Isolation, Characterization and Annotation of Bacteriophages Kanely and Big 3 at Virginia Union University

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ABSTRACT

During the 2016-2017 academic year, the Howard Hughes Medical Institute's Science Education Alliance - Phage Hunters Advancing Genomics and Evolutionary Sciences (SEA-PHAGES) course replaced one section of the traditional General Biology laboratory curriculum at Virginia Union University (VUU). Specifically, in Fall 2016, twenty students collected 40 environmental samples (both soil and water) from Northern, Central and Northern Neck, Virginia. Utilizing the bacterial host *Mycobacterium smegmatis* mc^2 155, a total of 11 phages were isolated, 2 via direct isolation and 9 via enriched isolation. One representative from either isolation protocol (2 total) was selected for purification and characterization. Kanely was identified through the direct isolation method from a Champlain, VA soil sample, and Big3, another soil sample, was identified by enriched isolation from Richmond, VA. Each phage was visualized via electron microscopy and exhibited a Siphoviridae morphotype. Following DNA extraction, each phage was characterized using restriction enzyme digestion and agarose gel electrophoresis. Kanely and Big3 genomes were sequenced at the University of Pittsburg. In Spring 2017, the SEA-PHAGES class worked to annotate the genomes of both phages utilizing DNA Master genome analysis software and bioinformatics tools including, NCBI BLAST, the Conserved Domain Database (CDD) and HHPred to determine putative protein function. Both, Kanely and Big3 are members of Cluster A, and A1 sub-cluster. Kanely is 52,539 bp in length and predicted to have 93 genes, whereas Big3 is 53,442 bp in length and has 90 predicted genes. Overall, the SEA-PHAGES course-based undergraduate research experience has provided an opportunity to successfully implement an authentic research opportunity to first year students.

