



Mycobacteriophages “DreamCatcher” and “Legolas,” Two Siphoviridae Collected in Lincoln, NE

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Abstract:

The SEA-PHAGES program grants undergraduates the opportunity to isolate a novel mycobacteriophage from the environment and sequence its DNA. Phages DreamCatcher and Legolas were isolated at Nebraska Wesleyan University in Lincoln, Nebraska. “DreamCatcher” and “Legolas” were classified as Siphoviridae as supported by plaque morphology and electron microscopy. DreamCatcher has 97 putative genes comprised of 53,408 base pairs. Whereas, Legolas has 104 putative genes comprised of 68,555 base pairs. Based on morphology as seen by electron microscopy DreamCatcher has been classified as a cluster A1 mycobacteriophage, and Legolas as a cluster B1 mycobacteriophage. The genome of both of these viruses were sequenced and then annotated using a variety of computer software and online tools.

Introduction:

Mycobacteriophages are viruses that infect bacteria and are ubiquitous on Earth. Mycobacteriophages have specific host ranges allowing them to infect specific groups of bacteria; phages can therefore carry important genetic information about their host cell genome (Asai, et al. 2013). Mycobacteriophages are relevant in discussions about the manipulation of Mycobacterium tuberculosis because their ideal host bacteria is Mycobacterium smegmatis, MC²155, a model organism for M. tuberculosis. Using PhagesDB, characteristics, plaque images, gel electrophoresis, electron microscopy pictures, DNA Master, HHpred, Blastp, Blastn, and Phamerator, our phages could be compared to other phages that have already been analyzed. When analyzing the DNA sequence using DNA Master, comparison of DreamCatcher and Legolas were made possible after annotation.

