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Phage Hunting at the University of Mary Washington: Genome Annotation of Hari and JackRabbit

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Background and Objective

Background: Relatively few *Bacillus* bacteriophages are reported in GenBank, although those numbers are increasing. The genus *Bacillus* is a heterogeneous group, with some interesting members such as *B. cereus* and *B. anthracis*. These two species, along with *B. thuringiensis*, make up the Cereus group and are fairly closely related. However, this group is less related to other species of *Bacillus*. We are interested in whether phages isolated on *Bacillus thuringiensis* subsp. Kurstaki (BTK) infect the other two or a wider range of *Bacillus* species.

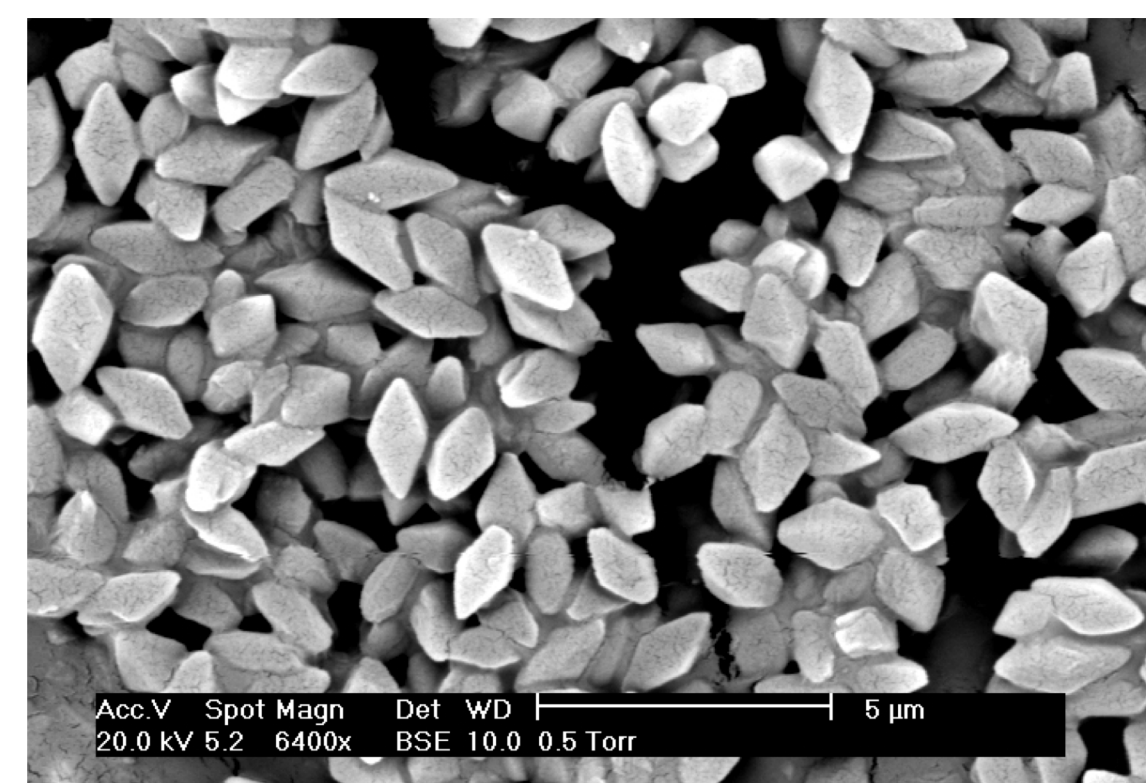


Figure 1. *B. thuringiensis* under a microscope.

Objective: To isolate and characterize the genomes of new and unique phages using *Bacillus thuringiensis* subsp. Kurstaki as a host, in order to increase knowledge about *Bacillus* phages.

