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11th Annual SEA Symposium Abstract

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A BLAST Result from the Past: Isolation and Analysis of Archived Cluster A6 Phage Hexamo

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The student participants in the 2014-15 year of the SEA-¬PHAGE project were members of the interdisciplinary program Introduction to Natural Sciences, a year-¬long, full-¬time learning community with integrated instruction in biology, chemistry, and science process skills. During the fall quarter students collected and purified phages using enrichment cultures of *Mycobacterium smegmatis* mc² 155 at 37 C. During this cycle 23 phages were isolated from local soils, purified, and entered into the PhagesDB collection. During fall 2017, during a DOGEMS (Deconvolution of Genomes after En Mass Sequencing) run a new A6 phage was assembled and PCR experiments identified this phage as the archived Hexamo from this collection. As relatively few A6 genomes had been completely annotated at that point, we used the supplied total sequence to fully annotate the genome of this phage.

This set of phages had their DNA purified and analyzed by restriction enzyme digestion and gel electrophoresis. Hexamo was visualized by transmission electron microscopy after negative staining with uranyl acetate. This resulted in a clear image which appeared to be *siphoviridae*. DNA from these phages was sequenced using the Illumina process at the Pittsburg Bacteriophage Institute. The sequence of Hexamo revealed a 52,359 bp linear double stranded DNA genome with a sticky ten bp 3’ overhang and with a GC content of 61.4 %. Analysis of the sequence of this phage confirmed that it was a siphoviridae in the A6 subcluster. BLASTn results indicated its closest relative was Artemis2UCLA. The genome was analyzed for potential protein coding open¬‐reading frames using Glimmer and GeneMark, and protein functions were predicted by BLASTp and HHPred, as well as examining synteny with related phages using Phamerator. Predicted starts were also examined using Starterator. Preliminary results suggest the presence of 105 protein coding genes and a predicted translational frame shift. Particular care was taken in the region around the minor tail proteins and head-tail connector region to use best available data to annotate this region. All expected genes for a temperate replication mode were present. We identified 3 tRNA genes using ARAGORN and tRNAscan-SE. The final annotation passed quality control and is now in GenBank as accession MK 359360.