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## Abstract:

Bacteriophage isolation from environmental samples has been performed for decades using principles set forth by pioneers in microbiology. The isolation of phages infecting *Arthrobacter* hosts has been limited due to the low success rate of many previous isolation techniques. This has resulted in an underrepresented group of *Arthrobacter* phages available for study. The enrichment technique developed at Cabrini College, unlike many others, uses a filtered extract free of contaminating bacteria as the base for indicator bacteria growth, *Arthrobacter sp.* KY3901, specifically. By first removing soil bacteria the target phages are not hindered by competition with native soil bacteria present in initial soil samples. This enrichment method has resulted in fourteen unique phages that belong to six different clusters from several different soil types. Different types of phages were even produced from the same enriched soil sample isolate. The growth characteristics of these phages have been examined and they have a variety of ideal growth temperatures and calcium chloride concentrations. These phages have also been examined with several genome analysis tools including DNA Master and Phamerator from the Department of Biological Sciences at the University of Pittsburgh. The results of this analysis may shed light on the genes that function in virulence, bioremediation or growth characteristics.

## Background:

Various species of *Arthrobacter* are ubiquitous in soil and display pleomorphic, Gram variable rods or cocci when grown in aerobic culture. *Arthrobacter* is a highly diverse genus of bacteria and is known for its ability to survive in harsh conditions and degrade nitrogenous environmental toxins (Mongodin et al, 2006). Of particular interest is *Arthrobacter*'s ability to break down atrazine and other s-triazine rings commonly used as herbicides and pesticides (Strong, 2002).

Previous attempts at isolating *Arthrobacter* phages from soils have been met with limited success. Past enrichment strategies included lengthy incubations or studies resulted in little characterization of the phages found with these methods (Germida & Casida, 1981). *Arthrobacter* phage isolation done in the past has been focused mainly on developing methods for phage typing of *Arthrobacter* soil isolates or for control of microbial growth in industrial processes (Brown et al, 1978; Petrovski et al, 2011). No research has been done until now to broadly characterize the genomic diversity of *Arthrobacter* phages, which, could lead to practical bioremediation applications due to the discovery of novel genes.

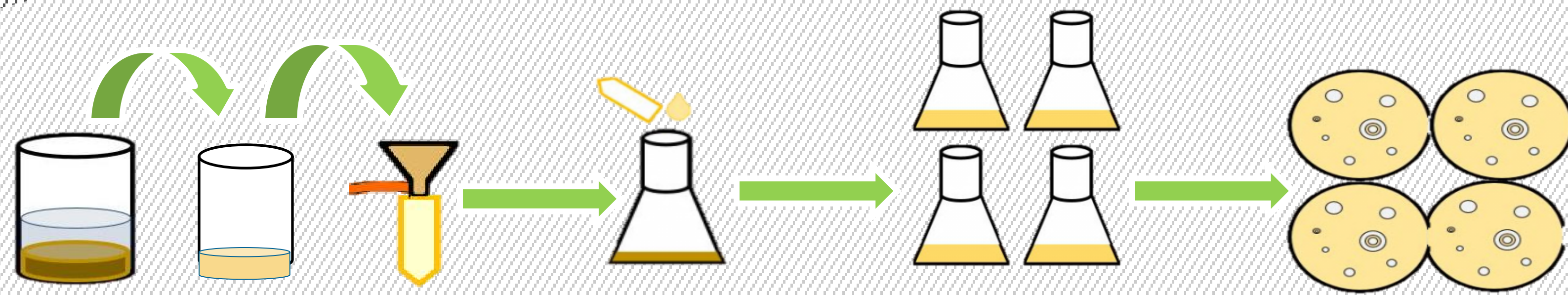


Figure 1: A visual representation of the novel isolation technique developed at Cabrini College.

