

Protein purification is a crucial procedure in order to analyze the behavior, structure, and function of a select protein. The Orf8 protein is an increasingly mutable accessory protein found within the SARS-CoV2 virus, however the complex and unpredictable nature of this protein has been met by its inability to be purified thus far. By purifying the model proteins, PaIVY1 and PaIVY2, we believe that we can denote a sound purification method to apply to the Orf8 protein. In our procedure, we transformed BL21(DE3)pLysS cells and inoculated them in LB media. The cells were then placed into a centrifuge to isolate the pellets and the substance was homogenized. We then used a microfluidizer to lyse the cells and used Immobilized Metal Affinity Chromatography (IMAC) to perform a His 1. To remove the 6-His tag from the N-terminal end of the protein, we completed a dialysis and TEV cleavage. Finally, we used IMAC a second time to complete a His 2 and Size Exclusion Chromatography (SEC) to ensure that our protein was pure. We successfully ran an SDS-PAGE gel and imaged it. The PaIVY1 and PaIVY2 samples were visible on the gel. The process that we used for purifying PaIVY1 and PaIVY2 is applicable to the Orf8 project as it entails a baseline purification method that produces a significant amount of protein for analysis.