

Welcome to the laboratory component of Introduction to Cell Biology. This course will give you “hands on” experience with some of the technology used in modern cell biology research, as well as solidify some of the concepts you are learning in the lecture course. The centerpiece of the lab course is a project involving Green Fluorescent Protein and protein localization. In this project you will use recombinant DNA techniques to genetically engineer yeast cells to express a novel gene encoding the Green Fluorescent Protein (GFP) fused to an endogenous yeast protein called Cdc10. You will use fluorescence microscopy to observe localization of the Cdc10 protein in living yeast cells.

Instructor: Dr. Kurt Toenjes. Office: 133, Lab 103
Phone: 896-5940
Email: ktoenjes@msubillings.edu

Reading Assignments: There is no assigned lab manual for this course. Reading assignments from Essential Cell Biology will provide some technical and conceptual background to the lab exercises (see below). The reading assignments are given with each lab protocol.

Lab Protocols : The protocols for the lab project are available to download and print from the department web site.

To find the handouts, go to: D2L Click on Biol 273. Go to content and then please print out the protocols and bring them to each lab.

Lab Notebook: You are expected to keep an up-to-date lab notebook. For each lab period, the notebook should include protocol, what you did during each lab period and your results. The notebook is essential to understanding the overall flow of the project. I will collect the lab notebooks for grading at the end of the semester.

Assessment: Your grade in this lab will be based on the following items:

1. Attendance (15% of your grade)
2. Final Exam (35% of your grade)
3. GFP Report (25% of your grade)
4. Lab notebook (25% of your grade)

These assignments will test your understanding of the technical and conceptual aspects of the lab project.

Attendance: Attendance is mandatory, as it is generally impossible to make up missed labs.

Academic Honesty: All students are expected to adhere to the highest standards of academic honesty and to refrain from any actions that are dishonorable or unethical. In all

exams, papers, labs, etc., you are expected to turn in your own work entirely. Cheating or aiding another in cheating in any manner will result in a grade of “F” for the class.

Tentative Schedule of Labs

<u>Date</u>	<u>Topic/Lab</u>
Sept. 17	Intro
Sept. 24	Lab #1 Purification of Genomic DNA
Oct. 1	Lab #2 PCR Amplification of DNA
Oct. 8	Lab #3 Analysis of PCR Product
Oct. 15	Lab #4 Construction of Recombinant DNA
Oct. 22	Lab #5 Bacterial Transformation
Oct. 29	Lab #6 Plasmid DNA Purification
Nov. 5	Lab #7 Molecular Analysis of Plasmids
Nov. 12	Lab #8 Yeast Transformation
Nov. 19	Lab #9 Fluorescence Microscopy
Nov. 26	Thanksgiving
Dec. 3	Flex day/ GFP report due
Dec. 10	Final Exam (Turn in lab book)

Biol 273 GFP Report Instructions

Objectives

Become familiar with the many different ways that GFP is used in cell biological research.

This exercise will expose you to some simple computer tools for gathering information about a particular gene or protein over the internet.

Assignment

Launch your web browser and go to the National Center for Biotechnology Information (NCBI) web site: <http://www.ncbi.nlm.nih.gov/>

This is the home page for the National Center for Biotechnology Information (NCBI) administered by the National Library of Medicine and the National Institutes of Health.

On the page that appears, there is a button labeled **Search** in the upper left corner. Next to this button is a pop-down menu. From the Search menu, choose **PubMed**. PubMed is the largest scientific journal database in the world.

Search PubMed for “Green Fluorescent Protein” or “GFP”

You should pull up thousands of articles (only the title and journal reference will be displayed). All of these studies use GFP in one way or another. Amazing! Begin looking over this list to find potentially interesting uses of GFP (you can view the abstracts of an article by clicking on the article title). Your assignment is to pick one article and write a short report (approximately one page) describing how GFP was used to study some aspect of cell biology.

Due Date: Dec 3, 2009.

Biol 273 Final Exam Study Guide

Know the following techniques

PCR

Gel Electrophoresis

Function of restriction endonucleases and DNA Ligase

Important functional features of plasmids

Plasmid purification from *E. coli*

Transformations of *E. coli* and Yeast

Fluorescence microscopy

Biological Concepts

GFP (source, size of ORF, size of protein, function)

Basic concepts in gene structure (promoter, ORF, etc.).

Basic concepts in gene expression.

Protein transport into the nucleus.