# MOLECULAR BIOLOGY AND BIOCHEMISTRY
# MBB 432-3

## Advanced Molecular Biology Techniques

### Spring 2012

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**Instructor:** Dr. D. Sinclair, SSB 6148

**Description/topics:** **Course Outline:** This course will give hands-on experience in sophisticated techniques that are used on a daily basis in both basic research labs and in the biotech industry. The techniques will be presented as a logical series of experiments on a small GTP-binding protein, Rac, which is a signaling molecule of great interest at present, due to the important cellular processes it participates in and its implication in cancer and other diseases. The work will involve various manipulations of the cDNA encoding the Drosophila version of Rac. The lecture component will consider some of the latest advances in molecular techniques including protein expression systems, transgenic animals, RNAi, live imaging with GFP, etc. One lecture and one 4 hour lab each week.

Some of the objectives of the experiments are listed below:

1. The Rac cDNA cloned into the pGEX expression vector will be used to prepare a GST-fusion protein. The preparation of GST-fusion proteins is one of the most widely used approaches to generating the protein product of a gene of interest. It allows one to do biochemical characterization of that gene's product as part of an exploration of gene function.
2. We will do site-directed mutagenesis on the Rac cDNA to create constitutively active and dominant negative mutant versions of the gene. Site-directed mutagenesis is a technique that has revolutionized molecular biology. It allows one to make specific changes in the coding sequence of a gene, thus altering its function, and is one of the foundations of gene engineering used in the biotech industry.
3. We will look at the use of transgenic animals to study gene function. Transgenic animals have made a very important contribution to understanding gene function in recent years and are emerging as important tools in biotech. We will subclone our mutant cDNAs into the Drosophila transgenic expression vector pUAST, and look at their expression during Drosophila embryogenesis using the GAL4-UAS expression system and RNA in situ hybridization.
4. We will use the yeast two-hybrid system to screen for binding partners for a Rac pathway member. The two-hybrid system is being extensively used throughout the molecular biology research community to find new binding partners for proteins, and to test predicted interactions. This technique has made a substantial contribution to understanding how proteins come together in complexes to regulate a range of cellular events.

**Grading:** 2 midterms (20% each) a report on work done in the lab (35%), lab quizzes and problem sets (15%), evaluation of lab performance (10%). Midterms will be held in class time.

**Required texts:** None, but a useful reference is the MBB 308 textbook: Howe, Christopher J.(2007). Gene Cloning and Manipulation (2nd Ed.).

**Prerequisite:** MBB 308 and MBB 331 (or BISC 331) or permission of the instructor.

**Notes:** -A non-refundable fee of $20.00 will be assessed for the lab manual and readings.