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Functional Analysis of Gene 89 in Mycobacterium phage Girr through Cytotoxicity and Immunity Assays

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F1 cluster with a genome of 103 genes. Gene 89 is a 237bp Hypothetical Protein with 82 members in the pham and currently no functional call based off HHPRED data. The purpose of this research is to investigate the potential cytotoxicity or immunity of genes within the Girr genome. The gene was isolated and amplified from the Girr genome through PCR and assembled into the pExTra inducible expression plasmid. pExTra contains Tetracycline-controlled transcriptional activation which allows for mediated expression of the gene as well as a Kanamycin resistance protein allowing for bacterial selection. The plasmid is transformed into E. coli cells and later electroporated into M. smeg in order to perform two phenotypic assays: cytotoxicity and immunity. The cytotoxicity assay involves M. smeg cells that have the gene-inserted plasmid plated on multiple Kanamycin plates each with varying levels of Tetracycline to measure influence of the gene on the bacteria. A positive result would be lysis of the bacterial cells resulting in a lack of growth of colonies on the spots. The immunity assay involves plates that contain the gene-inserted plasmid with spots of the Girr phage. If the gene is able to grant immunity to the bacterial cells then the phage would not be able to form a plaque and therefore no spots would be observed. From the two assays, Gene 89 showed negative results indicating that the gene has little cytotoxicity or immunity function. However, a negative result is not a definitive conclusion on the functional call of the protein. The gene was not sequenced meaning there could have been a detrimental mutation causing the protein to denature. The gene may be involved in cytotoxicity or immunity through a protein-protein interaction which it is deprived of in the plasmid. Also, both assays dilute the gene down to test for concentration, but it could be that the gene needs to be expressed at much higher concentrations than pExTra or the procedure is allowing. Western blotting or direct gene sequencing could solve two of these issues as a mutation could be detected and gene concentration could be monitored. To test for protein-protein interactions a 2-hybrid assay could be performed to test if the gene associates with any other Girr proteins. With 70% of all phage genes annotated being hypothetical, the potential for discovering genes of function with cytotoxicity or immunity is possible with these experiments. The impact of this research will give greater perspective of the identity of phage genes as well as improved annotation due to an influx of new functional calls. We thank Danielle Heller, Vic Sivanathan and the SEA GENES team for support of this project.