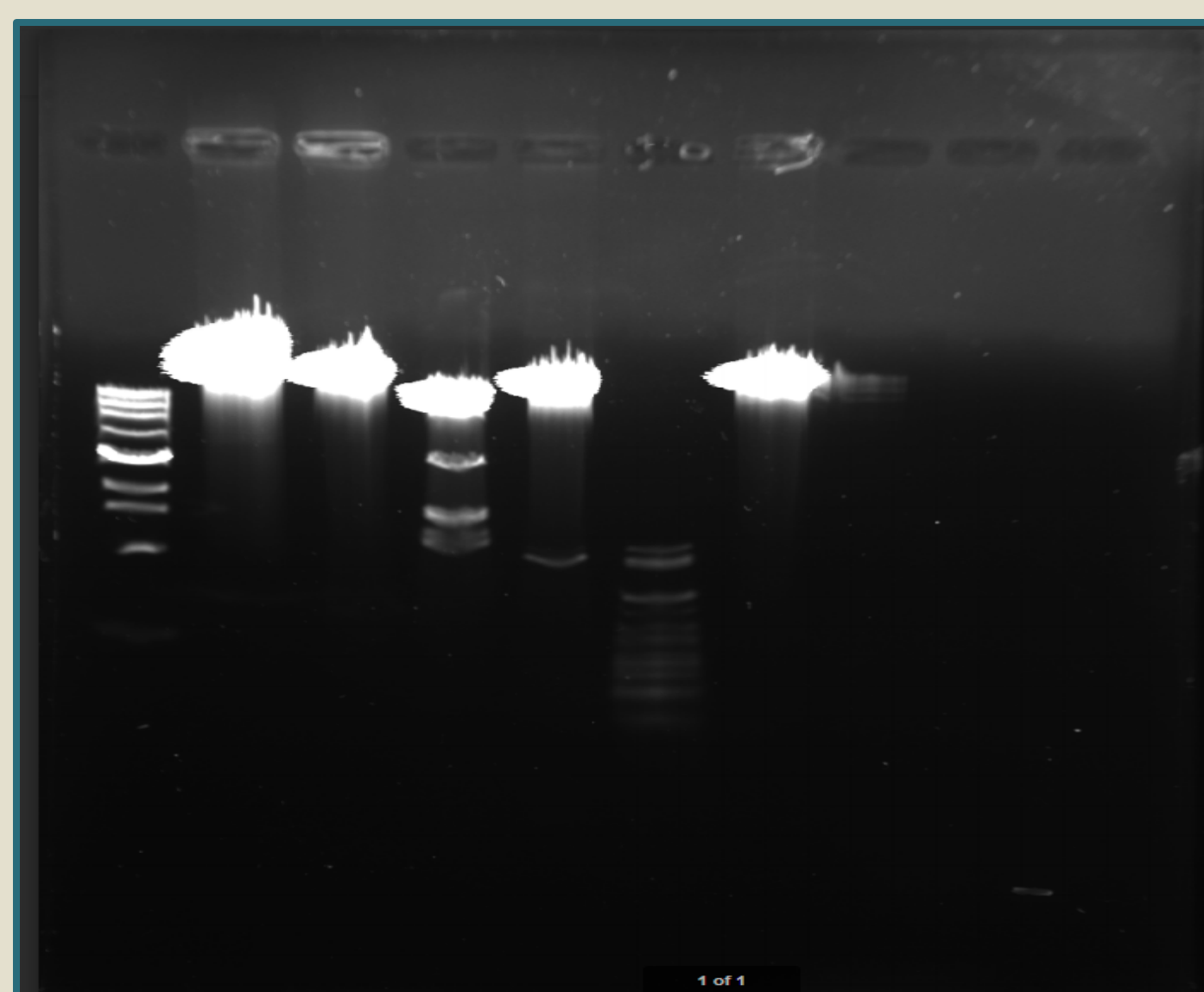


Figure 1. Gel Electrophoresis of Legolas. Cut with the following restriction enzymes: Bam HI, ClaI, EcoRI, HaeIII, HindIII.

