PROTEOMIC ANALYSIS OF AN E. COLI ORF135 KNOCKOUT MUTANT

Laura Filkins, *Dr. Suzanne O'Handley, *Dr. Thomas Kim, *lmf9140@rit.edu*

Orf135 expresses an enzyme that hydrolyzes CTP and dCTP. This enzyme may be involved in nucleotide and/or lipid metabolism based on its substrate specificity. Therefore, its role in cellular activity is being investigated. Two-dimensional gel electrophoresis is being utilized to analyze the differences in protein expression between wildtype *E. coli* and an Orf135 knockout mutant. The lack of the Orf135 enzyme in the knockout can cause the appearance of new proteins in the mutant, the down-regulation of proteins in the mutant that are present in the wildtype, as well as an increase or decrease in the concentration of specific proteins. Currently, the protein expressions of both the wildtype and knockout from pI 4-7 and molecular weight of 15-70 kDa are being compared. Seven proteins in this region have already been selected with high confidence as differentiated proteins. These proteins, along with two landmark proteins, are currently being prepared and sent out for identification by the Ohio State University Mass Spectrometry and Proteomics facility. The identification of those proteins unique to either the wildtype or knockout proteome or those that have significantly changed concentrations should help determine a broader role for Orf135 in *E. coli*.