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Research and investigation into the function and characteristics of Girr Gene 49

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Bacteriophage Girr was found in St. Louis, MO and genetically annotated at the University of South Florida. Located in the F cluster (F1 subcluster), Girr is a temperate, siphoviridae phage. Research on each of Girr’s genes has been conducted with the intention of investigating and understanding more about their functions and purposes, which may lead to potential applications in several different fields (medical, environmental, therapeutical, etc.). Girr Gene 49, annotated as a hypothetical protein, is 216 base pairs long with no known function. This research investigates Gene 49 and seeks to measure its level of function within Girr and possible cytotoxic or immune attributes. To conduct this research, several protocols were utilized with the goal of integrating Gene 49 into a pExTra plasmid that would allow for its expression in the different types of bacterial hosts used, E. coli and M. smeg. The pExTra plasmid was a crucial part of this research, as it possesses a site for insertion of Gene 49 and allows for it to be integrated within its backbone. Furthermore, it possesses an origin of replication for E. coli, and an origin of replication for M. smeg, allowing Gene 49 to express itself in these bacteria after transformation. Polymerase Chain Reaction (PCR), Isothermal Assembly, Plasmid Prep, E. coli Transformation, and M. Smeg Transformation through electroporation were all protocols used in achieving this goal. After these protocols were performed and Gene 49 was integrated into the pExTra backbone within these bacterial cells, Cytotoxicity and Immunity Assays were carried out to evaluate Gene 49’s ability to kill other cells. The results of these assays showed that Gene 49 did not possess any cytotoxic traits or immunity characteristics. Based on this research, this means Gene 49 does not possess the ability or function to kill cells, nor does it possess the ability to protect its bacterial host from infection by phages. Although a negative result was acquired for Gene 49, this is not conclusive, as future research can be conducted to obtain more data. Further investigation is suggested, and potential next steps include looking for possible protein-protein interactions, DNA sequencing to validate insert accuracy, and conducting a His Tag/Western Blot experiment. The impact of this research will be important to understanding the unique functions of bacteriophage genes and how certain characteristics may be useful in large-scale application. Learning more about the presence of cytotoxicity and immunity in phage genes may lead to research that can be applied to real-world problems in the medical field or environment and may contribute to breakthrough advances within these fields. We thank Danielle Heller, Vic Sivanathan and the SEA GENES team for support of this project.