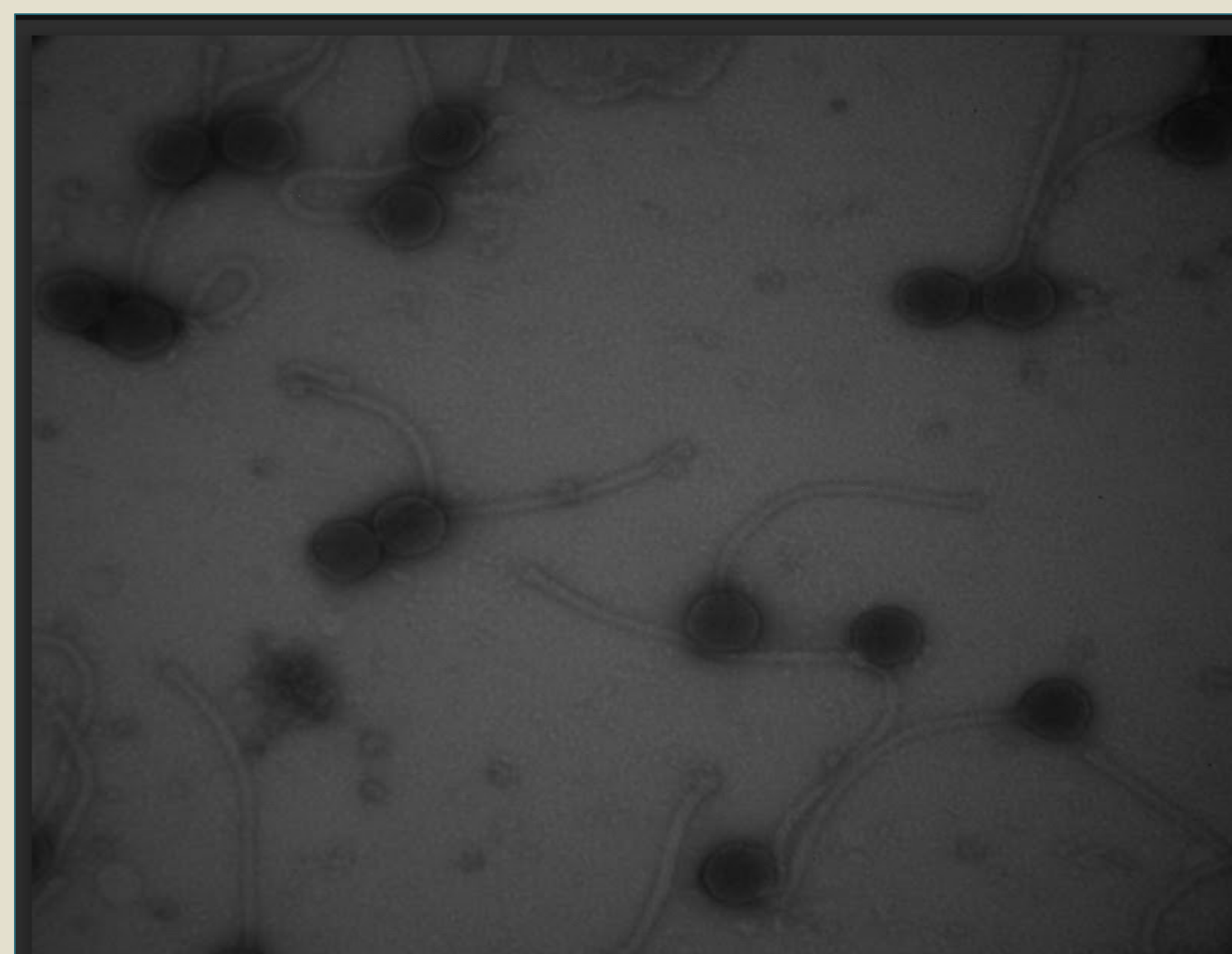