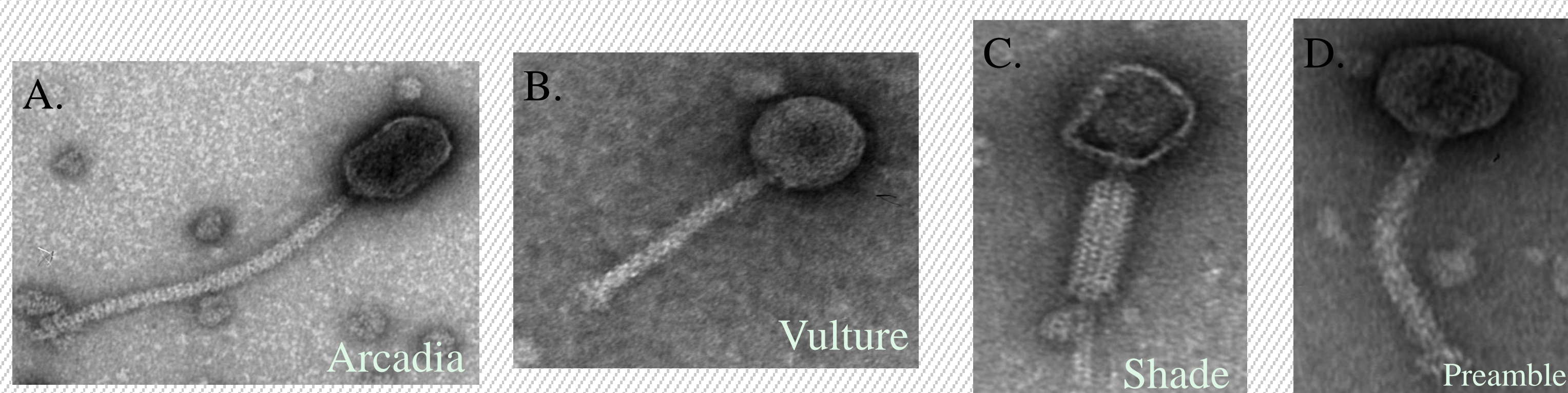


Figure 2: A. An electromagnetic picture of phage Arcadia. Arcadia is a prolate headed siphovirus. B. An electromagnetic picture of phage Vulture. Vulture is a siphovirus. C. An electromagnetic picture of phage Shade. Shade is a myovirus. D. An electromagnetic picture of phage Preamble. Preamble is a siphovirus.

## Acknowledgements:

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## References:

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## Results:

