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Phenotypic Analysis of Gene 43 Function in Mycobacteriophage Girr

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Girr is a phage with a 57,754bp genome and 103 genes located in the F1 cluster, and its host is M. smegmatis. This analysis is focused on gene 43 found in Girr. Gene 43 is part of the pham 19026, whose members are only found in the F cluster. It matches to hypothetical proteins in NCBI Blast, has no transmembrane domains, and it has no significant crystal structure hits in HHPRED, meaning it is currently designated as a hypothetical protein with an unknown function. However, this does not mean that it has no role in the phage’s life cycle. The goal of this research project is to determine if Gene 43 is involved in bacterial cell lysis or in preventing other phages from infecting a bacterial cell once Girr’s genome is inserted. To do this, multiple assays and tools were utilized. The most important one was pExTra. The pExTra plasmid is a 6,000bp plasmid that contains a Ptet inducible promoter, an mCherry reporter, a Kanamycin resistance gene, origin of replications for E- coli and for M. smegmatis, and a tet repressor that keeps the Ptet promoter off. This plasmid allows gene 43 to be replicated within E. coli and M. smegmatis bacterial cells. To start, the gene was amplified utilizing PCR. The primers had 10-15 bp that were specific to Gene 43 that were built off uniform pExTra sequences complementary to the pExTra plasmid. Then, gel electrophoresis was used to validate the PCR product. Then, it was purified and inserted into the plasmid utilizing isothermal assembly. E. coli cells were transformed with said plasmid via heat shock. They were then lysed to collect the replicated plasmid, which was then purified and used to transform M. smegmatis via electroporation. Then, cytotoxicity and immunity assays were performed to determine if gene 43 was cytotoxic and/or if it prevented further infection from other phages. When expressed for the Cytotoxicity assay, gene 43’s results did not differ much from the negative control, producing a negative result. When expressed for the Immunity Assay, gene 43 did not prevent infection from either other Girr phages or Larva phages, which are cluster K phages that also infect M. smegmatis. Due to these results, it is likely that gene 43 is not cytotoxic or involved in preventing infection from other phages, but this is not conclusive, as some changes can be made to produce more conclusive results. First, gene 43 might only be cytotoxic or provide immunity to further infection if it interacts with another protein, so studying protein-protein interactions would be useful. Additionally, gene 43’s protein expression levels were not measured during the project and doing so would be a good next step. Finally, gene 43 was not sequenced, meaning the insert could have has a loss-of-function mutation, but without it being sequenced, there is no way to know, so sequencing the gene insert would be an important next step. We thank Danielle Heller, Vic Sivanathan and the SEA GENES team for support of this project.