Summer Bridge Program/Research Experience for Undergraduates

STEM FYE program & Laboratory of Marine Genomics (RSMAS)

Laboratory 2 – DNA extraction

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When performing a laboratory experiment, it is important that you carefully read the protocol; you need to understand what you are doing and why you are doing it. This understanding is critical during troubleshooting if your experiment does not work (and experiments often fail). However, if you know what to expect during each step of a procedure as well as the critical parameters during that step, your experiment is much more likely to succeed. As you do more and more experiments, the steps that you perform will make more sense. For molecular biology experiments, think of an experiment as similar to cooking where you add specific ingredients for specific reasons. For instance, when you add a leavening agent (baking powder or baking soda), this makes your cake rise. In a molecular biology experiment, when you add a buffer, this maintains the pH of your reaction, and when you add for example, MgCl2, the Mg+2 is a necessary co-factor for an enzyme.

In today's laboratory, we will be isolating genomic DNA from tissue samples. As you know, genes in the genome code for many different proteins that are required to carry out the functions of the tissue. Our goal is to eventually sequence a gene encoded by the mitochondrial genome, the Cox I gene or cytochrome oxidase I gene. To do so, we need to isolate genomic DNA (this isolation includes the isolation of the mitochondrial genome) and enrich for the Cox I gene. We will enrich for the Cox I gene using an *in vitro* (in glass) enzymatic process called PCR. PCR stands for Polymerase Chain Reaction, and we will discuss PCR next week.

To isolate genomic DNA from a tissue, we need to release the DNA from the cells in the tissue. We can easily burst open cells (for instance, mechanically disrupt the cells, make cells hypertonic, etc.). However, when we burst open cells, we release everything else in the cells, not just the DNA. Everything else includes the proteins, cofactors, and enzymes that allow the cells to function. Included in this everything else are proteases and nucleases. Proteases degrade proteins and nucleases degrade nucleic acids, including DNA. Thus, when we burst open the cells, we need to protect the genomic DNA from these nucleases (in particular, DNAses). In the cell, DNAses are tasked with destroying any misplaced DNA (*e.g.,* DNA found outside of the nucleus).

In order to recover intact DNA, cell lysis must be performed under conditions that inactivate DNAses. These conditions include: proteinase K, SDS, and EDTA. Proteinase K is a protease (degrades proteins) and hence will degrade DNAses. SDS (sodium dodecyl sulfate) is a detergent that helps disrupt cell membranes (detergents disrupt fats) but that also often inhibits enzymes. However, SDS does not inhibit the enzyme proteinase K; in fact, proteinase K activity is enhanced by the presence of SDS. EDTA (ethylenediaminetetraacetic acid) sequesters (binds up) divalent ions from solution. Mg+2 is a divalent ion that is a necessary cofactor for many enzymes, including DNAses. Thus, by making Mg+2 unavailable, EDTA inhibits the action of any DNAses released during cell lysis.

For today’s laboratory we will follow the DNA extraction protocol in order to extract DNA from our sampled individuals. Remember to follow the protocol as well as the instructions of the professor and the T.A. Be extremely neat and always keep track of your samples. It is required to begin recording information in a laboratory notebook. For the notebook, record the date and title of the experiment as well as any valid observation worth noting for future analysis.