Soil Sample Collection Location

Bacillus phage Hari was isolated by Hannah Stottlemeyer and Arianna Chase, and was extracted from a soil sample from Elkton, Maryland with the GPS coordinates 39°32'27" N 75°54'16" W, shown on the left. Bacillus phage JackRabbit was isolated by Jayme Boudreau and Vallarie Burge, and was extracted from a soil sample from Blackstone, Virginia with the GPS coordinates 39°32'27" N 75°54'16" W, shown on the right.

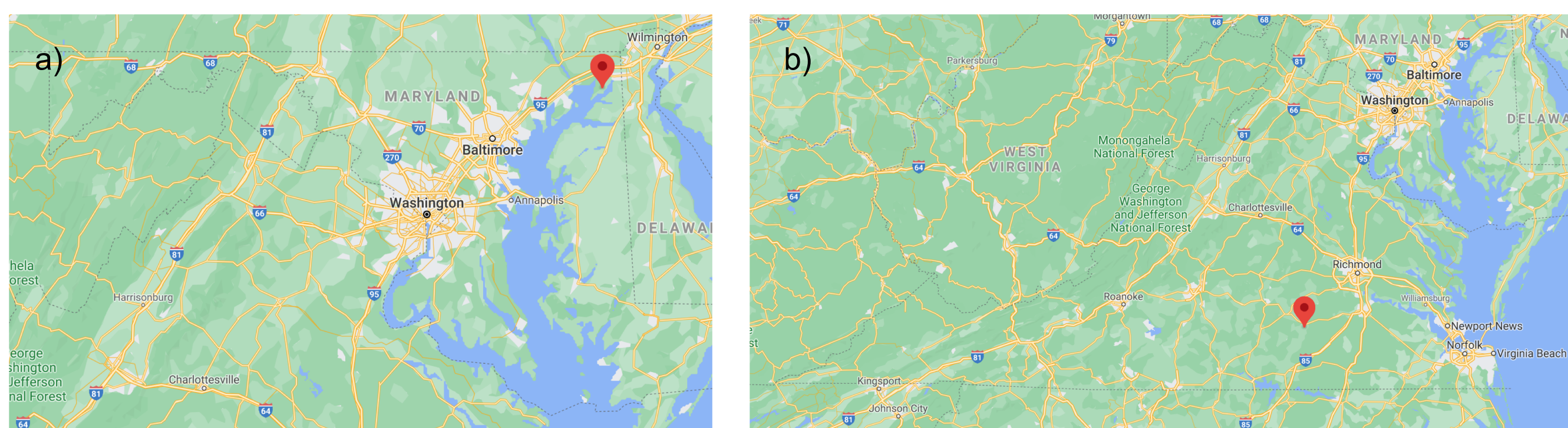


Figure 2. a) GPS location of Hari: 39°32'27" N 75°54'16" W Elkton, MD. b) GPS location of JackRabbit: 39°32'27" N 75°54'16" W Blackstone, VA.

