

Bio 437: General Overview and the three lab modules for Fall, 2009: from Prof Kranz

The magnificent boom in biotechnology since the 1970s is a direct result of the ability to manipulate and measure nucleic acids. These advancements have revolutionized research in health and the environment. This course is designed as a hands-on experience that will provide the student with an understanding of how research on nucleic acids is performed and that also may be of use in the biotechnology field.

- 1) **Cloning genes and sequence analysis.** We will PCR, clone and express genes that encode the putative cytochrome c synthetases that assemble c-type cytochromes. (The last two years' Bio 437 students cloned the cytochrome c genes that compose a microbial "nanowire" for electron transfer.) The genes cloned this year from various bacteria, *ccmF* and *ccsBA*, encode the synthetases that will allow overproduction and assembly of the nanowire cytochromes in *E. coli*. Moreover, as a prelude to the three dimensional determination of a protein (eg. enzyme or transporter) it is common to clone the gene from many different organisms and overproduce the recombinant protein product in *E. coli*; and then empirically determine which one can be overexpressed and crystallized. Students will use different expression vectors and genomes from different bacteria to optimize success in achieving cloning of the *ccmF* and *ccsBA* genes. The main scientific question: can we engineer, overexpress and ultimately purify, characterize, and crystallize the cytochrome c synthetases encoded by *ccmF* and *ccsBA* genes? Training rationale for these projects: learning to design and clone genes for expression is a hallmark of the biotechnology industry. (Both the CcmF and CcsBA proteins are integral membrane proteins which are particularly challenging to clone and overexpress.)TA: **Erica Fishel**
- 2) **Constructing knockout mutants (deletion of genes from the genome), and/or integrating recombinant DNA into the genome.** Students will "knock out" genes in *E. coli* and/or integrate recombinant DNA that may be involved in cytochrome c assembly. The homologous recombination we depend on is conceptually similar to those used to make "knockout" yeast or mice (i.e. to construct mutants in these eukaryotes). The main scientific question: what proteins or cofactors are necessary for assembling a cytochrome c ? Training rationale for these projects: learning the importance of mutant analysis and design to test a hypothesis. An overall goal is to better understand the impact that genetics can have on human health and environment. TA: **Brian San Francisco**
- 3) **Microarray study on mRNAs for gene expression profiling.** Students will carry out a separate project on analyzing the gene expression profile of all genes in a eukaryote (i.e. the plant *Arabidopsis thaliana*). Such microarray studies are now the gold standard for comprehensive understanding of what genes are involved (i.e. expressed) in certain conditions (or diseases). The main scientific question: what genes in a plant are specifically induced or repressed when the plant is starved for nitrogen (i.e. fertilizer)? Training rationale for this project: learning the details of mRNA analysis and global gene expression profiling. (eg. many studies on global gene expression profiling of human cancer cells are unraveling what genes are involved in the cancer state). TA: **Maggie Wilson**

Other learning goals: Although we will not sequence or annotate an entire genome, we will emphasize throughout the course how and why whole genome sequences have revolutionized biology and biotechnology. Note that there will be a couple papers to read, relevant to the research projects, which will be background for why and what the goals are for each module.

If time, we will examine *Arabidopsis* plants that are expressing the reporters LUC (luciferase) or GFP (green fluorescent protein). This will illustrate the usefulness of gene expression reporters. Plants like these will be further studied in module 3 on mRNA analyses.

There are many other methods of biotechnology (nucleic acid manipulation) which we will not use (eg. RNAi, yeast two hybrid, site-directed mutagenesis studies). The last two lectures will be dedicated to describing some of these in the context of the projects we have completed.

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