Environmental Samples

Types of samples	
Soil	25
Water	15
Total	40

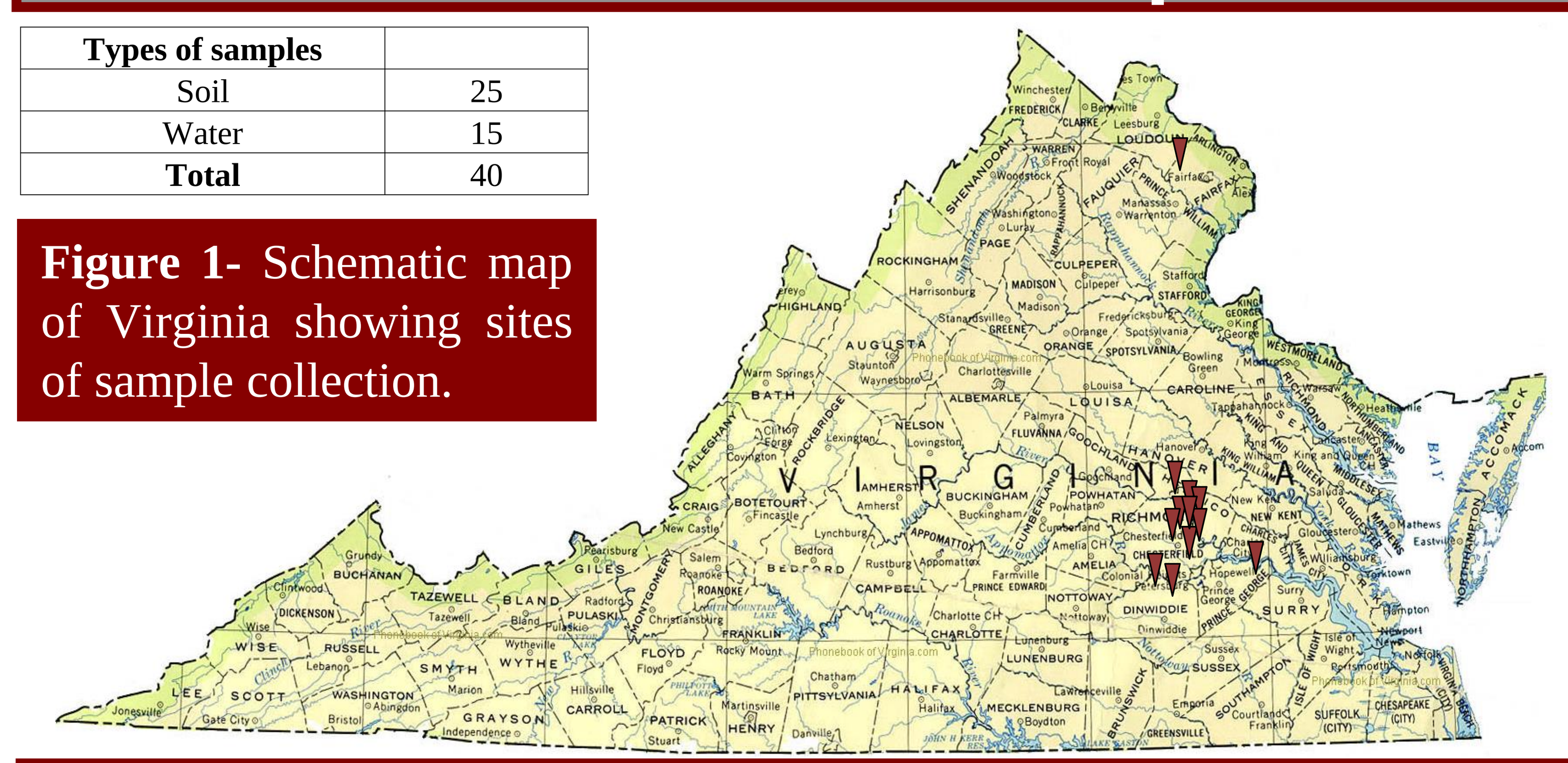
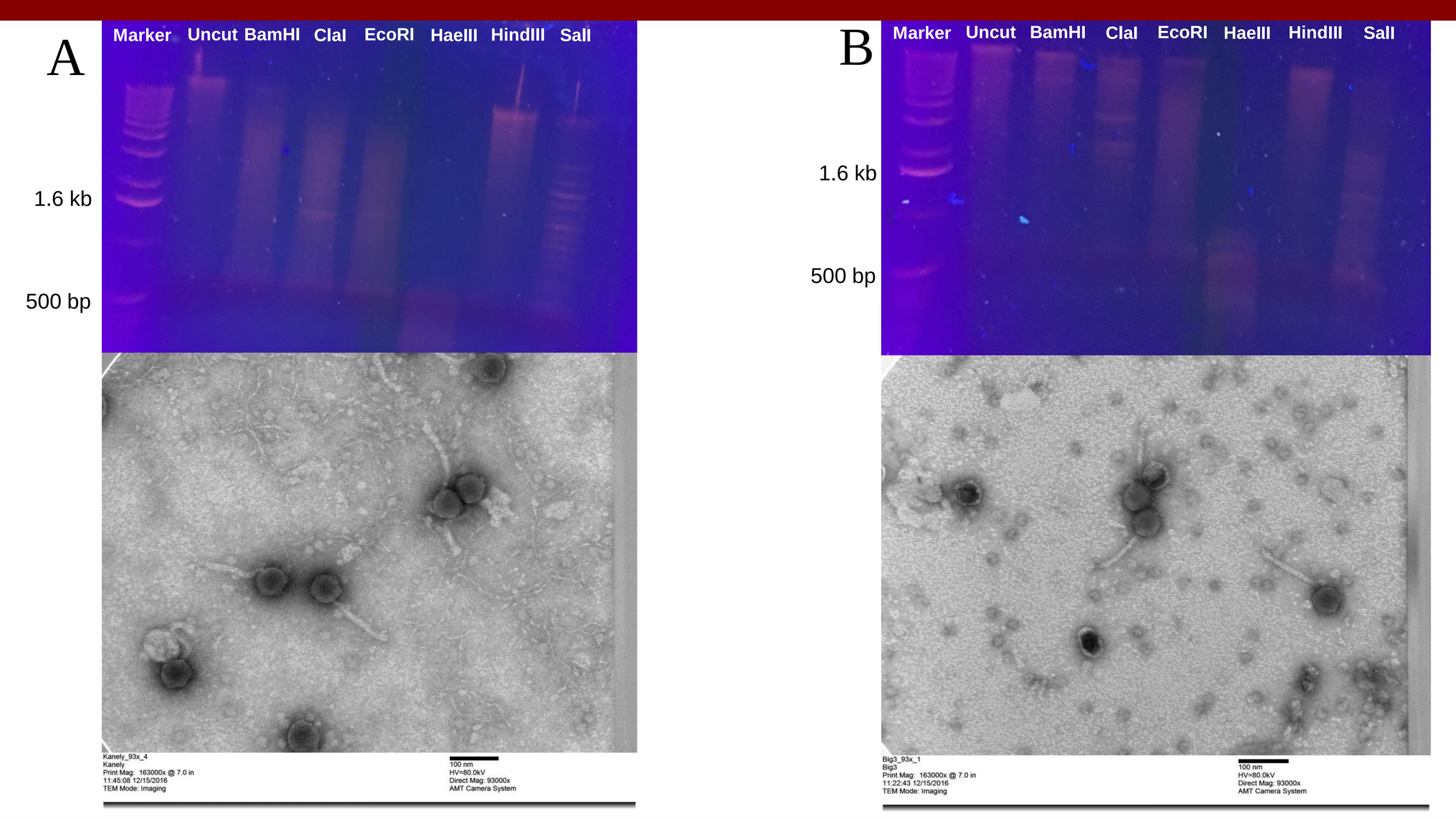


