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Predicting Evolutionary Clusters of Arthrobacteriophages Through PCR Analysis

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Bacteriophages, viruses that infect and kill specific strains of bacteria, are the most abundant biological entities on Earth, with diverse applications including phage therapy, food biocontrol, and wastewater treatment. Recently, phage research has gained increased attention as a possible treatment for the growing threat of antibiotic-resistant bacterial infections. A critical aspect of this research is understanding the genetic and evolutionary relationships of phages. While traditionally these relationships are defined through clustering via whole genome sequencing and analysis, this method can be time-consuming and resource-intensive. As a result, over 75% of discovered phages remain unsequenced, and thus unclustered, as of 2024, limiting their extent of application. This study explored the use of polymerase chain reaction (PCR) to predict the evolutionary clusters of unsequenced phages that infect *Arthrobacter globiformis*. Gepard dotplot analysis revealed high conservation in the tape measure protein (TMP) encoding gene across phage clusters, identifying this gene as a reliable marker for cluster prediction. For each cluster, TMP sequences were aligned using Mega11 software and PCR primers were designed in silico to target regions of 20 basepairs with high conservation. Phage samples were prepared using boiled lysate and analyzed via standard PCR protocols, followed by gel electrophoresis. By testing against three sequenced phages that infect *Arthrobacter globiformis* with known clusters, results demonstrated that the designed primers successfully identified the expected evolutionary clusters. This was assessed through the appearance of the expected PCR product size bands on the gels. Future work will involve expanding the primer library to cover additional phage clusters and incorporating this workflow into educational settings. This approach has the potential to streamline phage classification, enabling more efficient sequencing decisions and enhancing understanding of phage diversity.