Enrichment and Isolation

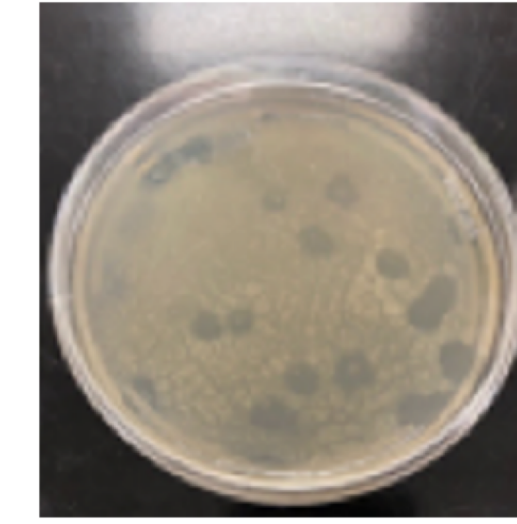
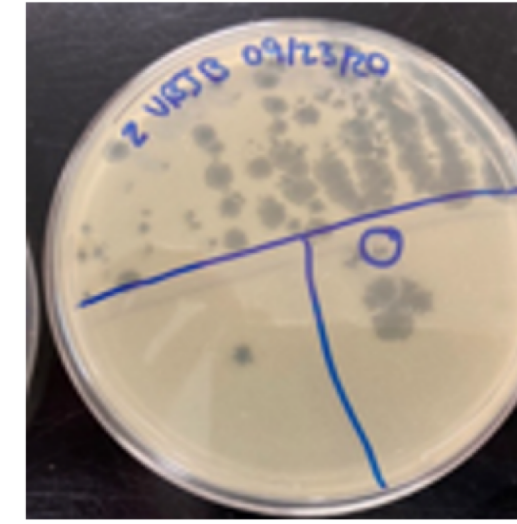
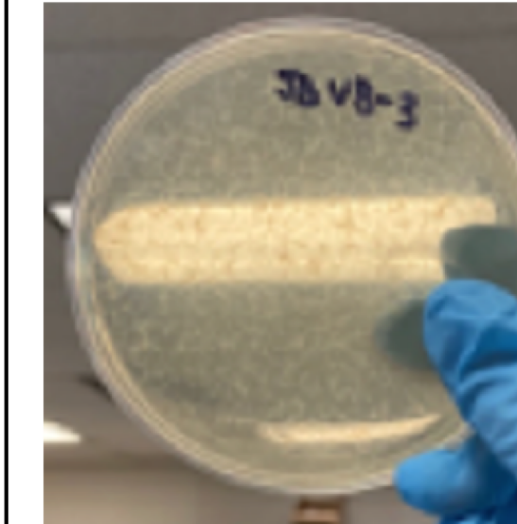


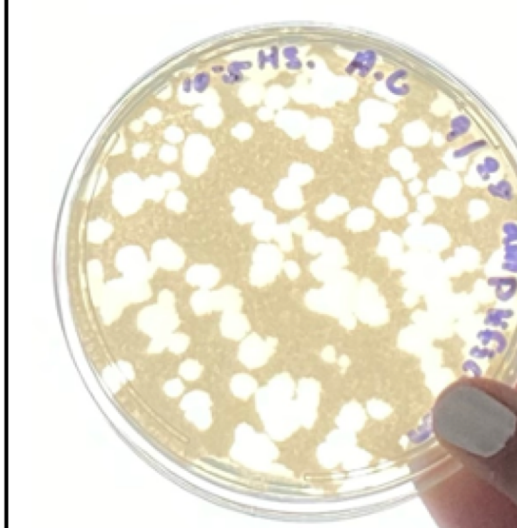
	Enrichment	T-Streak	Webbed
JackRabbit			
Hari			

Table 1. Comparison of agar plates throughout the enrichment and isolation processes.

TEM & Restriction Digest

The TEM and restriction digest of the two phages are shown below.