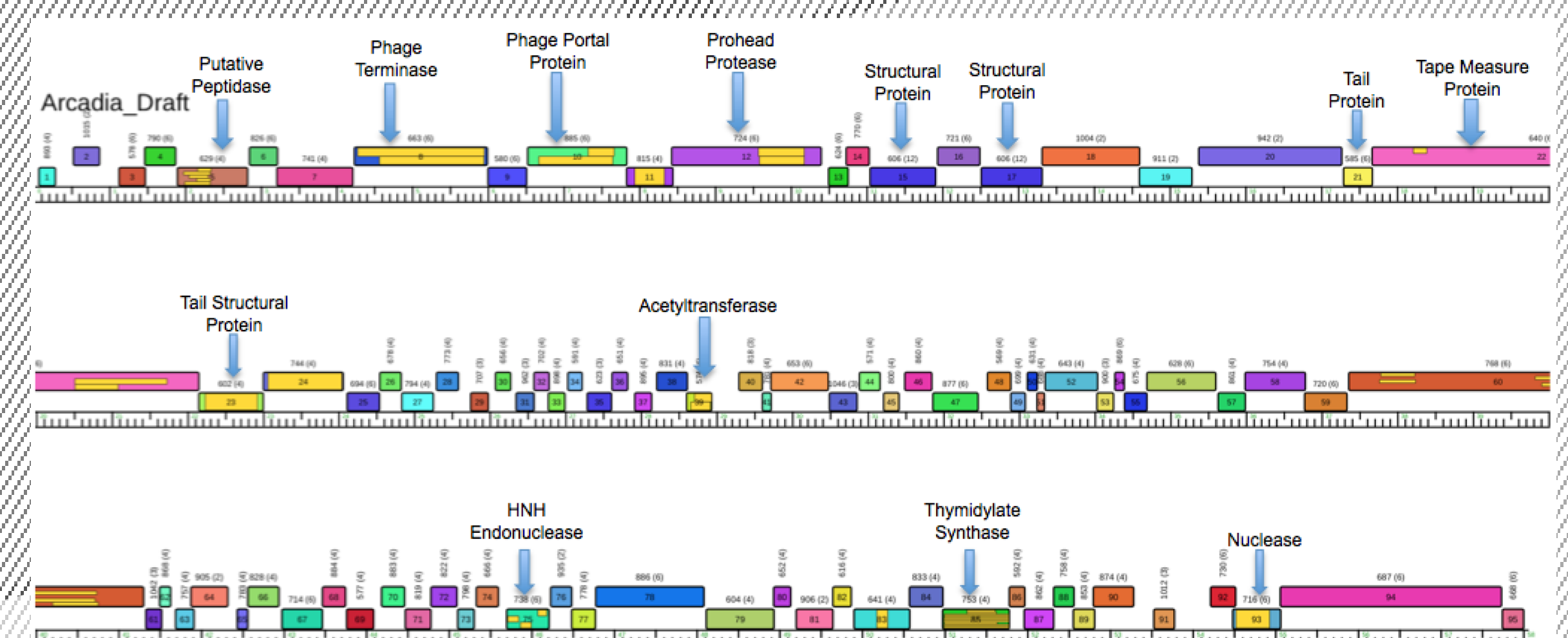


Figure 3: A Phamerator generated genome map of phage Arcadia. The yellow bars found within the genes represent conserved domains from closely related genes. Putative functions have been indicated above genes with conserved domains.

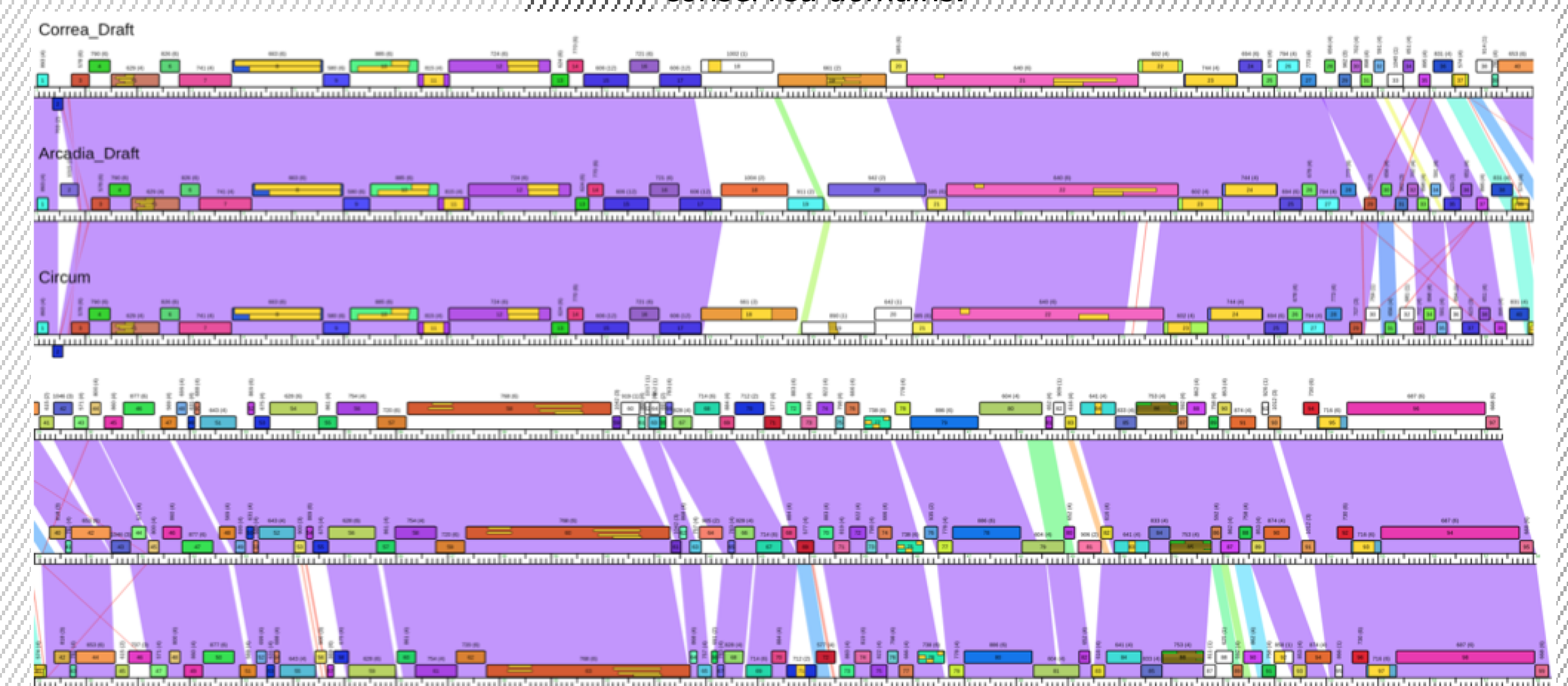


Figure 4: A Phamerator generated comparison map of phages Arcadia, Circum and Correa. Note the red lines indicating conserved sequences midway through the map. Highly conserved sequences are shown in violet. Colored boxes represent genes.

