

Laboratory Schedule

Monday - June 16 Protein Chemistry

I. Sample Preparation

Protein extracts from coral samples will be prepared from frozen tissue by first grinding in liquid nitrogen. Powdered tissue will then be extracted using four different extraction buffers to demonstrate critical features in sample preparation.

Tuesday – June 17 Protein Chemistry

II. Protein Concentration Assays

Protein concentrations will be determined using the Ghosh et al method as modified by Downs et al. Students will receive instructions in features of this assay to best achieve accuracy and precision, such as reproducible pipetting, type of filter paper, types of reagents and manufactures, etc. Students will also be introduced to NIH Image for assay quantification.

Wednesday – June 18 Protein Chemistry

III. Protein Gel Electrophoresis (SDS PAGE) and Western Blotting

Students will use samples prepared on days one and two to conduct western blotting. Students will learn how to prepare a polyacrylamide gel, load the protein sample and receive tips parameters that influence the protein gel electrophoresis process. Once proteins are separated, the students will prepare the gels for western transfer and immunostaining using chemiluminescence staining. Gel images will be digitally captured.

Thursday – June 19 DNA Chemistry

Students will use a portion of the frozen tissue powder prepared on day one for DNA analysis. DNA from frozen tissue will be extracted using a Dojindo Molecular Technologies DNA isolation kit (Get pureDNA Kit-Cell, Tissue, GK03-01). DNA purity will be assessed by spectrophotometry readings at 260nm/280nm ratios. DNA will be quantified using Molecular Probe's Picogreen Quantification Kit. DNA lesions will be measured using the Dojindo AP Site Assay kit.

Friday – June 20 DNA Chemistry

The AP Assay will be completed and data analyzed by the students.