Hari

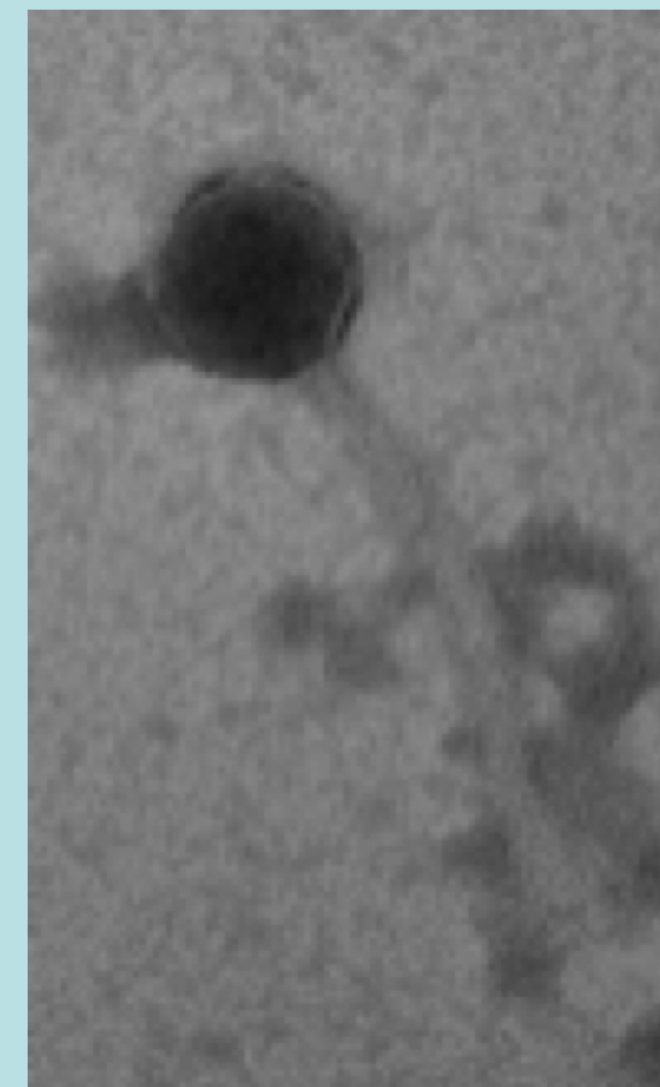


Figure 3. Hari TEM
Avg. capsid diameter: 25. nm
Avg. tail length: 95. nm

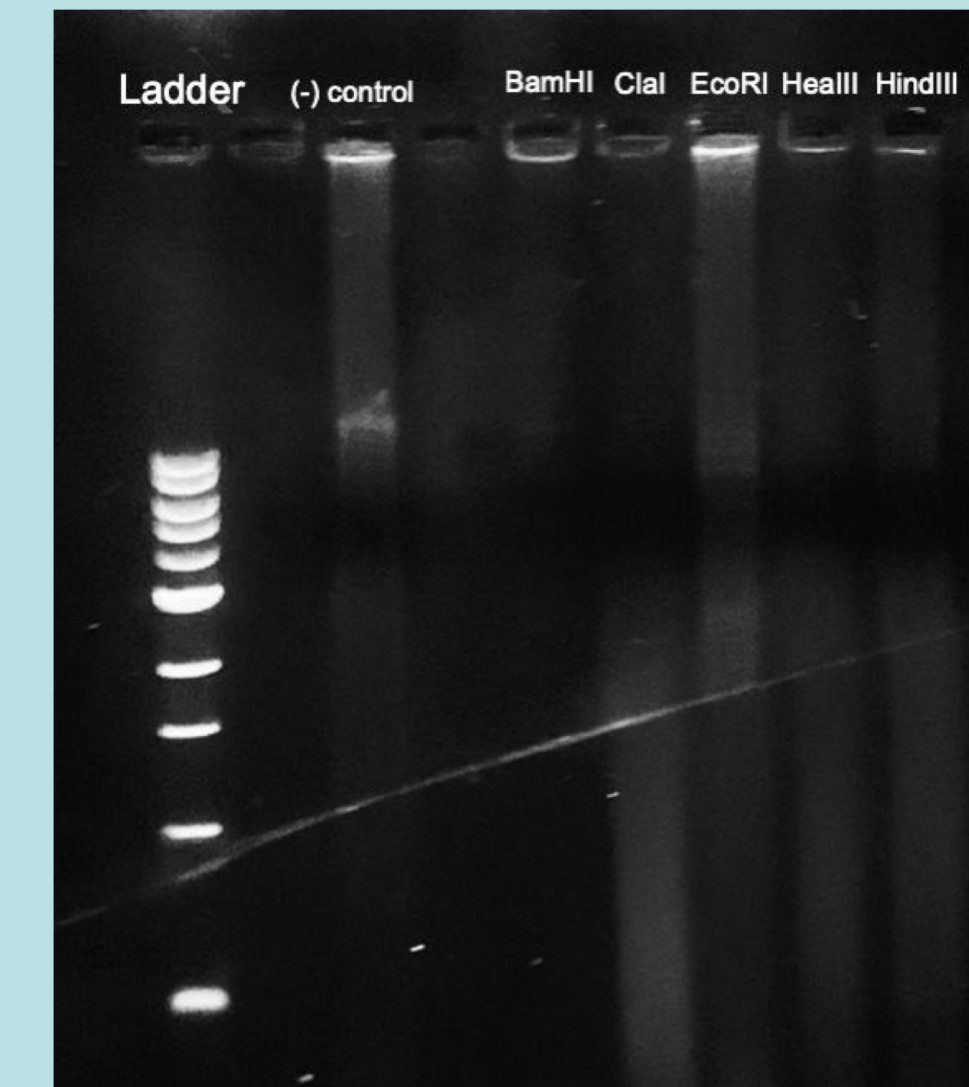


Figure 4. Wells from left to right: 1- DNA ladder; 3-undigested DNA; 5- BamH1; 6-Clal; 7-EcoR1; 8-HeaIII; 9-HindIII

