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Identification of genes from phage Lebron that are toxic upon overexpression in Mycobacterium smegmatis

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Bacteriophages engage in complex dynamic interactions with their bacterial hosts and their genomes display high genetic diversity. Bioinformatics tools have been used to assign function to many of the genes but the vast majority of genes have unknown function. Determining the functions of phage genes will help elucidate the mechanisms of infection. To this end, the GENES class at La Sierra University studied Mycobacteriophage Lebron. Lebron, which infects *Mycobacterium smegmatis*, is a temperate phage with a 73,453 bp genome and contains 120 putative protein-coding genes, 31 of which had been previously assigned a function, and 9 tRNAs. In order to begin to elucidate the gene functions of other, the first 66 genes of the genome were amplified by PCR and cloned into plasmid pExtra-01. After verifying by PCR and gel electrophoresis that each plasmid had the right insert, the plasmids were introduced into *Mycobacterium smegmatis* via electroporation. Using cellular toxicity of phage gene overexpression as an assay, the expression of 10 genes resulted in inhibition of *M. smegmatis* growth either by reducing colony number and/or size. Out of the 10 genes, 7 of them have not been assigned a function. Combining the cytotoxicity data with future defense assays and phage-host interactions will provide further insight into the role of those genes in the phage life cycle and possibly identify novel phage therapies.