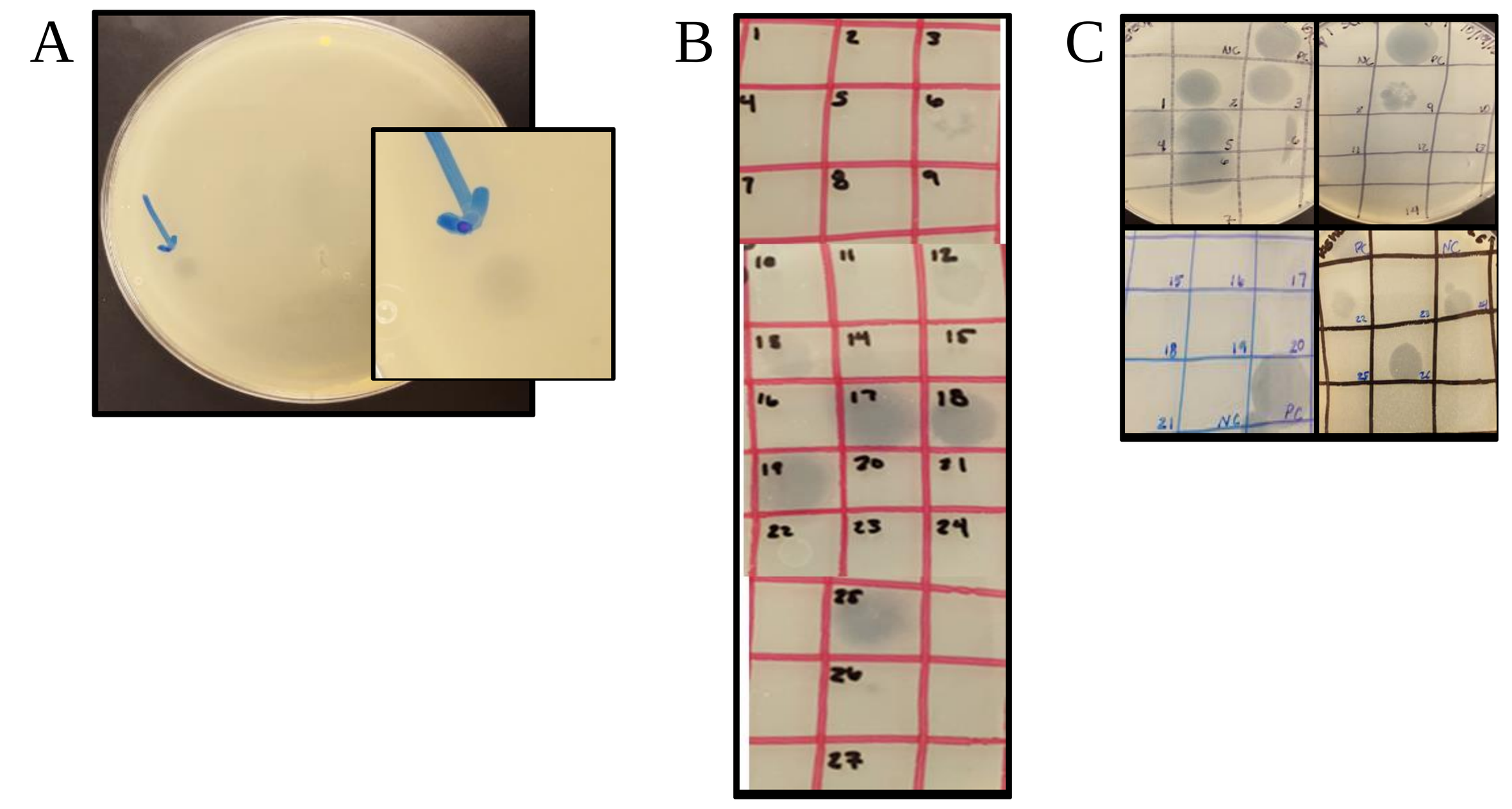
Figure 1- Schematic map of Virginia showing sites of sample collection.

