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2023 SEA Symposium Abstract

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M is for Methods, Membrane proteins and Manipulation of Microbacterium foliorum phages

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Using the host *Microbacterium foliorum* NRRL B-24224, students at Drexel University isolated and purified 45 novel bacteriophages in Fall 2022. All but five soil samples were collected in Philadelphia, PA near Drexel’s campus. The other five were from surrounding Pennsylvania suburbs. The Pittsburgh Bacteriophage Institute generated genomes using Illumina Sequencing for three of these bacteriophages: PhunaPhoke (cluster EF), Rapheph (subcluster EA1), and PuffyCat (cluster EE). All demonstrated to be in the lytic life cycle. During bioinformatic analysis, these genomes were found to have 99% or greater similarity to previously annotated phage genomes in their respective clusters/subcluster. Two of the four student sections annotated PhunaPhoke and the other two sections annotated Rapheph. All four sections annotated PuffyCat in a third annual “Phage Cup” where each section participates in a friendly competition among the others using a point system to see who can annotate the genome the quickest and most accurately. All aspects of the annotation and competition are student-led. Students produced high quality annotations and self-reported not only enjoyment for the activity but also learning gains from being able to employ their knowledge from annotating their first, instructor-guided, genome annotation. Students identified genes as membrane proteins, utilizing the web-based programs TMHMM (Transmembrane Helices Hidden Markov Model) and SOSUI (Classification and Secondary Structure Prediction of Membrane Proteins) in all three of their novel bacteriophage genomes and drew several insights into the value of being able to identify these proteins. It was noted that with the addition of calling membrane proteins, the percentage of genes with identifiable functionality characteristics increased. For example, in the PhunaPhoke genome, 28% of genes (23 of 82 genes) have probable identifiable functions. With the addition of six membrane protein identifications, the determination of gene functionality rose to 35.4% of genes. Additionally, it was noted that often the membrane proteins are found clustered together, further lending insight to their function. Students have identified their Spring quarter research projects investigating various aspects of these novel phages. These projects will investigate effects of varying experimental factors such as exposure to varying chemical compounds (copper and iron sulfate, tetrazolium red, salinity, mutagens, antibiotics, disinfectants, sugars, Vitamin C, nitrogen and phosphorous, spices, caffeine) and physical factors such as UV levels and temperature, with the intention to test phage efficiency, conversion between the lysogenic and lytic cycles, effects on phages with circularly permuted vs. linear DNA, etc. These projects will garner insights and implications for phage biology, particularly in projects utilizing our sequenced genomes due to their high sequence similarity with other EF, EA1 and EE phage genomes.