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4-27-2022

The Discovery and Analysis of Mycobacteriophage "Rita"

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Introduction

Mycobacteriophage "Rita" was isolated on Mycobacterium *smegmatis* $mc^{2}155$ from an enriched soil sample from North Easton, Massachusetts. As Rita infects Mycobacterium smegmatis, further study of the phage was completed in order to determine its ability to be utilized in phage therapy for infections caused by pathogenic Mycobacterium, such as Mycobacterium tuberculosis and Mycobacterium abscessus. Therefore, analysis through experimentation and genome sequence annotation was necessary in order to further understand the phage's genomic diversity.

Figure 7: **Phamerator** Map Genome

map of phage Rita and similar phages of the same cluster.

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Figure 1: Purification

Mycobacteriophage Rita was isolated from an enrichment culture and purified by three rounds of purification.



Figure 2: Electron Microscopy Image of the phage particle shows that Rita is a Siphoviridae with a long contractile tail.



Figure 3: Isolated DNA

Analysis of Rita DNA, isolated by phenolchloroform extraction, using a microvolume spectrometer shows that the DNA is of high quality.



Figure 5: Sequencing

Sequencing confirms assignment to the F1 cluster.

Isolation Temperature	37°C	1000	
Genome Length (bp)	58,771		
Overhang Sequence	CGGAAGGCGC	500.1	
GC Content	61.6%	500 6	
Sequencing Facility	Pittsburgh Bacteriophage Institute	300 bj	
Shotgun Sequencing Method	Illumina Sequencing		

Figure 4: Determination of the Phage Cluster by PCR

Rita was determined to belong to cluster F and subcluster F1.

Primers + Rita Control rimers + Rita rimers + Rita Primers + Rita Dontrol B Ladder	KB Ladder
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Figure 6: Annotation

Bioinformatics tools DNA Master, PECAAN, Starterator, and Phamerator were utilized to complete a genome annotation. Below is an example annotation for a single gene on DNA Master.

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