

CHEMISTRY 346 - SPRING 2008

LABORATORY SCHEDULE

Week	Date	Experiment
		Isolation & Characterization of plasmid DNA
1	1/29	Class introduction/safety issues/prep for week #2
2	2/5	Isolation & purification of plasmid DNA
3	2/12	Analysis of plasmid DNA - spectroscopy
4	2/19	Analysis of plasmid DNA – gel electrophoresis
5	2/26	Restriction analysis of recombinant plasmid DNA
		Comparison of Methods of Plasmid Preparation
6	3/4	Plasmids Preps – Alkaline Lysis & Qiagen Prep
7	3/11	Plasmid Preps – Alkaline Lysis & Qiagen Prep
---	3/18	SPRING BREAK
---	3/25	Instructor Prep
		Isolation & Characterization of recombinant protein
8	4/1	Transformation of <i>E. coli</i> with recombinant plasmid
9	4/8	Expression of protein/Bioinformatics
10	4/15	Preparation of cell lysate /Design of purification scheme
11	4/22	Purification & analysis
12	4/29	Purification & analysis
13	5/6	Presentations on purification project

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Course Overview

In this course you will explore two major biochemical techniques:

- Isolation, purification and analysis of plasmid DNA
- Expression & purification of recombinant proteins

The ability to manipulate plasmid DNA is a major driving force in biotechnology. This laboratory course is designed to help you develop technical proficiency in some of the more common techniques used to study the physical, biochemical and molecular biological properties of DNA.

We will begin our study of plasmid DNA by learning how to isolate it from bacterial cells. Bacterial systems are ideal for the propagation of large numbers of plasmid samples because they grow efficiently under a variety of conditions. We will then analyze the plasmids using some of the common biochemical and biophysical methods that you are likely to encounter as you move beyond the classroom.

The second major project will involve comparison of two different methods of plasmid preparation. This exercise is designed to help you develop the following skills:

- Develop critical and analytical skills
- Learn how to apply basic theoretical knowledge to new situations
- Effective time management & planning to accomplish a goal.

The third major project involves the expression and purification of a heterologous[foreign] protein in bacteria. This is another very common technique used in modern biochemistry. It will provide experience in dealing with a second type of biomolecule.

The course consists of the projects described below. Each has been spread over a period of several weeks to accommodate our course schedule. For the first two projects there will be a lab report in the form of a journal article and one or more homework assignments designed to help in the preparation of the report. The final project will require an oral presentation. There is a schedule of assignments on the following page.

I. Isolation and characterization of plasmid DNA

- Extraction of plasmid DNA from bacteria and purification by chloroform extraction and ethanol precipitation.
- Characterization/analysis of purified DNA – each group will be given a different plasmid and charged with the task of identifying the plasmid
 - **Assignment:** Written description of the procedure in the form of a journal article

II. Comparison of Plasmid Preparation Methods

- Extraction and analysis of plasmid DNA from bacteria using two different methods of plasmid preps
 - **Assignment:** Written description of findings in the form of a 3-page business memo

III. Purification of recombinant GFP/BFP

- Transformation of *E. coli* with a recombinant plasmid containing the gene for either GFP or BFP
- Selection of transformants and subsequent expression of protein
- Purification of desired protein from cell extract
 - **Assignment:** Oral presentation

Remember the Honor Code

Suspected violations of the Honor Code will be referred to the Academic Honor Board. For a full description of the code and what constitutes a violation of the code, refer to the Goucher Handbook or online at www.goucher.edu/x1292.xml

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Assignments

Throughout the course of the semester, there will be writing and homework assignments that will be due as outlined during the course of the semester. You will have one oral presentation as well. Guidelines for writing assignments will be handed out by the instructor. The audience you should write for will be your classmates.

Although you may discuss your paper with classmates, the writing assignment must be an individual effort. If you intend to take CHE 346 for writing proficiency within the major, please let the instructor know at the beginning of the semester.

Homework assignments are designed to help you gain a deeper understanding of the laboratory by helping you to make connections between theory and practice. **You are encouraged to discuss the assignment with one another, but again each of you must hand in an assignment that was done individually.**

Homework assignments will not be accepted after the due date; other assignments will be downgraded 2% for each day late and will not be accepted more than one week after the original due date except by prior arrangement with the instructor.

In addition to these scheduled assignments, lab performance will also be evaluated. The lab performance grade will be based on attendance, punctuality, preparation and proficiency in lab skills and understanding of lab techniques and procedures. A portion of your grade will also depend upon proper lab notebook maintenance. Each unexcused absence from lab will result in a 10 pt deduction from the lab performance grade. I reserve the right to give quizzes if it appears that levels of preparedness are less than what is expected.

Your final grade will be based on your total points as a percentage of 470 pts.

Assignment	Points
HW Assignments [2 @ 35 pts each]	70 pts
Writing assignment #1 – Purification/Analysis of plasmid DNA	100 pts
Writing assignment # 2 – Comparison of Plasmid Preparations	50 pts
Oral presentation – Purification of GFP/BFP	100 pts
Lab Performance	100 pts
Notebook	50 pts