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Uncovering Gene Functions of the Mycobacteriophage Phayonce

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Bacteriophages are viruses that infect bacteria and the genomes of phages discovered and annotated by SEA-PHAGES represents a vast repository of genes. Because the functional significance of many genes is lacking, SEA-GENES aims to analyze multiple phage genomes to identify cytotoxic genes and protein interactions in an unbiased manner. During our first semester of SEA-GENES, we analyzed all 77 genes from the P5 cluster phage Phayonce using a series of protocols to determine whether expression of each gene is cytotoxic to the host bacteria, *Mycobacterium smegmatis*. First, we attempted PCR to amplify each gene and clone the ORF into the shuttle vector pExTra, which allows for inducible expression of cloned ORFs in *M. smegmatis*. Verified clones were transformed into *M. smegmatis* and assessed for cytotoxic effects by measuring relative cell growth on inducing and control media. We tested 24 different genes and observed cytotoxic effects of five phage genes, two of which have putative functions previously inferred by molecular homology. This includes Phayonce\_33 (immunity repressor) and Phayonce\_77 (adenylate cyclase). Interestingly, induction of Phayonce\_77 was also cytotoxic, suggesting Phayonce\_76 and Phayonce\_77 might have similar biochemical functions during infection. Although we were unable to test cytotoxicity of all genes, our results can be compared with other SEA-GENES schools to discover cytotoxic functions of specific phage gene phamilies.