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The Cloning and Evaluation of Mycobacterium Phage Girr Gene 28

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Bacteriophages are viruses that infect strains of bacteria. Mycobacterium phage Girr is a phage that uses Mycobacterium smegmatis as a host; Girr is also an F1 cluster phage with 103 genes and a size of 57754 base pairs. Although over 3,000 phages have been discovered through the SEA-PHAGES/SEA-GENES programs, 70% of annotated phage genes have a hypothetical function. In order to potentially increase the amount of functional genes within the database of phage genes, a series of tests and protocols were performed to determine if a gene has functional capabilities such as toxicity in bacteria. Gene #28 of Mycobacterium phage Girr is a gene that underwent this series of tests; Gene 28 has a size of 135 base pairs of mRNA. Gene 28 was initially determined to be a protein without function due to poor results from evaluation through HHPred. However, gp28 has transmembrane helices; this suggests that the gene may cause lysis or be involved with host immunity. The research question was: what is the role of Mycobacterium phage Girr gp28 in improving the operation of Girr through impacting its ability to have toxicity as a function of gp28? A series of protocols were performed to evaluate gp28. A PCR reaction was first performed with a following purification; then, the gene sample underwent an isothermal assembly reaction to construct a plasmid that can express gp28. The pExTra-Girr28 plasmid was produced with the backbone DNA of the pExTra plasmid. The pExTra plasmid has a size of 6000 base pairs, holds origins of replications that allow for replication in M. smegmatis and E. Coli, and produces a resistance protein to the Kanamycin antibiotic. After transforming the pExTra-Girr28 in E. Coli, the pure plasmid was then electroporated in M. smegmatis to prepared for the phenotypic assay protocols. The Cytotoxicity Assay protocol was used to determine whether gp28 had the ability to be toxic to the host M. smegmatis, while the Host Immunity Defense Assay protocol was used to determine if gp28 could protect infected M. smegmatis cells from infection by other phages. These protocols served the purpose of preparing and purifying a gene into a plasmid, infecting the plasmid into bacterial hosts of the phage, and testing the ability of the plasmid to lyse or protect a bacterial host. After performing the cytotoxicity and immunity phenotypic assays, the results concluded that gp28 was not toxic and did not give the host immunity against reinfection. A negative result for the cytotoxicity and immunity assays is not conclusive because the gene evaluated could be mutated or could require a protein-protein interaction with at least one other gene to be functional. In order to get more data, more tests can be done to evaluate whether gp28 has a mutation or requires protein-protein interactions to phenotypically show that it is toxic or can give its host immunity. We thank Danielle Heller, Vic Sivanathan and the SEA GENES team for support of this project.