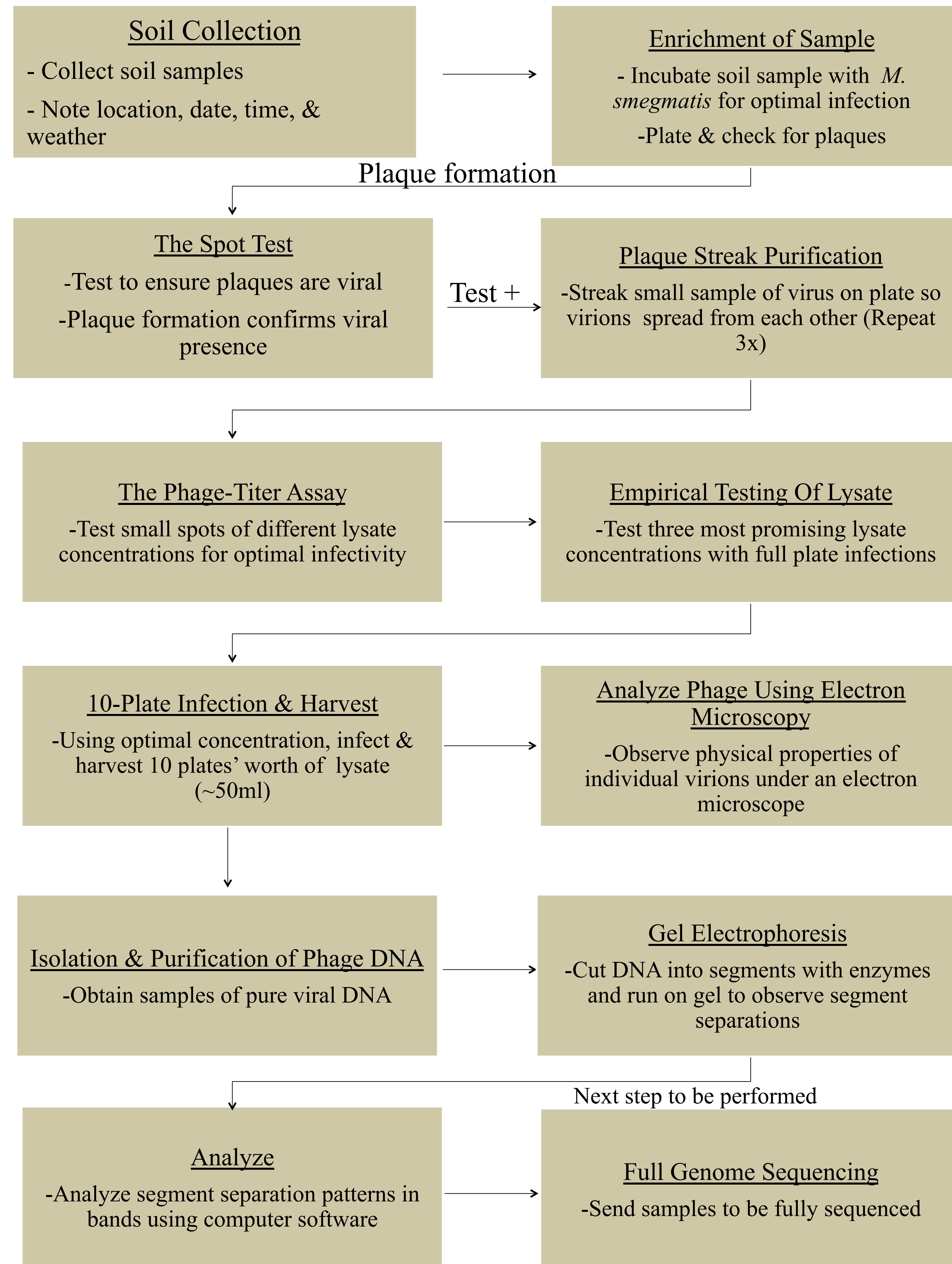


