THE ANNOTATION OF THE PARTIAL GENOME OF BACTERIOPHAGE *STAGNI* WITH THE USE OF PROGRAMS DNA MASTER AND VIRTUAL MACHINE

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The Siphoviridae mycobacteriophage *Stagni* was isolated and annotated from a soil sample using the principles and guidance of the HHMI SEA-Phage program. The SEA Phages program allows undergraduate students worldwide to get involved in the studies of genetics through the observation and annotation of bacteriophage genomes. The importance of this research lies in the attempt to combat multidrug resistant strands of bacteria. Since mycobacteriophage are a form of virus that targets bacteria cells specifically, the use of phage therapy is being considered to help combat the rise in antibiotic resistance. *Stagni*'s initial gene placement was predicted through the auto annotation program DNA Master after its structural data was received from University of Pittsburgh. Using additional information provided by coding files from the Glimmer and GeneMark programs as well as online BLAST databases (for comparisons to known proteins from other phage), we were able to check the predictions and placements made from DNA Master and Virtual Machine in order to reaffirm or modify the annotated gene. Out of the ninety sequenced genes picked up by DNA Master, forty-four were successfully annotated (from sections A and C), having genes modified and added into the program after comparisons with other research groups. Although the structures of *Stagni* have been determined, the functions of these proteins are currently unknown, opening up further explorations into other research opportunities such as the infections of other bacteria.

Introduction

- Mycobacteriophages are viruses that infect mycobacteria species
- *Stagni* is a lytic Siphoviridae mycobacteriophage (digests *M. smegmatis*)
- *Stagni* was isolated at Nankin Lake Livonia, MI
- Extracted DNA was sent for complete sequencing
- The genome from *Stagni* was broken into sections
    - Sections A and C were fully annotated
    - Section A included potential genes1 through16
- The goal was to fully annotate the genome of *Stagni*

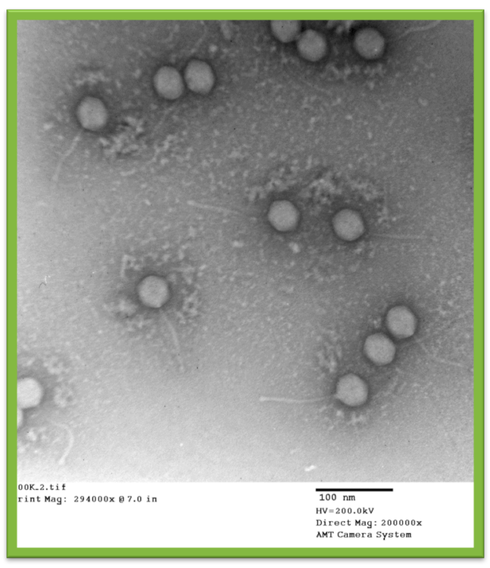

Figure 1: (Left) The TEM Image of *Stagni* showing structure, capsid size and shape, as well as tail length

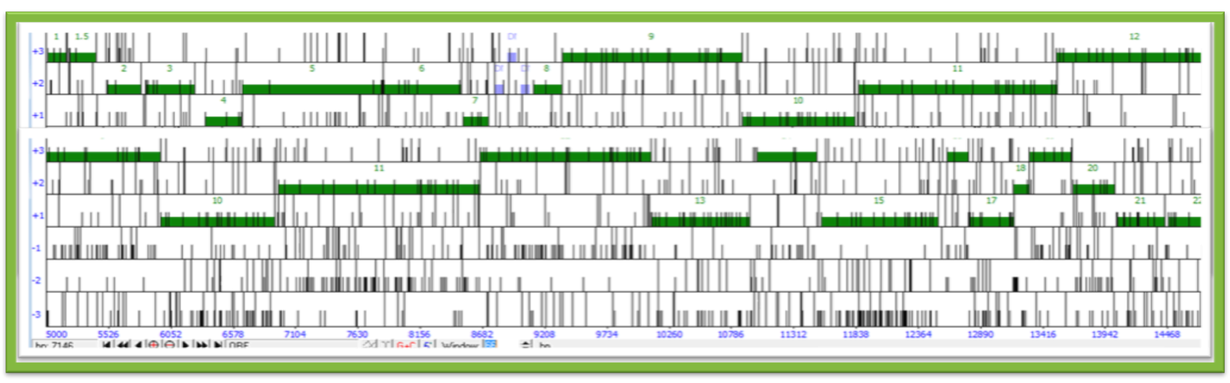

Figure 2: (Above) Open Reading Frames from Section A of *Stagni* (genes 1-16)

Methods

- Searched for large gaps between genes in the DNA Master Frames
- Checked GLIMMER and GeneMark files for possible coding potential
- Inserted the gene as gp1.5 in DNA Master
- Ran an external BLAST of the given product
- Observed the Shine Dalgarno (SD) scores
- Compared the genome of *Stagni* to BLAST results using Phamerator

