings through written / oral methods

### **Course Staff:**

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### **Class Meeting Time and Location:**

Class will meet in the 10A time slot. Additionally, we will meet during the 9S block as a discussion section on Tuesdays to discuss the lecture material and answer reflection questions. There are no planned activities for the X-hour, but students will have the option to use the lab space at that time (if they would like to repeat an experiment for example).

### Course Calendar (an overview):

Most of each class period will consist of performing experiments, but some time will also be taken for general discussion and instruction pertaining to the experiments being performed. The X-hour will be used as needed to complete or repeat certain experimental procedures.

<b>Class</b>	<b>Lab Activity</b>	<b>Lecture Topic</b>
1.1	Bioinformatics Tools	DNA, Gene Structure
1.2	Construct Design	Introduction to Cell Biology
2.1	Polymerase Chain Reaction	DNA Replication
2.2	Run gel of PCR product, PEG purification	Gene expression and transcription
3.1	HiFi vector prep, bacterial transformation	Techniques for DNA modification
3.2	Mini prep, restriction digest	RNA structure and function
4.1	Run gel of digest, pick clones for sequencing	Translation, protein folding
4.2	Analyze sequencing results	Protein structure
5.1	Recap – what have we achieved so far?	Protein function
5.2	Transfect mammalian cells	Techniques for working with proteins
6.1	Fix cells for imaging, stain with phalloidin	Cell biology pt 2 – Immunology
6.2	Initial imaging	Intro to Microscopy
7.1	Imaging (continued), initial image analysis	Cytoskeleton and Motors
7.2	Transfect mammalian cells	Cell Biology Continued
8.1	Fix cells for imaging, stain with phalloidin	Cellular communication
8.2	Initial imaging	Presenting data to other scientists
9.1	Imaging (continued), initial image analysis	Image Analysis
9.2	Poster prep	Scientific communication
10.1	Poster presentations	

## **Topics and Schedule:**

### **Project Overview:**

This offering of Bio 9 will examine the roles of specific cell signaling proteins in T cell biology. T cells are a subset of white blood cells (leukocytes) that help to keep the body healthy by participating in immune responses. T cells are able to remove virally-infected cells and tumor cells. In addition, they direct other cells of the immune system to become activated to eliminate pathogens. Understanding the mechanisms that govern T cell function is therefore critical to understanding the immune response and for generating novel therapies.

We will examine the roles of candidate cell signaling proteins in the ability of T cells to adhere to a stimulatory surface and the localization of these proteins before and after T cell stimulation. T cell adhesion to their cognate antigens is critical for naïve T cells to become activated by antigen presenting cells. In addition, T cells must bind to target cells to carry out their effector functions of the immune system. We have chosen this cell type as they are easy to culture, well amenable to genetic modifications by DNA transfection and RNA interference and have well established protocol for stimulation *in vitro*. Importantly, T cells have an easily-identifiable adhesion phenotype that can be used as a readout for identifying novel regulators of T-cell activation.

In this course, students will select from several T cell proteins (pre-selected by the instructors) whose role in T cell adhesion is currently unknown. Students will design constructs expressing fluorescently tagged protein of interest (POI) or small hairpin RNAs that downregulate the mRNAs encoding the POI. They will then transfect the T cells with these constructs to examine the localization of the fluorescent fusion POI as well as the role of POI in maintaining a stable interaction of the T cells with a stimulatory surface.

### **Part 1 (Week 1 – Week 5): Molecular Cloning**

In the first half of the term, students will become familiar with the model system, gain a working knowledge of the structure and function of DNA, RNA and proteins, and learn about basic cell biology and immunology. Students will also be provided with the necessary background information to understand the overall design and the rationale for the experiments they perform in lab. Laboratory work in this module will consist of using online bioinformatics tools to analyze protein structures and protein interaction networks, creating fluorescent fusion proteins and RNAi hairpin constructs, and verifying these constructs by restriction enzyme digestions and DNA sequencing.

#### **➤ Week 1: Introduction to Molecular Cloning – Tools and Techniques**

##### **Class 1.1**

Intro Lecture in Class: DNA, RNA, and protein: a brief introduction

In-Class Activity: Learn about tools for bioinformatic analysis of protein structures and protein interaction partners (<https://string-db.org/>).

After class pre-recorded Lecture: Overview of the Central Dogma of Biology, Introduction to the structure and properties of DNA, components and structure of a gene

### **Class 1.2**

Pre-recorded Lecture: Introduction to cell biology.

In-Class Activity: Design primers to generate a construct that will express a fluorescent fusion protein or an RNAi hairpin in a mammalian cell.

### **Week 1 Discussion**

- Using bioinformatics to learn about genes and proteins.
- Predicting protein structure based on primary sequence.
- Discuss the components of a plasmid, relating back to lecture 1.1 with the role of promoters, and how cDNA can be used in the construct to replace the native gene containing introns and exons.

