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2022 SEA Symposium Abstract

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Determination of gene function in Cluster P1 mycobacteriophage Brusacoram

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Cluster P1 mycobacteriophage Brusacoram contains 78 protein coding genes, 65% of which have no known function. Previous mass spectrometry experiments performed by our students have determined that at least 34 of these genes are expressed within the first 3 hours of infection, including at least 12 with no known function. This suggests possible roles during the Brusacoram life cycle for several genes with no known function. As part of the SEA-GENES program, a series of phenotypic assays were performed to further elucidate gene function across the Brusacoram genome. Each protein coding gene was targeted for PCR amplification and then cloned into the pExTra expression vector through isothermal assembly. In total, 51 of the 78 Brusacoram genes were successfully cloned into pExTra and were then moved on to sequential analysis in phenotypic assays, including cytotoxicity and defense assays. To date, 27 gene constructs have been successfully electroporated into *M. smegmatis*. In the process, we have identified 12 toxic or slightly toxic genes, 11 with no known function and some of which were detected via mass spectrometry. Our defense assays were less successful, as we encountered a tenacious contamination problem that interfered with the successful completion of many of our experiments. For the few successful assays, we have not yet identified a gene that confers homotypic defense. In the future, we plan on completing defense assays for the remaining genes that were previously successfully electroporated and moving on to protein interaction experiments.