Mycobacteriophages: Kanely and Big3

Figure 2- Each mycobacteriophage, Kanely and Big3, was characterized utilizing restriction enzyme digestion to verify successful DNA extraction, and electronic microscopy to visualize the viral structure (Siphoviridae). (A) Kanely was isolated from a boating dock in Champlain, VA (38.010007N, 77.004354W) via Direct Isolation at 37ºC. (B) Big3 was found in the Treehouse Apartment community in Richmond, VA (37.5N, 77.42W)) and extracted via Enriched Isolation at 37ºC.



Isolation and Purification



D

	Direct Isolation	Type	Eriched Isolation Day 2	Type	Enriched Isolation Day 4	Type
Positive	2	Soil	8	6 soil 2 water	9	8 soil 1 water
Total	26		23		26	
Efficiency	7.70%		34.78%		34.62%	

Figure 3- Plaques identified through direct isolation and enriched isolation. (A) Shows a single plaque isolated through direct isolation. The inset is a magnified view of the area of clearing. (B) Plaques were identified in samples 6, 12, 13, 17-19, 25 and 26 of the day 2 enriched isolation. (C) Plaques were identified in samples 2-6, 9, 22, 24, and 26 of the day 4 enriched isolation. (D) Shows the efficiency of isolation between direct isolation and enriched isolation (days 2 and 4).

