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Toxicity Analysis of Mycobacterium Phage Girr’s Gene 29

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Girr is a 57754-base pair, Cluster F phage that infects Mycobacterium smegmatis. It contains 103 genes, which approximately 60% of them produces a product with no known function. This includes gene 29. Gene 29 is 222 base pairs long. It is found in Pham 53380, which contains phages’ genes across multiple clusters, which none of them called a function during their annotation process. Gene 29 has poor HHPred hits, and no transmembrane helices were detected. Will the expression of Girr’s gene 29, a hypothetical protein, be toxic towards the bacterial host, M. smegmatis? First, pExTra plasmid was constructed with gene 29 by PCR, column purification, and isothermal assembly. Using pExTra plasmid is crucial as it contains the origin of replications for E. coli and M. smegmatis, mCherry reporter to validate expression of the gene, kanamycin resistance gene to select for bacteria that contain this plasmid, and Ptet (promoter) and tet repressor to control the gene’s expression. Second, pExTra plasmid with gene 29 (plasmid DNA) stock is generated by E. coli transformation and thermo geneJET plasmid miniprep. Finally, plasmid DNA is introduced into M. smegmatis with transformation by electroporation to determine toxicity by a cytotoxicity assay and a defense assay. The cytotoxicity assay and defense assay revealed the expression of Girr’s gene 29 is non-toxic towards M. smegmatis. However, this negative result is not conclusive. It is possible that DNA mutation(s) occurred; therefore, DNA sequencing could confirm whether the plasmid DNA has the correct nucleotide sequence for gene 29. Even a single nucleotide change could affect the toxicity results. It is also possible that the gene is expressed, but M. smegmatis is killing off the protein and this could be evaluated by running affinity chromatography. We thank Danielle Heller, Vic Sivanathan and the SEA GENES team for support of this project.