Chem 567L - Biochemistry, Cell & Molecular Biology Laboratory II
Fall 2010

Instructor: Anca Segall
Office/lab: LS 331, 619-997-4490
e-mail: asegall@sunstroke.sdsu.edu

Co-Instructor: Jason Rostron
Lab: LS 331, 619-594-6528
e-mail: 1220jer@gmail.com

LS420 4:00-6:40 pm (optimistically – you will spend a lot more time in this lab)

Class Objectives
This course is designed to teach techniques commonly encountered in graduate school and biotech. The class is divided into three sections, and 3 research projects. In the first segment, you will learn about genomic sequencing approaches and using multiplex PCR to “close” genomes. In the second segment of the class, you will perform a research project combining genetics, molecular biology, cell physiology, and biochemistry. In the third section you will 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 - 4, but you are expected to develop your own skills.

The overall goal of the course is to train you to become independent in your thinking and in the application of your skills.

Section 1 - Characterization of microbial communities for Bacteria by 16S rDNA cloning and sequencing
1 - Isolate genomic DNA from bactéria
2 - PCR 16S rDNA as a positive control of primer sets of 12 – 24 primers to identify segments that bridge gaps in a bacterial genome sequence
3 – Purify segments and clone with TOPO-TA kit
4 - Sequence clones
5 – Perform alignment of sequences with rest of genome

Brief Lectures
The 16S rDNA loci and uncultured microbes
Principles of DNA isolation
Principles of primer design – multiple strategies for different uses
Newest methods of DNA sequencing
Cloning - plasmids, cosmids, BACs, enzymes
PCR and sequencing (potential trip to SDSU sequencing facility)
Bioinformatics - BLAST, NCBI, ribosomal database, the SEED – Ramy Aziz or Anca Segall
Other useful databases and sites
Practicals
Calibration of pipettors
Solution preparations
Autoclave and plate pouring
PCR
Polyacrylamide gel electrophoresis
TOPO and SLIC cloning
Bioinformatic analyses of 16S rDNAs

Section 3 - Design and conduct independent research.

You will discuss your ideas for this segment with Anca Segall or Jason Rostron, and get help with designing an experiment that will give meaningful results. However, you will be expected to think about and come up with ideas on your own first.

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 (20 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:
You will obtain up to 30 points for the practicals

Write-up of Second section project
This write-up will serve as the practice for the final project report. It is worth 30 pts.

Presentation and write-up of Final Project:
10-12 minute powerpoint presentation AND a report of your independent project (100 points). Presentations can be done as a group; reports are independent efforts.

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 or you will fail.
Grading Scale:
94 - 100%  A  74 - 76%  C+  60 - 63%  D
90 - 93.9%  A-  70 - 73%  C  below 60%  F
84 - 89.9%  B+  67 - 69%  C-
80 - 83%  B  64 - 66%  D+
77 - 79%  B-  60 - 63%  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 10µl of mammalian RNA, respectively.
Future: I will change X in order to get better results; or, I will do Y to answer the question that arises from my last experiment.

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....

DO NOT ERASE OR WHITE-OUT MISTAKES; cross out with a line or an X
DO NOT COVER OVER ANY TEXT

Schedule for Fall 2010 (Monday and Wednesdays)
Week 1  - Check in and Starting Quiz
  - Autoclave, pour plates with antibiotics
  - Genomic DNA isolation
  - Pipettors, calibrations, making solutions, and dilution problems

Week 2 – 5  - First project: closing a genome using multiplex PCR

Week 5 – 9  - Second project, starting with SLiC cloning
(note overlap)

Week 8 – 9  - Students will plan and discuss independent projects with instructors

Week 10 – end  - Independent (third) project

Last day in lab and check-out: December 8;

Dates for presentations to be determined; all independent 3rd project reports due December 16