Figure 2. Electron Microscopy of DreamCatcher. The phage's head is dark, indicating a large amount of DNA in this phage.

Methods: Isolation & Purification of Phage



Results:

The enrichment technique was used to isolate both of these phages. Through many steps, a pure single phage population was obtained. Both of these viruses had plaques that were fast growing and circular with blurred rings around each individual plaque. Using electron microscopy DreamCatcher and Legolas were found to have a large head of DNA and a long tail. DreamCatcher has 97 putative genes comprised of 53,408 base pairs with GC content of 63.36%, which makes it cluster A1. DreamCatcher has 57 known gene functions and Legolas has 33. Legolas has 104 putative genes comprised of 68,555 base pairs with a GC content of 66.4%.

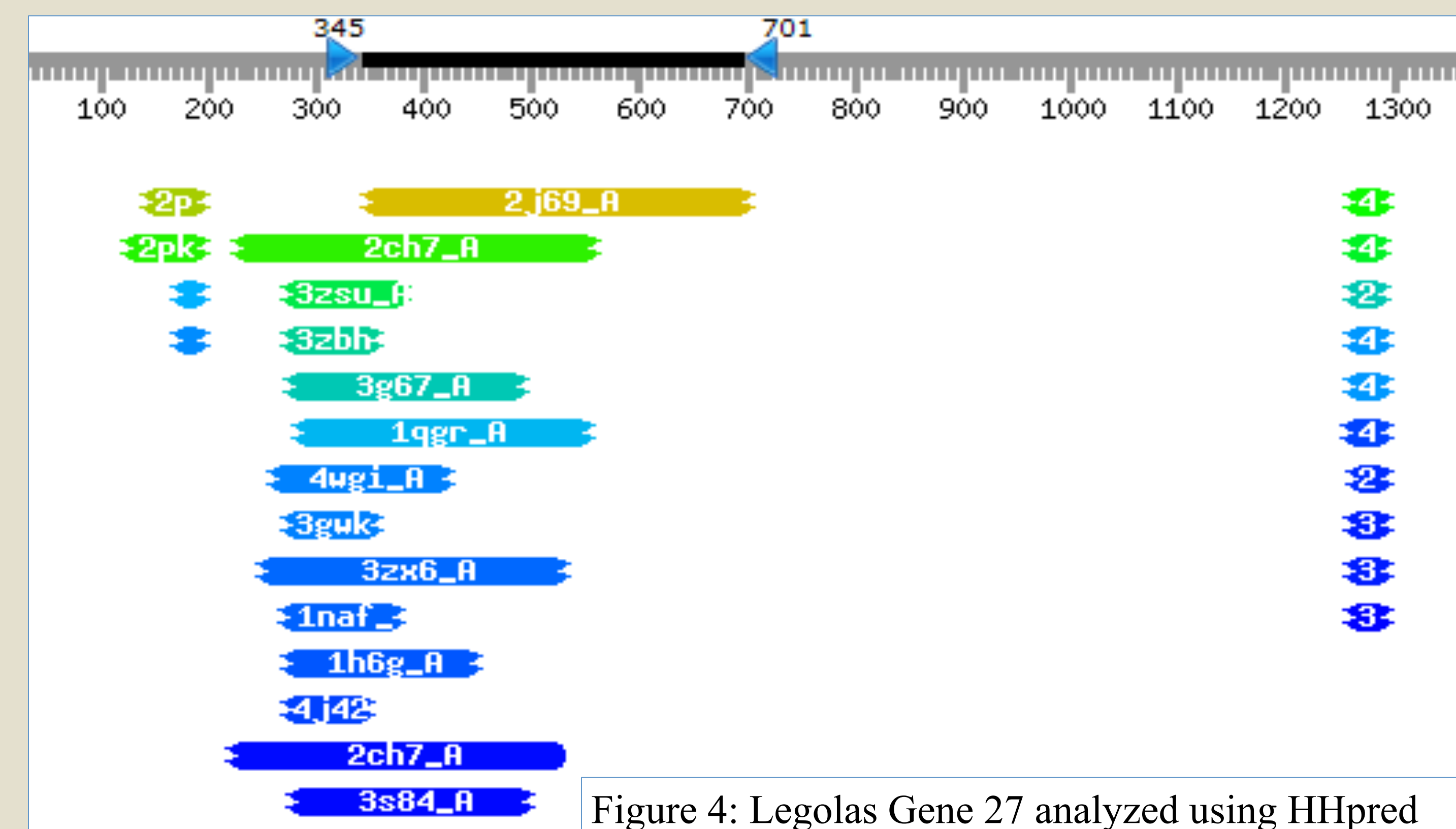


Figure 4: Legolas Gene 27 analyzed using HHpred

Conclusion:

DreamCatcher and Legolas were relatively similar sized genomes. DreamCatcher has 52,821 base pairs and Legolas has 68,555 base pairs. The majority of isolated phages are between 50,000 and 80,000 base pairs. Even though DreamCatcher and Legolas are found in different clusters, they have similar numbers of base pairs, as well as being siphoviridae.

Next Step (After Bioinformatics):

Further experimenting would include determining specific functions of genes found in DreamCatcher and Legolas.

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Bioinformatics Methods:

