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2021 SEA Symposium Abstract

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Analysis of bacteriophage Girr gene 86 properties

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The relevance of bacteriophage research in today’s society is of increasing significance, as bacteriophages can infect and kill bacteria that may be deleterious to human health. Therefore, by isolating bacteriophages along with annotating bacteriophage genomes, the research process can be advanced to researching the functions of genes and their roles in reference to infection. For this research, bacteriophage Girr’s genome is studied, specifically gene 86. Bacteriophage Girr is a siphoviridae bacteriophage that has a genome of 57,754 base pairs, possesses 103 genes, is a member of the F cluster and is categorized in the F1 subcluster. Girr infects Mycobacterium Smegmatis and appears to have a lytic life cycle. Girr’s gene 86 has 180 base pairs and has been previously identified to have no known function, it belongs to a pham (a categorization method grouping genes with similar characteristics and relative positions in the genome) in which there are 163 other genes and matches 100% to gene 85 in bacteriophage SiSi. This research investigates the terra-incognita of bacteriophage Girr’s genome and furthermore studies the properties of individual genes to reveal biological functions such genes may possess, such as the cytotoxic or defensive functions of a gene, more specifically, research is conducted on Girr’s gene 86. The methodology for this project includes performing a PCR to amplify gene 86, utilizing gel electrophoresis to confirm the gene, purifying the gene and utilizing isothermal assembly to proceed to prepare the plasmid (which is pExTra for this process), then proceeding to diagnostic procedures with E. Coli, once confirmed, the host bacteria Smegmatis is then transformed with the plasmid with gene 86, and a cytotoxicity assay is performed, followed by the immunity assay. The cytotoxicity assay utilizes serial dilutions and a control of different pExTra plasmids that contain varying amounts of molecule anhydro-tetracycline (aTc), to induce expression of the gene, or lack thereof, and with such allows the determination of if a gene is nontoxic (in which results would show no changes in growth) or toxic. The immunity assay determines if a gene inhibits infection of another bacteriophage and is performed by serial dilution and utilization of bacteriophage Larva in this research. The observations recorded for gene 86 is that this gene does not present cytotoxic properties based on the cytotoxicity assay performed. Furthermore, the observations of the immunity assay reveal that gene 86 does not appear to possess properties that protect its infected host cell from a secondary bacteriophage. Advancements from these observations can include immunity assays on gene 86 compared with other phages, as well as utilizing other research methods to explore what properties this gene possesses. We thank Danielle Heller, Vic Sivanathan and the SEA GENES team for support of this project