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Research and Analysis of the Function of Gene 30 in Mycobacterium Phage Girr

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Girr is a Temperate Siphoviridae Mycobacterium Phage in the F Cluster and F1 subcluster which is known to infect the Mycobacterium Smegmatis. It was isolated from a sample taken in St. Louis, MO, and was annotated by the SEAPHAGES cohort at the University of South Florida. It contains 103 genes and is 57754 base pairs long. In this year’s SEAGENES cohort every one of Girr’s genes is being researched to learn more about their possible functions and develop a better understanding of the field. Gene 30 is a hypothetical protein that is 174 base pairs long in Pham 6905 with no known function. This research seeks to explore the effects of Gene 30 when inserted into the genome of bacteria and assessing possible cytotoxic or immunity characteristics which could provide more insight into its function or role. In order to carry out this research multiple protocols were required in order to insert the desired gene into the genome of the targeted bacteria so the gene could be expressed, and analysis could be carried out. To accomplish this the Gene needed to be isolated via Polymerase Chain Reaction (PCR), then inserted into a prepared plasmid, pExtra, via Isothermal Assembly. pExtra is a custom-made plasmid designed with sites for gene insertion as well as origins of replication that allow it to be integrated and expressed by M. Smeg and E. Coli. It was a crucial part of the research and would not be possible without it. Following Plasmid prep, the plasmid containing the gene was inserted into target bacteria through E. Coli and M. Smeg Transformation via shaking or electroporation respectively. After the completion of these protocols Gene 30 was assimilated into the bacterial cells via pExtra and Cytotoxicity and Immunity assays were carried out to test to potential toxic or protective effects of Gene 30. Despite the insertion of Gene 30 there were no differences from the controls of either assay showing that it did not kill the host cells or protect them from infection by phages. Regardless of the negative results it is not conclusive as there is still more testing that would be required to validate them. Further research would include the investigation of potential protein-protein interactions and sequencing the DNA to validate the accuracy of the insert, as a single mistake in the insert anywhere along the line could alter the results drastically. The findings of this research could have widespread ramifications which could contribute to the medical field as well as paving the way for advances in the war against anti-biotic resistant bacteria. We thank Danielle Heller, Vic Sivanathan and the SEA GENES team for support of this project