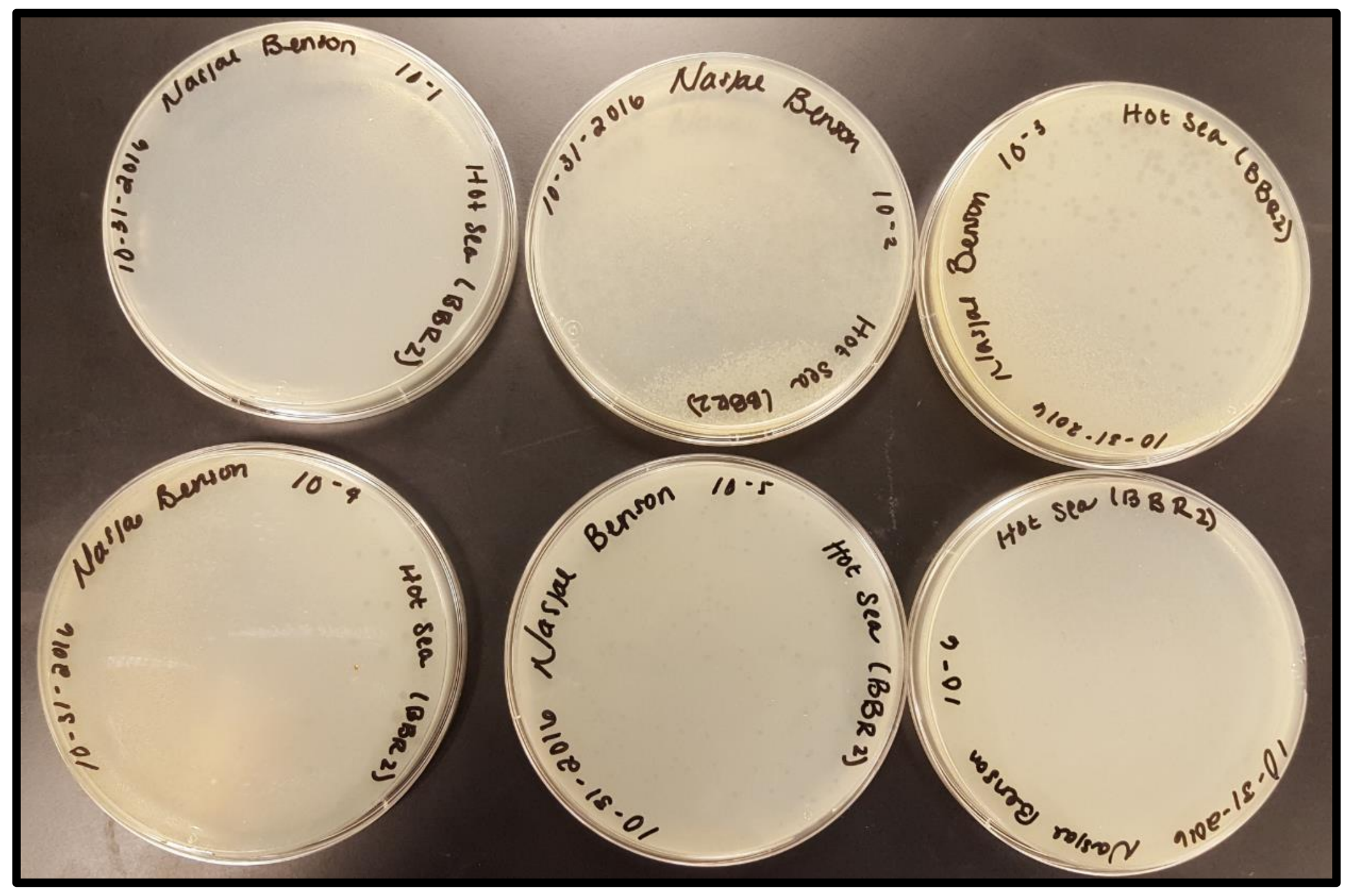
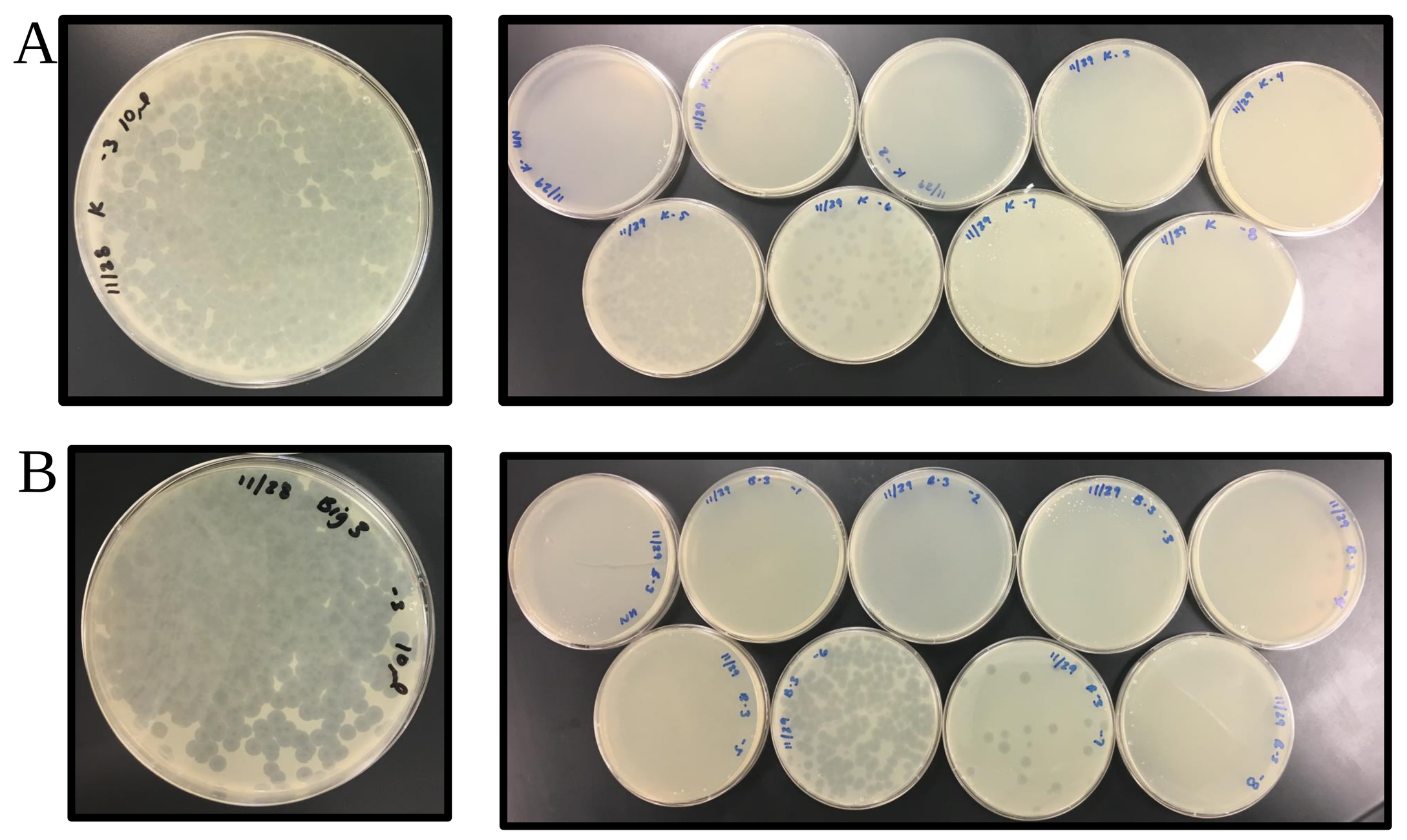


