INTRODUCTION

In the past, prokaryotes were used extensively to describe the basic properties of genetic material - what it is, how it is organized, how it works. At the same time, the basis of molecular biology was laid. Techniques were developed to allow us to dissect and analyze genes, chromosomes, and most recently entire genomes. The relative simplicity of prokaryotes allowed the delineation of the ground rules for the basic processes of replication, recombination and regulation of genetic material. Now we are moving on to apply these rules and techniques to more complicated organisms and problems - such as development, brain research, etc., In many cases the same techniques that were developed for the prokaryotes can be applied, with some modifications, to eukaryotic systems.

The aim of Bi180 is two-fold: to acquaint the student with the basic techniques used in molecular biology and to foster basic genetic research skills by allowing each student to carry out an original research project. This year's projects will involve magnetotactic bacteria. To date, there has been relatively little genetic characterization of these organisms, so that much of what we do will be done for the first time.

Course Content

In the first part of the course we will review a number of current molecular genetic techniques and in the second part each student will apply those techniques to a research project of general interest. The techniques that we will cover in the first part include:

Preparation of genomic DNA
Isolation of transposon-induced mutants
Restriction, ligation and electroporation
Preparation of plasmid DNA
Standard gel electrophoresis
Southern blot
Sequencing and computer searches for homologies
Gene mapping by hybridization
Isolation of genes by PCR
Pulsed-field gel electrophoresis

as well as standard microbiological techniques involving bacteria and bacteriophages.

The research projects will be concerned with the genetic characterization of a relatively unstudied group of organisms, magnetotactic bacteria, which synthesize and store crystals of magnetite, a magnetic form of iron. As a result of the presence of magnetite, the organisms align themselves with the earth's magnetic field, presumably because that helps them to find the right nutrient environment. The projects, therefore, will be directed toward the isolation, characterization, and mapping of genes that are involved in or regulate the uptake, storage, and utilization of iron. Currently we are also involved with the assembly and annotation of the complete genome sequence of the strain MS-1. Last years' projects included:

Isolation and characterization of non-magnetic strains of AMB-1.

Gene transfer by electroporation of AMB-1.
Isolation of conserved gene clusters from AMB-1**.**
Assembly of the MS-1 genome.
Connecting scaffolds with long PCR.

Schedule

Bi 180 is a 12-unit course consisting of 8 hr of lab, 2 hr of lecture, and 2 hr of homework. It meets three afternoons a week. For the first five weeks the schedule will be MW at 1:00 in 101K for an hour of discussion, followed by 2 hr of lab in 317K, and F from 1:00-4:00 in 317K. The remaining 2 hr of lab will be used to cover individual, unscheduled labtime, necessary to carry out longer procedures or experiments. The last five weeks will be spent entirely, 1:00 - 4:00, in 317K.

Requirements

Bi 180 is graded "pass-fail". The requirements for passing are:

1. Attend the discussions and carry out all the class exercises.

2. Keep a detailed lab notebook describing the exercises and subsequent project. Save the first 2-3 pages for a table of contents. Record all exercises. Include worksheets and results. See below for more details.

3. Carry out a small original project. This will be decided on together with the instructor, during the course and will be summarized at the end of the course by a written report. The written report should follow standard scientific format: ask a question, propose an experimental approach, describe the result, and draw a conclusion. It should supply enough detail so that next year's students can continue the project, if necessary.

Notebooks.

Write up each exercise as if it were a small project. For example, using the first exercise: start by writing a question, e.g. “What is the relationship between optical density and concentration of viable organisms ?” Then, write a “Results” section, e.g. “we measured the optical density of an overnight culture of *E. coli* strain MS-4100 grown in LB broth following the instructions in the lab manual and got the following results”. Make a table for the data where possible. Then continue with “we also measured the concentration of viable bacteria by colony assay” and again tabulate your results. Finally make your calculations and draw your conclusions. If the exercise did not work, suggest why and what you would do to “trouble shoot”. If it did work, critique the procedures or discuss how the results might be improved upon.

Reading.

There is no required textbook. The following general references will be kept on reserve in the Caltech Library.

"*Microbial Genetics*" by Maloy, Cronan and Freifelder (Jones and Bartlett).

"*Molecular Genetics of Bacteria*", Snyder and Champness (ASM Press).

"*Molecular Cloning*", Sambrook, Fritsch and Maniatis (CSH Press).

"*PCR Protocols*", Innis, Gelfand and Sninsky (Academic Press)

"*An Introduction to Genetic Engineering*", Nicholl (Cambridge)

"*Pulsed Field Gel Electrophoresis*", Birren and Lai (Academic Press).