### ➤ **Week 2: Polymerase Chain Reaction (PCR)**

#### **Class 2.1**

Pre-recorded Lecture: DNA replication and its application in the PCR technique

Activity: Become familiar with pipetting. Set up the PCR reaction mixture and the proper reaction program on the thermo cycler based on the primer parameters determined during Week 1. Begin the PCR reaction (instructors will store the PCR products after the reaction).

#### **Class 2.2**

Pre-recorded Lecture: Gene expression and transcription

Activity: Run the PCR product on the agarose gel and purify the product using Polyethylene glycol (PEG) precipitation method.

### **Week 2 Discussion**

- Using knowledge of DNA replication (and thermostable polymerases) to create useful laboratory procedures.
- Compare gene expression in a cell versus in a test tube. When would/wouldn't you want to use a constitutively active promoter to drive gene expression?

### ➤ **Week 3: Plasmid Construction and Amplification**

#### **Class 3.1**

Pre-recorded Lecture: Techniques for DNA modification

Activity: Use NEB HiFi system to insert DNA into host vector, transform plasmids into bacteria. (Instructors will pick colonies and inoculate the in culture medium 24 h before next class).

#### **Class 3.2**

Pre-recorded Lecture: RNA structure and function

Activity: Perform miniprep to purify the constructs, followed by restriction enzyme digestion to confirm the constructs.

### **Week 3 Discussion**

- Harnessing enzymes for use in molecular cloning (similar to discussion from week 2)
- Discuss how to make cDNAs.

### ➤ **Week 4: Confirming the DNA constructs**

#### **Class 4.1**

Pre-recorded Lecture: Translation and protein folding

Activity: Analyze the results of restriction enzyme digestion by gel electrophoresis and select positive clones for sequencing.

#### **Class 4.2**

Pre-recorded Lecture: Protein structure and how properties of amino acids determine secondary structure.

Activity: Analyze sequencing results to confirm the constructs.

At this point, if students do not have a useable plasmid, they can use one generated by instructors prior to the start of the class.

### **Week 4 Discussion**

How the structure of a protein determines its location in the cell (the role of hydrophilic/phobic interactions, protein domains, protein-protein and protein-lipid interactions).

### ➤ **Week 5-1: Summary of molecular cloning**

#### **Class 5.1**

Pre-recorded Lecture: How the function of a protein is dependent on its shape.

Activity: Recap – what have we achieved so far?

### ***Part 2 (Week 5 – Week 10): T Cell Transfection and Functional Assays***

In the second module, students will use the constructs they generated in the first part of the class. Plasmids containing fluorescent fusion proteins or RNAi hairpins will be transfected into mammalian T cells (Jurkat – human T cell line). Students will then stimulate cells on anti-TCR coated coverslips. Cells will then be fixed and stained for imaging assays. Students will examine localization of tagged proteins and cell shape as a readout for adhesion.

### ➤ **Week 5-2: T cell transfection**

#### **Class 5.2**

Pre-recorded Lecture: Techniques for working with proteins such as SDS-PAGE, Western blot, immunofluorescence (IF) staining

Activity: Transfect constructs into human T cells. Return cells to incubator for cell recovery and protein expression over the weekend.

### **Week 5 Discussion**

- Based on the sequence and known binding partners of your protein, where might it be localized?
- Caveats to expressing a fusion protein from a plasmid (e.g., overexpression, effects due to tag).

➤ **Week 6: Stimulation, Staining and Imaging of the Transfected Cells**

**Class 6.1**

Pre-recorded Lecture: Introduction to immune cell biology

Activity: Add transfected cells to stimulatory glass coverslips, fix, stain for actin with phalloidin.

**Class 6.2**

Pre-recorded Lecture: Introduction to light microscopy, covering parts of the microscope and the light path for imaging

Activity: Imaging fixed cells by fluorescence microscope (day 1).

**Week 6 Discussion**

- Discussion of how cells respond to stimulatory surface and how this response might mimic their role in a live organism.
- Tips for using microscopes: how to focus and acquire high-quality images.

➤ **Week 7: Stimulation, Staining and Imaging of the Transfected Cells (cont.)**

**Repeat Transfection (Round 2)**

**Class 7.1**

Pre-recorded Lecture: How the cytoskeleton and motor proteins are responsible for transport and controlling cell shape.

Activity: Imaging fixed cells by fluorescence microscope (day 2); begin image analysis.

**Class 7.2**

Pre-recorded Lecture: T cell biology – how signaling affects cell shape to support T cell activation?

Activity: Transfect mammalian T cells

**Week 7 Discussion**

- Choosing cells for imaging, refresher on focusing and acquiring images
- The importance of repeating experiments, how replicating a result can support your findings.

➤ **Week 8: Repeat Stimulation, Staining and Imaging of the Transfected Cells**

**Class 8.1**

Pre-recorded Lecture: Cellular communication by cell-cell contact or secreted signals

Activity: Add transfected cells to stimulatory glass coverslips, fix, stain with phalloidin

**Class 8.2**

Pre-recorded Lecture: Presenting data to other scientists (I): How to make figures.

