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Phase-Two of SEA-GENES Research Project: Investigating Mycobacteriophage Kumao Gene Functions at Lehigh University

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Kumao is a Singleton mycobacteriophage identified from a soil sample collected by a Lehigh University undergraduate student in 2015. Its genome was sequenced at the Pittsburgh Bacteriophage Institute and subsequently annotated in 2017 by students from Lehigh University’s SEA-PHAGES program and a participant from the University of Pittsburgh’s Phage Hunters Integrating Research & Education (PHIRE) initiative. The Kumao genome comprises 70,373 base pairs and encodes 115 protein-coding genes, along with a single tmRNA gene. Interestingly, 35 of its genes are orphams, lacking identifiable homologs in other phages. Kumao being a singleton, coupled with the presence of orphams constituting over 30% of its genome, motivated its selection for functional characterization in our SEA-GENES research project. In the initial phase of our SEA-GENES study, 99% of Kumao genes were successfully amplified, approximately 63% were cloned into the pExTra vector for tet-inducible expression, and ~10% of the genome was phenotyped to assess cytotoxicity and potential defense mechanisms in *Mycobacterium smegmatis*. Building on previous efforts, we successfully achieved PCR amplification of all but three remaining protein-coding genes in Kumao this year and cloned a subset into the pExTra vector for phenotypic analysis. Certain genes consistently failed to be cloned and were therefore excluded from further investigation. As a result, our focus shifted toward completing the phenotypic characterization of genes that remained uncharacterized from the prior year. To date, 100% of Kumao’s protein-coding genes have been amplified, and 85% of its genome has been successfully cloned, revealing that 6 out of 21 tested genes exhibit cytotoxicity in *M. smegmatis*. Our ongoing work aims to expand cytotoxicity assessments for all cloned Kumao genes by the end of the semester. Additionally, we will conduct defense assays to determine whether the overexpression of specific Kumao genes confers resistance to *M. smegmatis* against Kumao and other bacteriophage infections. These findings will provide valuable insight into the functional roles of singleton phage genes, particularly those lacking known homologs.