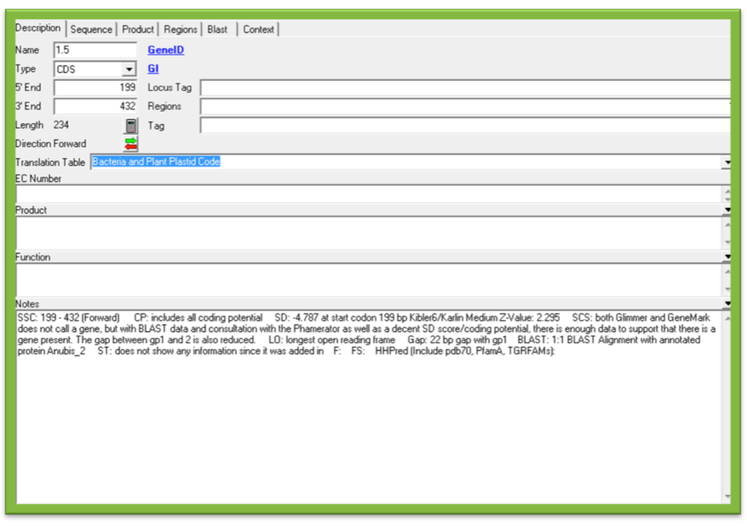

Figure 3: (Right) The description window from DNA Master of gene 1.5

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Results

- SSC: (199-432) Forward Strand
- CP: Includes all possible coding potential
- SD: Z Value: 2.295 Final Score: -4.787 (highest score) Kibler6/Karlin Medium
- SCS: Glimmer: does not call a gene GeneMark: does not call a gene
- LO: yes, longest open reading frame
- Gap: no predicted overlap, 22bp gap with gp2
- BLAST: BLAST Alignment 1:1 with several annotated proteins including Anubis_2 and Wooldri_2

```
>Wooldri_2, function unknown, 67
         Length = 67

 Score =  120 bits (302), Expect = 3e-28
 Identities = 56/65 (86%), Positives = 58/65 (89%)

Query: 1  MSLMWLLSHSRGPATLDPKVSPTIGCRGKREGNLRRGERWGLALARPSEEPPPKRLPSGS 60
          MSLMWLLSHSRGPATLDPKVSPTIGCRGKREGNLRRGERWGLALARPSEEPPP   P G+
Sbjct: 1  MSLMWLLSHSRGPATLDPKVSPTIGCRGKREGNLRRGERWGLALARPSEEPPPSGSPPGA 60

Query: 61 ARSAP 65
           + P
Sbjct: 61 PGALP 65



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Sbjct: 1  MSLMWLLSHSRGPATLDPKVSPTIGCRGKREGNLRRGERWGLALARPSEEPPPSGSPPGA 60

Query: 61 ARSAP 65
           + P
Sbjct: 61 PGALP 65
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Figure 4: (Above) BLAST data from the two closest matches with the predicted gene 1.5

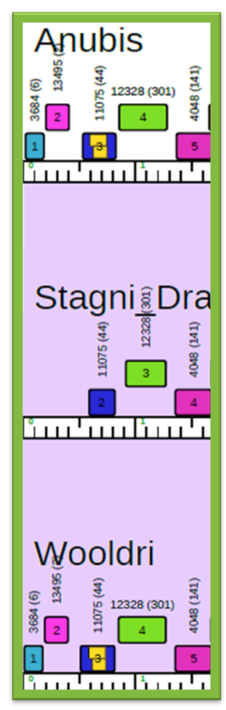

Figure 5: (Right) Results from Phamerator showing the similarities between Wooldri, Anubis, and Stagni at the location for a possible addition of gene 1.5

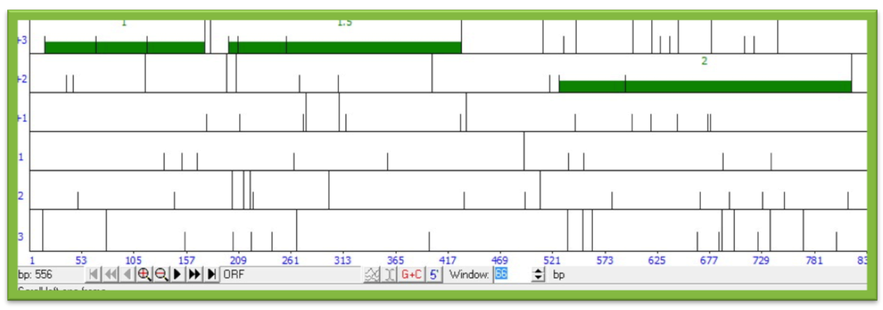

Figure 6: (Above) The inserted gene 1.5 showing its relation to gene 1 and gene 2

Discussion

- The data provided by the external BLAST and Phamerator concludes that there was an unpredicted gene present (gene 1.5)
- The new gene discovery allows for a more complete phage database
- Unique phage discoveries can lead to more possible phage therapy on bacterial strains (as opposed to antibiotics)
- Future project: *In vitro* and *ex vivo* experimentation of *Stagni* genes

Acknowledgements

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- Jacob D. Kagey, Joshua Thomson, and C. Knight for instruction and assistance during the annotation process of the genome
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