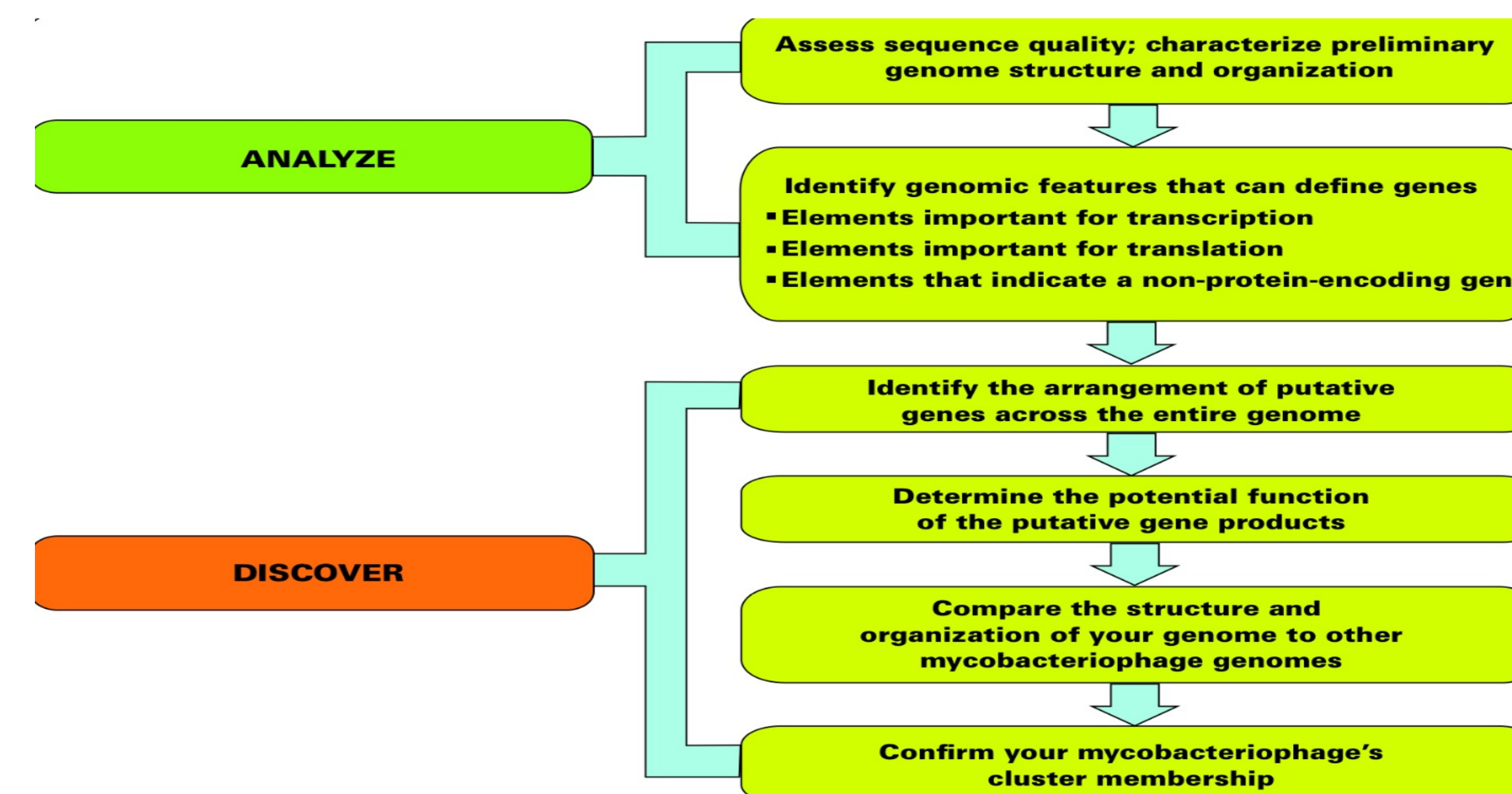


Figure 3. Bioinformatics Flow Chart.

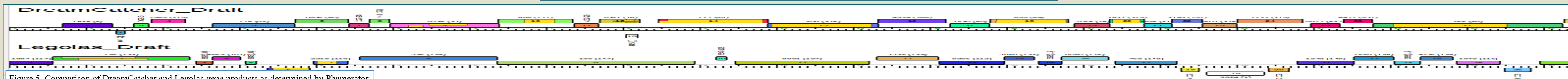


Figure 5. Comparison of DreamCatcher and Legolas gene products as determined by Phamerator.