JackRabbit

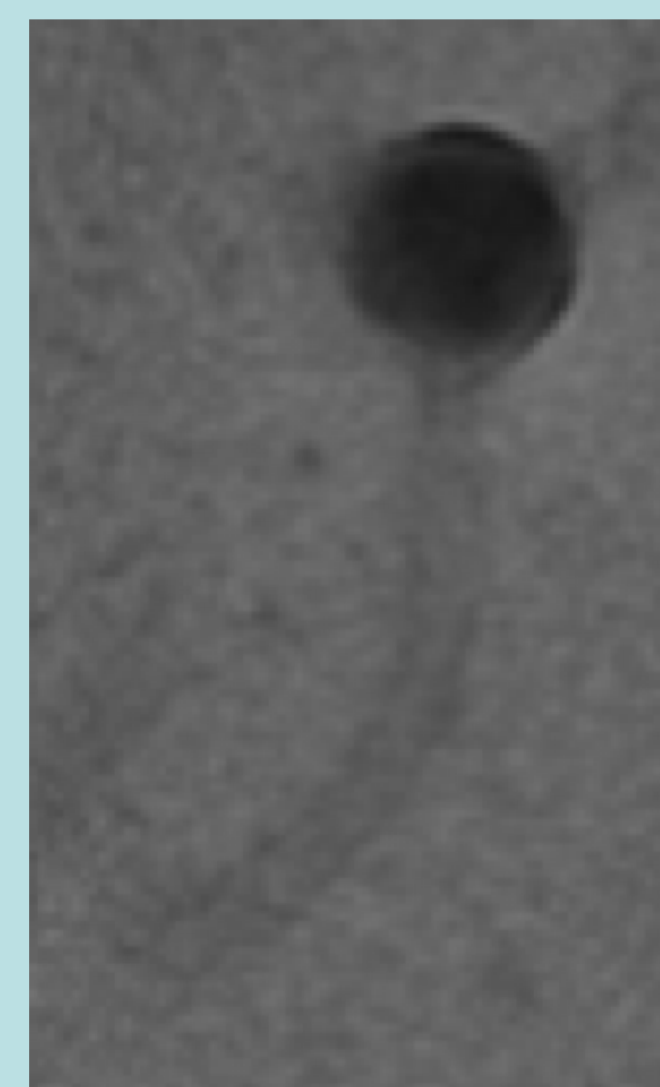


Figure 5. JackRabbit TEM
Avg. capsid diameter: 18. nm
