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# Group 3, Final Presentation

— Madalyn Alm, Kathryn Atherton,  
Barbara McAnulty, Sarah  
McGinness —

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# Introduction

- This research aims to produce an annotated genome of JewelBug, a newly discovered species of mycobacteriophage, for the SEA-PHAGES project.
- Mycobacteriophage are virions that infect mycobacteria in order to replicate.
- The SEA-PHAGES project is an international effort of students characterizing mycobacteriophage found in the environment to discover new genes and their functions.

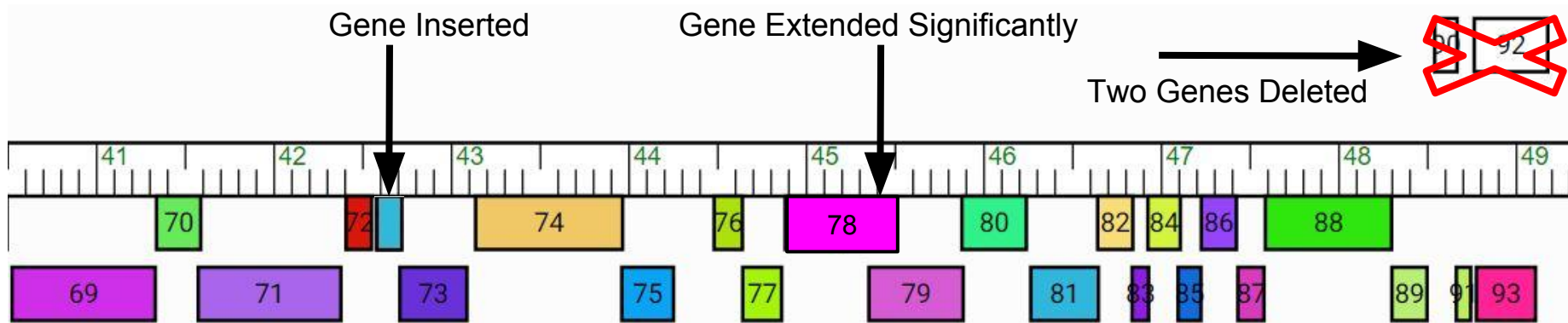



JewelBug's plaques after infecting  
mycobacterium smegmatis

# Why this annotation is important

- Viruses are the most genetically diverse group of biological material as well as the least studied. Therefore, they contain the largest number of novel genes.
- Expanding the databases of viral genomes allows for more efficient and higher-quality future annotations.
- The databases provide the groundwork for research into the evolution and adaptation of viruses which also provides information about evolution, adaptation, and susceptibility of bacteria. Two paramount concerns for human medicine.
- Mycobacteriophages have specific applications in human medicine since their hosts include *M. tuberculosis*.

# Genes 67-90: 40900-50341bp



24 genes originally called  23 genes in final annotation  
(two deletions and one addition)

# General Characteristics

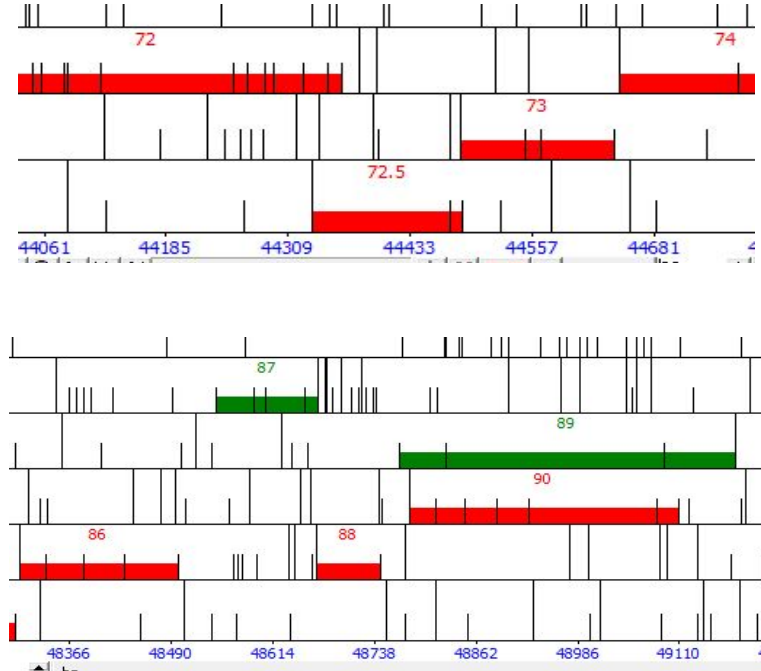
- All features were read in the backward direction in the complementary strand
- 61.6% GC
- Start codon frequency: 8.7% TTG, 37.1% ATG, 52.2% GTG
  - According to the Annotation Guide, TTG should be around 7% and GTG and ATG should be about 46.5% each
- Most BLAST results were hypothetical proteins

