

In this lab students will conduct two gel electrophoresis experiments. The SDS-PAGE experiment is used to elucidate protein structure. One of the basic ways to understand a protein is to know its mass. There are a few methods to learn the mass of a protein. In this exercise students will use SDS-PAGE gel electrophoresis to estimate the mass of the subunits of an unknown protein. In conjunction with information gathered from other methods, the number of subunits of the unknown protein also will be determined.

For the second experiment students will receive a portion of their amplified DNA sample from the lab earlier in the quarter. They will check for PCR product in two ways: by visualizing the product on an agarose gel and by checking the concentration using the spectrophotometer. Students also will learn how to use BLAST, ClustalX and Mega4, programs to be used in the analysis of individual sequences in lab 10.

Concepts: understanding protein structure; understanding protein size estimation methods; understanding qualitative and quantitative measures of PCR success; understanding MCRA and how to read phylogenetic trees; understanding the concept of the molecular clock.

Skills: proper use of micropipetters; loading an SDS-PAGE gel; creating a standard curve in Excel; determining the size of an unknown polypeptide using a standard curve; load and run an agarose gel; use of the spectrophotometer to determine DNA concentration; using BLAST to align two DNA sequences; estimating a divergence rate for a set of sequences; aligning multiple DNA sequences in ClustalX; drawing a phylogenetic tree in Mega4.