Avg. tail length: 54. nm

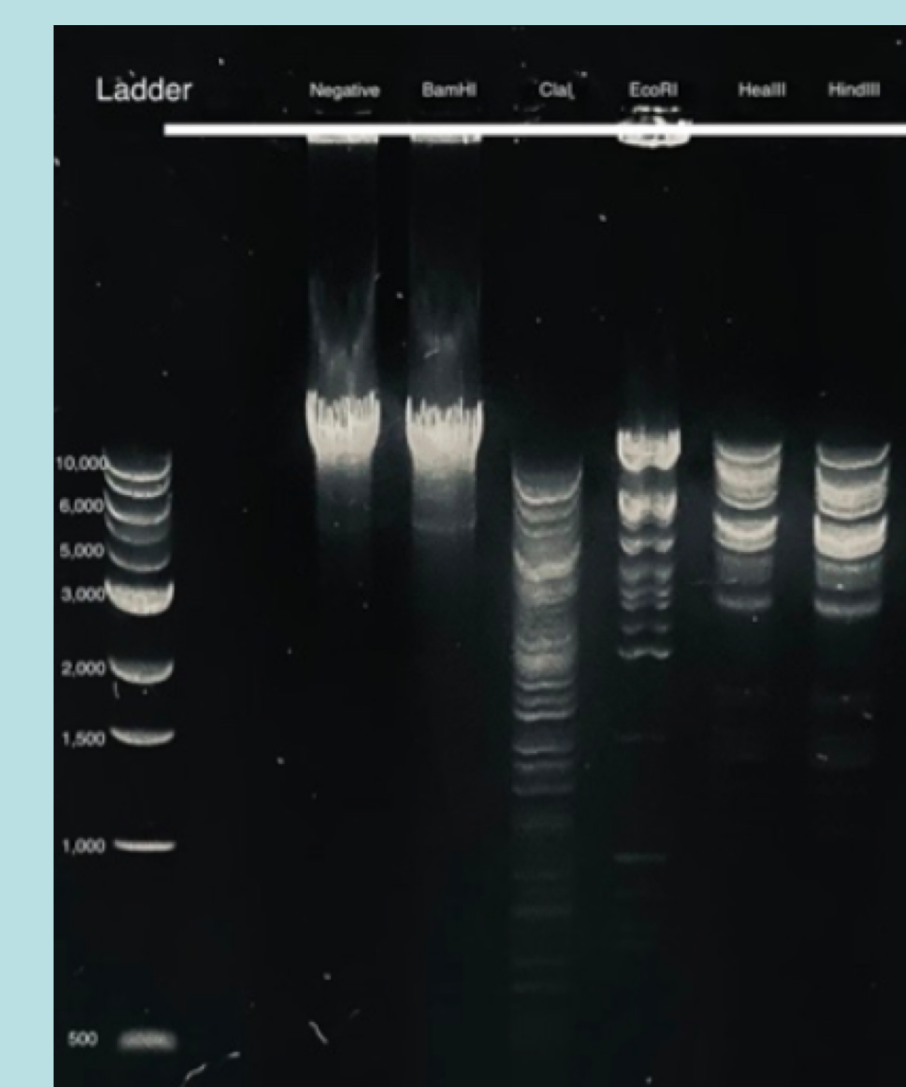
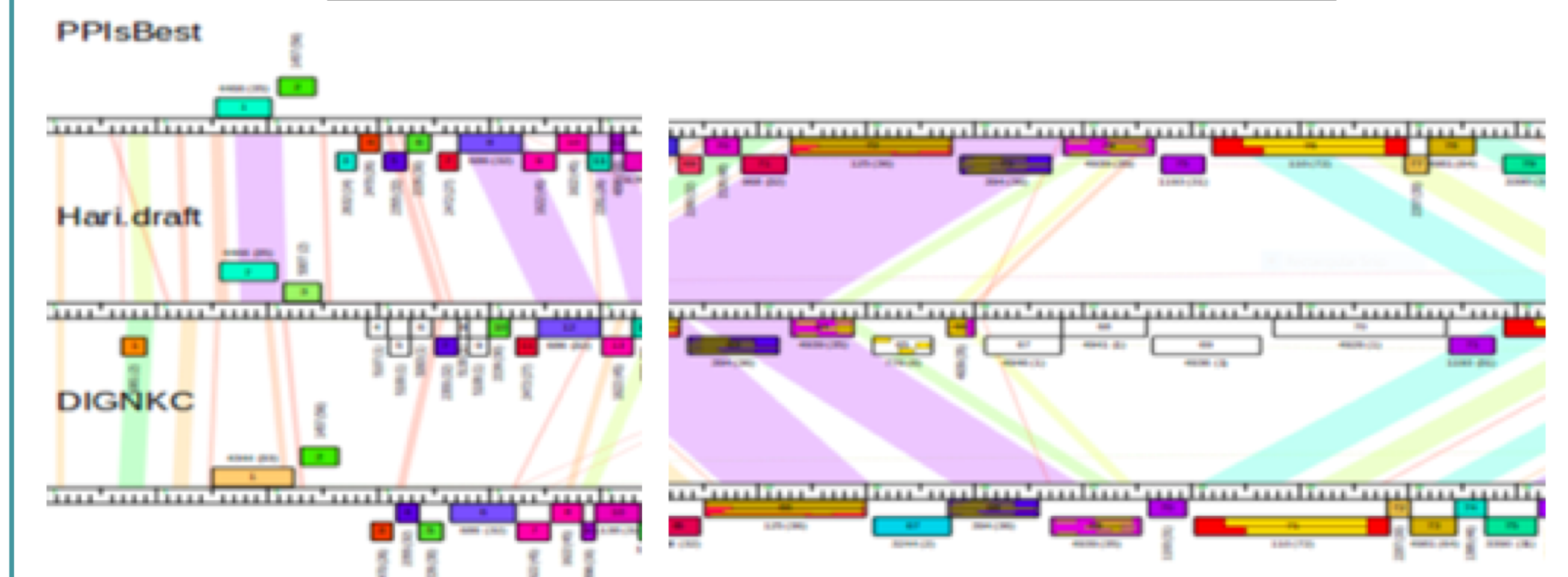