# General Characteristics

- A6 phages most closely related to JewelBug based on BLAST and Phamerator:
  - VOHMINGHAZI
  - ISIPHIWO
  - MCFLY
  - KAZAN
  - CLOUDWANG9
  - ARTEMIS2UCLA

# Major Changes in the Genome

- Adding a gene between features 72 and 73
  - There was a large 119 bp gap, GeneMark coding potential, and significant blast results once this feature was added
- Deleting features 87 and 89
  - Both features 87 and 89 were in the forward direction, even though all of the other features in this part of the genome were in the reverse direction
  - The features did not have strong BLAST results, if any, and there is not enough space between the surrounding genes to suggest a reversal of direction. Features 86, 88, and 90, on the other hand, have stronger evidence supporting their existence



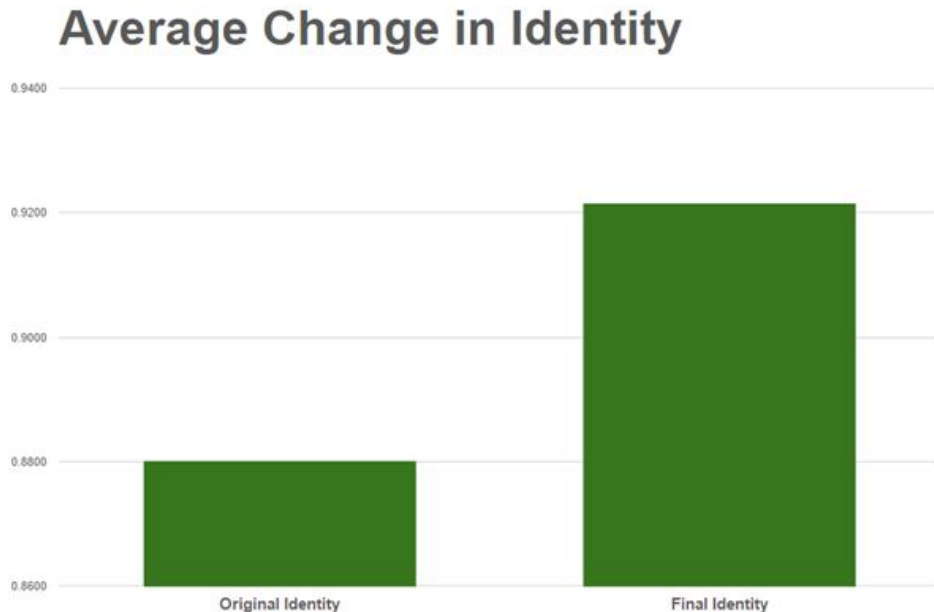
# Noteable Functions

- A significant proportion of our features had no known function after thorough analysis of the available evidence.
- The genes in our sections that did have supported putative functions were regulatory proteins (promoters and anti-repressors) or associated with DNA (DNA methylase and a helix-turn-helix domain).
- The majority of our features were similar with to features of other phages found within the A6 cluster with a few exceptions.



