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2025 SEA Symposium Abstract

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B2H Interaction Assay: Exploring the Mechanism of Interaction Between Phage Island3 and Host Mycobacterium smegmatis

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Island3 is a bacteriophage that infects Mycobacterium smegmatis (M. smeg). In past years, Northwestern College students have reannotated Island3’s genome, performed genetic screens, and performed interaction assays with it. Though extensive research has been done on this phage and its genome, there is still much that is not understood about the mechanisms through which it functions. To explore the mechanism of action for genes 35 and 36 (both known to be cytotoxic), I performed a bacterial-2-hybrid interaction assay with each of these genes, creating a plasmid containing hybrid gene with the target phage gene in place of the alpha subunit sequence of RNA polymerase through molecular cloning. An additional hybrid plasmid, containing a CI repressor and M. smeg genome fragment hybrid gene, was introduced to represent the host genome. Using this method, one potential interaction between Island3 gene 35 and an M. smeg host fragment has been found. Further trials are needed to verify this interaction, and repeat experiments are needed for gene 36.