Activity: Imaging fixed cells by fluorescence microscope (day 1).

### **Week 8 Discussion**

Check in – what have we learned so far? Begin working on poster outline.

### ➤ **Week 9: Repeat Stimulation, Staining and Imaging of the Transfected Cells (Cont.)**

#### **Class 9.1**

Pre-recorded Lecture: Measuring images: how to choose what to measure and how to perform the desired measurement.

Activity: Imaging fixed cells by fluorescence microscope (day 2) with a focus on protein localization and cell shape.

#### **Class 9.2**

Pre-recorded Lecture: Presenting data to other scientists (II): Poster preparation.

Activity: Image analysis and creating poster outlines.

### **Week 9 Discussion**

Check in with each group for guidance on image analysis and final wrap up of the project.

### ➤ **Week 10: Culminating project**

#### **Class 10.1**

Pre-recorded Lecture: Presenting data to other scientists (III): Poster presentation strategies.

Activity: Poster prep

Discussion: Check in with each group for guidance on poster content/organization

#### **Class 10.2**

Activity: Students present their data and conclusions in a poster format

## **Preparation for Class**

A pre-recorded lecture will be available before most classes (excluding the first class and the last two classes). It is your responsibility to watch the assigned lecture recordings and to read through the Lab Protocol prior to each day of class.

## **Assessment and Grading:**

Assessment will occur throughout the term and will allow the instructors to provide frequent constructive feedback. Graded assignments will include:

- Quizzes (25%) will cover background information presented in the lecture recordings and the lab protocol.
- Participation (15%) - will be based on the instructors' observations of your performance during lab
- Lab notebook (35%) – Your lab notebook will detail the rationale, methods, and results of the experiments you perform such that they could be replicated by another scientist. For grading, turn your lab notebook in to your instructor on the dates outlined in the calendar.
- Poster (25%) – You will present the results of your research through a culminating poster presentation at the end of the term.

## **Other materials:**

You will need a computer to access pre-recorded lectures as well as the digital laboratory notebook and laboratory manual.

## **Honor Principle:**

The faculty, administration, and students of Dartmouth College acknowledge the responsibility to maintain and perpetuate the principle of academic honor, and recognize that any instance of academic dishonesty is considered a violation of the [Academic Honor Principle](#).



### **Student Wellness:**

The academic environment is challenging, our terms are intensive, and classes are not the only demanding part of your life. There are a number of resources available to you on campus to support your wellness, including: the [Counseling Center](#) which allows you to book triage appointments online, the [Student Wellness Center](#) which offers wellness check-ins, and your [undergraduate dean](#). The student-led [Dartmouth Student Mental Health Union](#) and their peer support program may be helpful if you would like to speak to a trained fellow student support listener. If you need immediate assistance, please contact the counselor on-call at (603) 646-9442 at any time. Please make me aware of anything that will hinder your success in this course.

### **Title IX:**

At Dartmouth, we value integrity, responsibility, and respect for the rights and interests of others, all central to our Principles of Community. We are dedicated to establishing and maintaining a safe and inclusive campus where all community members have equal access to Dartmouth's educational and employment opportunities. We strive to promote an environment of sexual respect, safety, and well-being. Through the Sexual and Gender-Based Misconduct Policy (SMP), Dartmouth demonstrates that sex and gender-based discrimination, sex and gender-based harassment, sexual assault, dating violence, domestic violence, stalking, etc., are not tolerated in our community.

For more information regarding Title IX and to access helpful resources, visit Title IX's website ([sexual-respect.dartmouth.edu](http://sexual-respect.dartmouth.edu)). As a faculty member, I am required to share disclosures of sexual or gender-based misconduct with the Title IX office.

If you have any questions or want to explore support and assistance, please contact the Title IX office at 603-646-0922 or [TitleIX@dartmouth.edu](mailto:TitleIX@dartmouth.edu). Speaking to Title IX does not automatically initiate a college resolution. Instead, much of their work is around providing supportive measures to ensure you can continue to engage in Dartmouth's programs and activities.

### **Religious Observances:**

Dartmouth has a deep commitment to support students' religious observances and diverse faith practices. Some students may wish to take part in religious observances that occur during this academic term. If you have a religious observance that conflicts with your participation in the course, please meet with me as soon as possible—before the end of the second week of the term at the latest—to discuss appropriate course adjustments.

### **Student Accessibility and Accommodations:**

Students requesting disability-related accommodations and services for this course are required to register with Student Accessibility Services (SAS; [Apply for Services webpage](#); [student.accessibility.services@dartmouth.edu](mailto:student.accessibility.services@dartmouth.edu); 1-603-646-9900) and to request that an accommodation email be sent to me in advance of the need for an accommodation. Then, students should schedule a follow-up meeting with me to determine relevant details such as what role SAS or its [Testing Center](#) may play in accommodation implementation. This process works best for everyone when completed as early in the quarter as possible. If students have questions about whether they are eligible for accommodations or have concerns about the implementation of their accommodations, they should contact the SAS office. All inquiries and discussions will remain confidential.