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Amplification and Cloning of Mycobacteriophage Pixie and ThetaBob Genes

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Bacteriophages are rapidly becoming a topic of interest in the health community because of their use in combating antibiotic-resistant bacteria. The field of bacteriophage research is not fully developed yet, and only a small amount of knowledge has been gathered on the diverse bacteriophage population. A large portion of protein functions are still unknown to us, but this research helps us grow the library of known functions. Working through the SEA-GENES program, our group amplified, cloned, and PCR-verified 99 of 106 genes for ThetaBob, and sequence-verified 71 of 100 genes for Pixie. Continuing work with Pixie, we amplified 24 and cloned 18 genes for phenotypic assays to study their use against antibiotic-resistant bacteria. Using high-titer lysate DNA, genes were amplified by PCR, and products were verified through gel electrophoresis. PCR products were then purified by either column purification or gel extraction, depending on the banding patterns of the gel. The purified products were then ligated into the pExTra01 plasmid with isothermal assembly. Ligated plasmids were transformed into 5-alpha F’Iq *Escherichia coli*, extracted, and sequenced. Extracted DNA was electroporated into *Mycobacterium smegmatis*. Preliminary phenotypic assays with Pixie indicated 38 potentially cytotoxic gene products grouped in clusters.