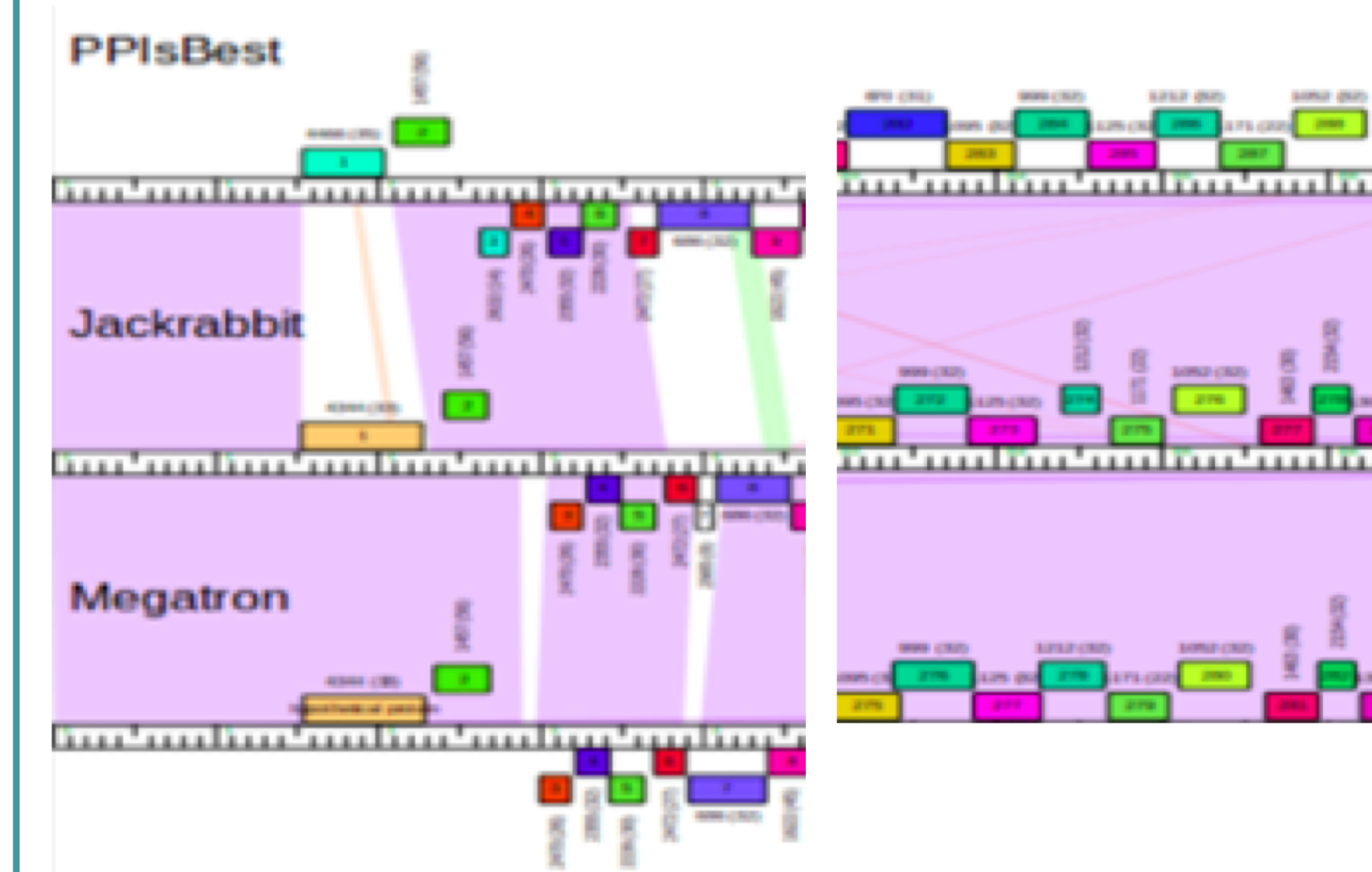
Figure 6. Wells from left to right: 1- DNA ladder; 3-undigested DNA; 4- BamH1; 5-Clal; 6-EcoR1; 7-HeaIII; 8-HindIII