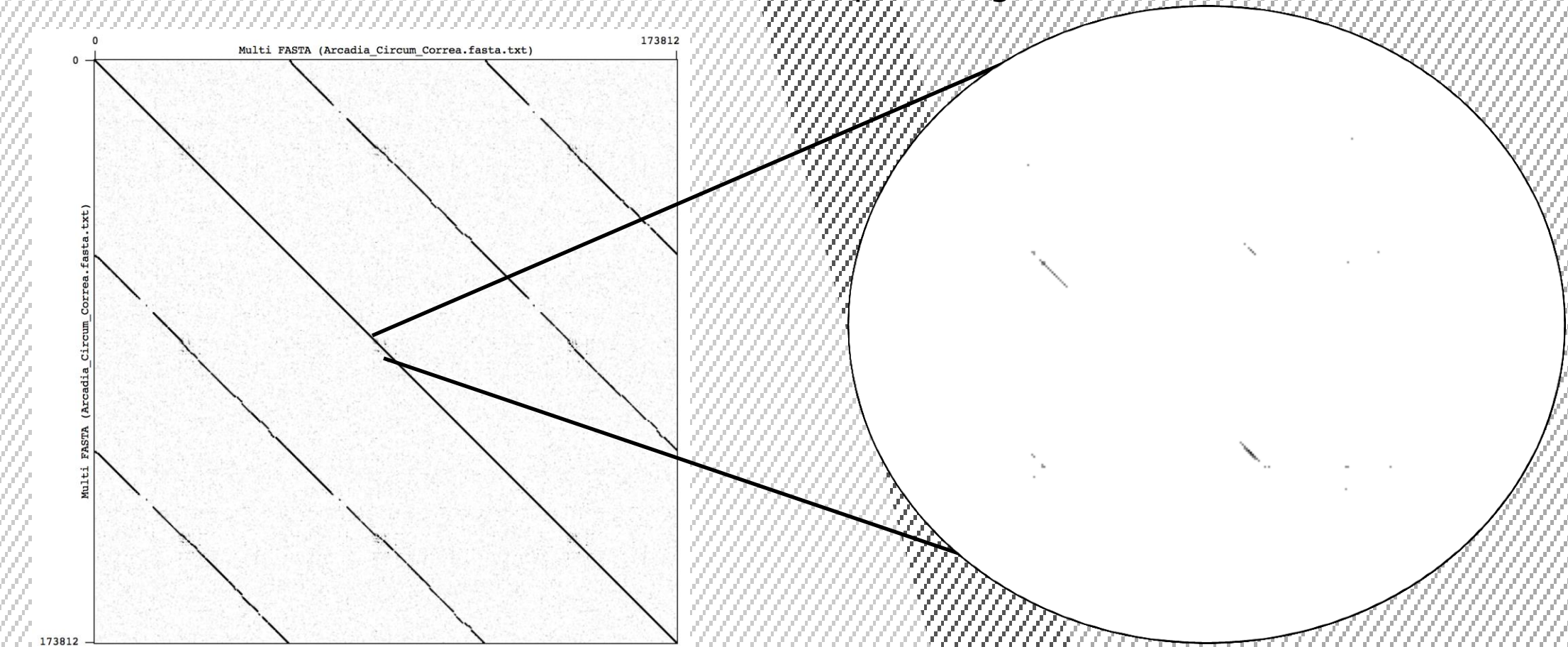


Figure 5: A Gepard generated Dotplot comparing phage Arcadia, Circum and Correa. The dark lines indicate sequence similarity. Note the dark spots that indicate highly conserved regulatory regions

## Conclusions:

This investigation has led to the discovery of 15 new *Arthrobacter* phage isolates which confirms the efficacy of this method for isolating multiple types of *Arthrobacter* phages without lengthy soil incubation while producing a high success rate. In addition to finding new *Arthrobacter* phages with this method, it is likely that  $\text{CaCl}_2$  dependency could be a factor in selecting for different phages. Our next step includes completing the genome annotations to then submit to GenBank for further analysis. Also, proteomics analysis will be conducted to confirm the presence of genes and their start sites.