Syllabus for MCB 3895 (Experiments in Molecular Biology)

This course encompasses a 75 min lecture / discussion period and a 3 h laboratory component that will be closely supervised by the instructor and a teaching assistant. Grading will be based on lab reports, participation in the laboratory exercises, and written exams to test the students’ knowledge base. The enrollment cap is 14 students.

Content of the laboratory component:

Lab 1: Recording of UV spectra of DNA (intact and digested), RNA (intact and digested), and free nucleotide bases to familiarize students with the theoretical foundations of nucleic acid quantitation procedures.

Lab 2: Preparation of total RNA from Arabidopsis leaves.

Lab 3: Quantitation of RNA via spectrophotometry; reverse transcription – polymerase chain reaction (RT-PCR) of mRNA from the UGE5 gene encoding UDP-glucose 4-epimerase using an initial primer set.

Lab 4: Agarose gel electrophoresis of the above RNA preparation and RT-PCR reactions; re-amplification of cDNA with a nested primer set to obtain a more defined product for molecular cloning purposes.

Lab 5: Agarose gel electrophoresis of products from the second round of PCR; topoisomerase-mediated insertion of cDNA into a cloning vector for PCR products; transformation of E. coli with the above construct.

Lab 6: Plasmid preparation from several independent clones; digestion with restriction enzymes to determine the presence and sizes of cloned fragments. This step will also verify the predicted structure of UGE5-derived cDNA.

Lab 7: Agarose gel electrophoresis of digested plasmid preparations. Purification of recombinant UGE5 via Ni-NTA columns; measurement of protein concentrations. The source of the recombinant UGE5 will be a liquid culture of an existing strain of E. coli that contains the UGE5 cDNA in an expression vector.

Lab 8: SDS-PAGE of crude extracts from E. coli and the purified UGE5 protein; transfer of protein to PVDF membrane for western blotting.

Lab 9: Native gel electrophoresis for size determination of the intact UGE5 protein that tends to form dimers and tetramers; development of the western blot via a colorimetric visualization system.

Lab 10: Time course of incubation of UGE5 protein with uniformly $^{13}$C-labeled UDP-glucose or UDP-galactose; hydrolysis of reaction products to release glucose and galactose from
the nucleotide sugars. This experiment is designed to familiarize the students with the concept of thermodynamic equilibrium that should be reached regardless of whether UDP-glucose or UDP-galactose is used as the substrate. The $K_{eq}$ value for the UDP-glucose / UDP-galactose pair is approximately 3.

Lab 11: Conversion of the released monosaccharides to alditol acetates for quantitation by gas chromatography – mass spectrometry (GC-MS).

Lab 12: Clean-up of products from Lab 11, and start of GC-MS data acquisition (separation of glucitol hexaacetate from galactitol hexaacetate, and recording of mass spectra to distinguish between monosaccharides from the labeled nucleotide sugars and potential unlabeled contaminants).

Lab 13: Analysis of data from the GC-MS runs (quantitation of the alditol acetates, and plotting their abundances as a function of length of incubation time).

Lab 14: Determination of UGE5 kinetic parameters ($K_m$, $V_{max}$, $k_{cat}$) via a coupled assay system using UDP-galactose as the substrate, and UDP-glucose dehydrogenase as the coupled enzyme to generate NADH from NAD$^+$ for quantitation via spectrophotometry.