Phamerator analysis



Hari

When compared to *Bacillus* phage DIGNKC and PPIsBest. This part of the phemerator shows that genes 65 through 70, where there are multiple white boxes. It seems to be an insertion in the gene, indicating that those specific genes are not present in any other *Bacillus* phages and are most likely due to evolution.



JackRabbit

When phages Megatron and PPIsBest are compared to Jackrabbit, genes 271 through 276 (right) displays the similarities between the phages given the size and color of the boxes with only a small shift of difference. Additionally, Jackrabbits gene 8, shaded in purple (left), is in close relation to genes 7 of Megatron and gene 8 of PPIsBest.

Genes with predicted function in Hari

Genes with identified functions in Hari using HHPred analysis include:

- Dihydrofolate reductase
- Outer capsid cell attachment
- Adenylate Kinase
- Reductase-thymidylate Synthase
- Ribosomal protein
- Nicotinamide phosphoribosyl transferase
- Ribose-phosphate pyrophosphokinase protein
- Endolysin
- HNH homing endonuclease
- Large phage terminase subunit
- Lipopolysaccharide assembly protein
- Major capsid protein
- Tape measure protein
- Baseplate protein
- Helicase
- DNA polymerase I
- HU protein
- DNA Polymerase I
- Homing endonuclease
- RNA Polymerase
- Holin
- DNA Double-strand break repair protein

Overall Phage Genome characteristics

	Genome Size	Top 3 BLAST Matches	Auto-annotated features
Hari	161,978 bp 2,633 bp terminal repeat	DIGKNC DirtyBetty PPIsBest	286
JackRabbit	161,552 bp 2,821 bp terminal repeat	Megatron PPIsBest Hakuna	288

Phage Hunters student are characterizing the phage genomes during the second semester.

Conclusions & Future directions

- In addition to Hari and JackRabbit, seven other *Bacillus* phages were isolated in the Fall semester by other students in the Phage Hunters class.
- All nine phages that were isolated this year, all were myoviridae
- The two phages sequenced and annotated this year are similar to several that have been found in the past
- Future work will focus on placing these phages in Clusters.