

Chem 567L - Biochemistry, Cell & Molecular Biology Laboratory II

Spring 2009

Instructor: Forest Rohwer
Office: LS 301, 619-857-1336
email: forest@sunstroke.sdsu.edu

Co-Instructor: Linda Wegley
Office: LS 301
email: lwegley@gmail.com

Co-Instructor: Matt Haynes
Office: LS 302
email: mhaynes@projects.sdsu.edu

LS420

1:00-3:40 pm - Section 1

4:00-6:40 pm - Section 2

Class Objectives

This course is designed to teach molecular biology techniques commonly encountered in graduate school and biotech. The class is divided into two research projects. First, you will characterize environmental microbial communities by 16S rDNA cloning and sequencing. In the second part of the class, you design and conduct your own experiments. Unlike other laboratory courses, you will be responsible for making solutions, troubleshooting protocols, and interpreting the results. You will work in groups of 2, but you are expected to develop your own skills.

Section 1 - Characterization of microbial communities for Bacteria by 16S rDNA cloning and sequencing

- 1 - Isolate DNA from environmental sample
- 2 - PCR 16S rDNA
- 3 - Clone with TOPO kit
- 4 - Sequence 5 clones with 27F
- 5 - Bioinformatic analyses of sequences
- 6 - Production of *Taq* DNA polymerase
- 7 - Make lambda-phage DNA ladder

Brief Lectures

The 16S rDNA loci and uncultured microbes
Principles of DNA isolation
Cloning - plasmids, cosmids, BACs, enzymes
PCR and sequencing (trip to SDSU sequencing facility)
Bioinformatics - BLAST, NCBI, ribosomal database
Libraries - PubMed and Web of Science
Lambda DNA ladders

Practicals

Calibration of pipettors
Solution preparations
Autoclave and plate pouring
PCR
Agarose gel electrophoresis
TOPO-cloning
Bioinformatic analyses of 16S rDNAs

Section 2 - Design and conduct independent research.

Possible research questions/goals:

- 1) Culture and molecularly characterize ammonium-oxidizing Archaea from reef-building corals.
- 2) Clone and express rhodopsin from saltern phages.
- 3) Completely sequence the genome of a novel virus from mosquitoes.
- 4) Isolate novel antibiotic resistant genes in cystic fibrosis microbes.
- 5) Develop a real time PCR for autotrophic versus heterotrophic bacteria.
- 6) Your ideas?

Required Materials:

Lab notebook – a normal compositions book will be fine
Lab coat
Fine-tip Sharpie
Calculator

Lab notebooks:

Laboratory notebooks will be periodically checked as part of your subjective grade (50 points total). These notebooks can also be used during the practical exams, so it is to your advantage to do a good job.

Short write ups and pop quizzes:

Pop quizzes and short write ups will be given throughout the course of the semester on material already covered and also on new material that you should have read before coming to class (up to 100 points).

Practical Exams:

There will be one practical worth 50 points.

Presentation of Final Project:

Ten minute powerpoint presentation of your independent project (100 points).

Attendance:

You must attend each class meeting. If you miss a class, let me know why and when. If you miss 2 classes you will be dropped.

Grading Scale:

95 - 100%	A
90 - 94%	A-
84 - 89%	B+
80 - 83%	B
77 - 79%	B-
74 - 76%	C+
70 - 73%	C
67 - 69%	C-
64 - 66%	D+
60 - 63%	D
57 - 59%	D-

Laboratory Notebooks:

Notebook Table of Contents: Experiment title, date, and page numbers

Title: State the title of the experiment.

Purpose: State the purpose of the experiment. Why are we doing the experiment?

Data/Calculations/Results: Include all data: figures, tables, calculations, etc. Introduce each result section.

Discussion/Conclusions: State any conclusions you have regarding data and why or why not expected results were obtained. Example: As expected, relatively pure, undegraded RNA was isolated from mammalian cells as seen in Figure 1, Lanes 1-3, which represent 1, 5 and 10 μ L of mammalian RNA, respectively.

Tape protocols, disks with data, Post-It notes into your notebook. The main purpose of the notebook is to be understandable at a latter date....

**Schedule for Spring 2009 (Tuesday and Thursdays)
Section****January**

- Jan 22rd (H) - Check in and Quiz
- Autoclave, pour plates with antibiotics
- Jan 27th (T) - Pipettors, calibrations, making solutions, and dilution problems
- Jan 29th (H) - Intro to 16S rDNA and DNA isolation with kits

----TURN IN REPORT ON 16S rDNA PRIMARY PAPER----

February

- Feb 3rd (T) - Intro to PCR and setup 1st PCR
- Feb 5th (H) - Electrophoresis plus Intro to cloning and TOPO
- Feb 10th (T) - Taq DNA Polymerase protein isolation (**FLR Gone**)
- Feb 12th (H) - PCR with own Taq; set up for lambda isolation

----EVERYONE NEEDS TO GET A SAMPLE FOR THE NEXT CLASS----

- Feb 17th (T) - Independent sample processing; lambda DNA ladder
- Feb 19th (H) - Independent sample processing; lambda DNA ladder

Feb 24th (T) - Independent sample processing; lambda DNA ladder
Feb 26th (H) - Intro to Sequencing and trip to SDSU-MCF

March

Mar 3rd (T) - Practical #1 (**FLR Gone**)
Mar 5th (H) - Bioinformatics demo and analyze own sequences
Mar 10th (T) - Catch up
Mar 12th (H) - **TURN IN REPORTS ON 16S RDNA ANALYSES**
- Discussions of Independent Projects
Mar 17th (T) - **PRESENTATION OF PLAN FOR INDEPENDENT PROJECT**
Mar 19th (H) - **WRITE UP OF PROJECT AND ORDERING INFORMATION**

March 24th -April 30th is INDEPENDENT RESEARCH
FLR Gone March 22-April 25

Spring Break - March 30-April 3

FINAL PROJECT PRESENTATIONS MAY 7TH AND 12TH

Last Day in Lab is May 12th
All reports and assignments are due on May 15th