Figure 4- Serial dilution from the 1st round of purification. Plates were diluted from 10^{-1}- 10^{-8}. 10^{-7} and 10^{-8} had no plaques and were omitted from the figure. (Sample: Enriched Isolation #6 Fig. 2 B and C)



Webbed Plates

Full Plate Titer Serial Dilutions

C

	Average plaque size	Titer (pfu/mL)	>10^9?
Kanely	0.425 cm	1.125×10^{10}	yes
Big 3	0.6 cm	2.3×10^{10}	yes

Figure 5- Webbed plate and serial dilution from purified phage samples. Full plate titer lysates were diluted from 10^{-1}- 10^{-8}. (A) Kanely, a direct isolate from soil and (B) Big3, an enriched isolate from soil. (C) Shows the concentration of high titer lysates each purified phage.

Genome Annotation

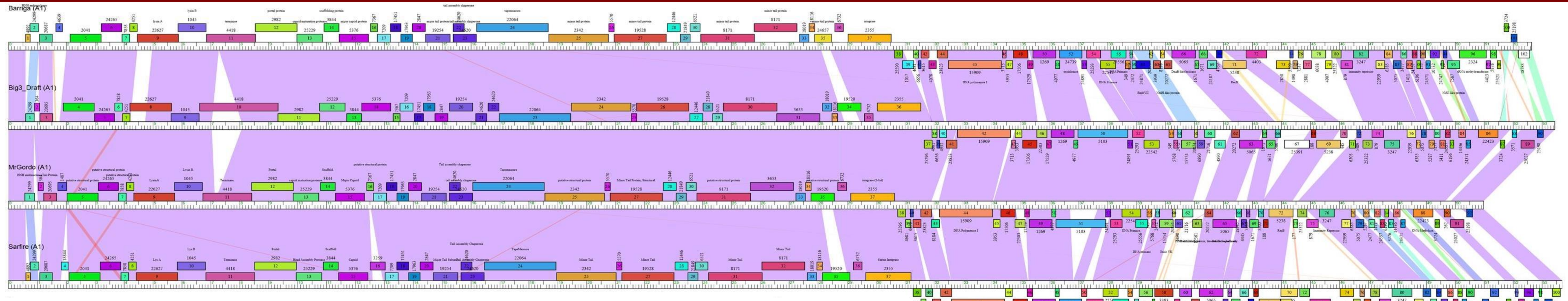


Figure 6- Genome comparison utilizing phamerator to demonstrate sequence conservation between Big3 and the most closely related mycobacteriophages, Barriga, MrGordo and Sarfire. Regions of high synteny are maintained on the left arm of the genome (structural genes) and more genetic diversity in the left arm.

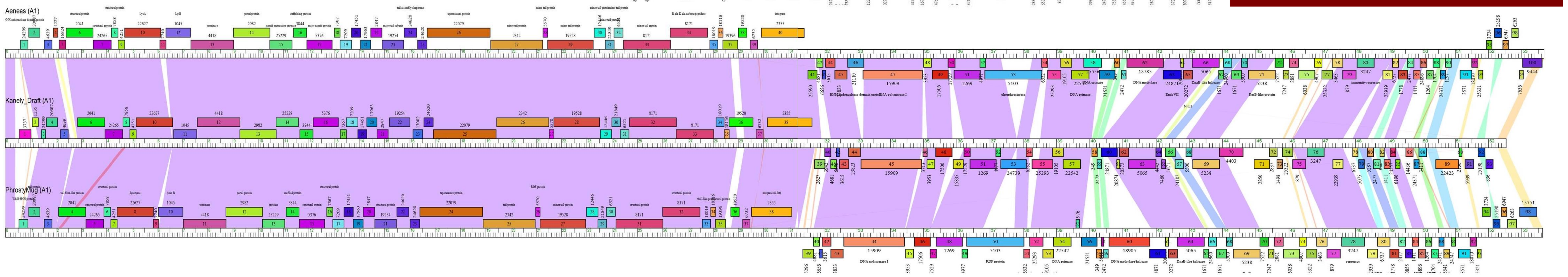


Figure 7- Genome comparison utilizing phamerator to demonstrate sequence conservation between Kanely and the most closely related mycobacteriophages, Aeneas and PhrostyMug.

Acknowledgements

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References

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