# Improvements from auto-annotation

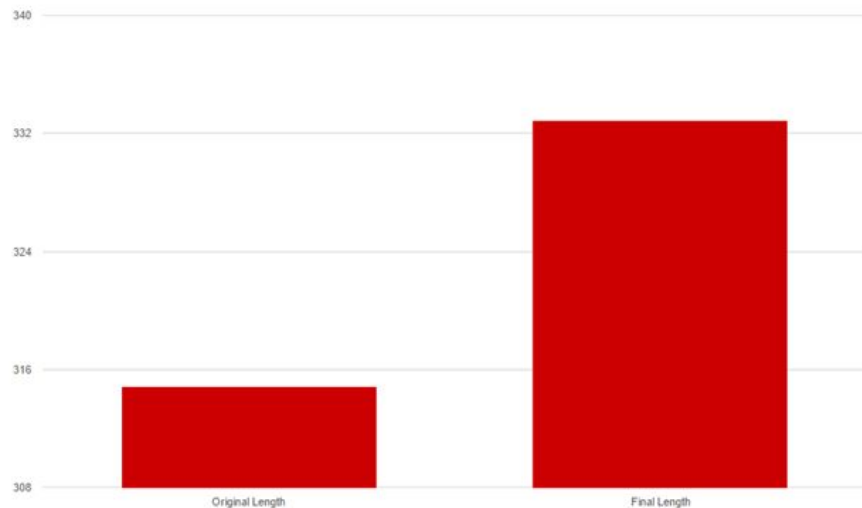
By moving a few start sites, the team was able to improve identity of JewelBug's proteins with proteins in the BLAST database by 4.7%.



# Improvements from auto-annotation

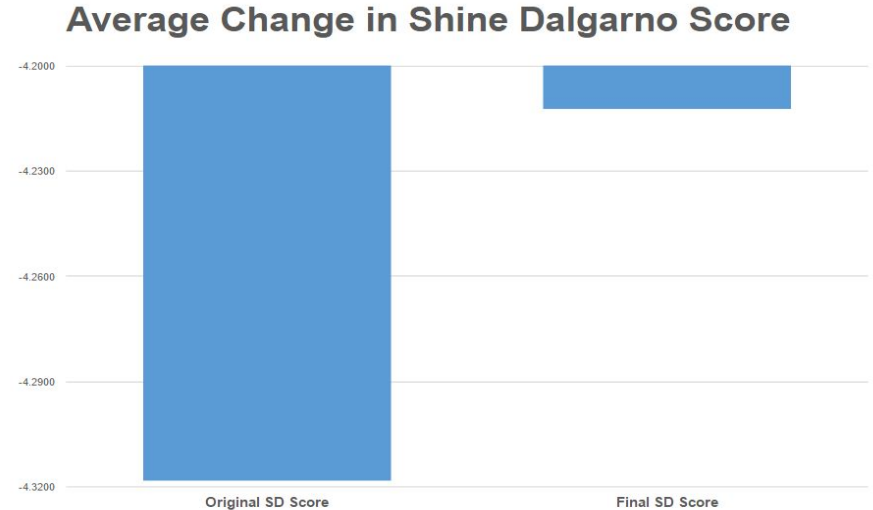
By moving a few start sites, the team was able to improve the length of genes by 5.7%, which reduces the gap between genes.

Average Change in Length of Gene



# Improvements from auto-annotation

By moving a few start sites, the team was able to improve Shine Dalgarno scores, a measure of the ability of ribosomes to initiate protein synthesis. The SD scores became 2.5% less negative. Although SD scores are not the most important measure, the guiding principles do call for a score closer to zero.



# Conclusions

- The auto-annotation by DNAMaster utilizing GeneMark and Glimmer gene call algorithms required several revisions including two gene deletions, one gene addition, and many start adjustments on its 40900 - 50341 base pair section.
- Wet lab evidence suggests that the JewelBug is a temperate phage. If this is the case, the genes found in this section of the genome are likely instrumental in the regulation of the JewelBug's life cycle as well as determining its host range.

# Future Work

- Send the completed annotation to University of Pittsburgh Howard Hughes Medical Institute quality control for wet lab verification of the gene calls
- Determine the function of the proteins that currently do not have a known function
- Determine the host range of JewelBug
- Compare to the rest of the A6 cluster to determine possible evolutionary path

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# Thanks for a Great Semester!

— Special Thanks to Dr. Clase and —  
Yi!!!

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