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Identification of cytotoxic genes in mycobacteriophage Brusacoram

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Cluster P1 mycobacteriophage Brusacoram has 78 putative protein coding genes in its 47,618 base pair genome. Consistent with other annotated phage genomes, 46 of these genes in Brusacoram have no known function. Previous RNAseq and tandem mass spectrometry experiments at our institution have confirmed the expression of the majority of these genes, including those with no known function. Through participation in the SEA-GENES program, we are in the process of elucidating the role Brusacoram genes with no known function may play in host cell infection. In the SEA-GENES project, Brusacoram genes are first PCR amplified and cloned into the pExTra expression vector for use in a series of phenotypic assays. To date, we have successfully PCR amplified nearly all 78 Brusacoram genes and cloned over 60 into pExTra constructs. We have then employed these constructs in phenotypic assays to determine cytotoxic or homotypic defense effects of Brusacoram gene expression in Mycobacterium smegmatis. In these assays, we have identified genes that exhibit varying levels of cytotoxicity. No genes that exhibit defense against phage infection have been identified, although our dataset is incomplete. We are also in the process of examining a select few toxic genes for effects on host cell morphology using fluorescent microscopy.