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Anna Fakhri

Providence College

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The Discovery and Analysis of Mycobacteriophage “Rita”

Anna Fakhri and Kathleen Cornely

Department of Chemistry and Biochemistry, Providence College, Providence, RI



Introduction

Mycobacteriophage “Rita” was isolated on *Mycobacterium smegmatis* mc²155 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 1: Purification

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

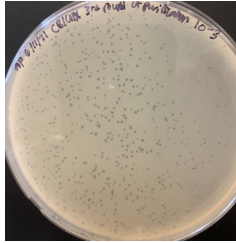


Figure 2: Electron Microscopy

Image of the phage particle shows that Rita is a Siphoviridae with a long contractile tail.

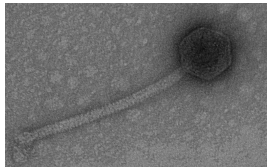


Figure 3: Isolated DNA

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

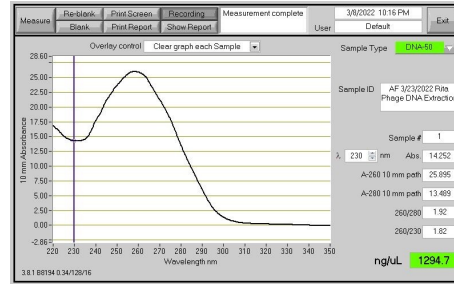


Figure 5: Sequencing

Sequencing confirms assignment to the F1 cluster.

Isolation Temperature	37°C
Genome Length (bp)	58,771
Overhang Sequence	CGGAAGGCGC
GC Content	61.6%
Sequencing Facility	Pittsburgh Bacteriophage Institute
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.

1 KB Ladder
 KI Primers + Rita
 KI Control
 H Primers + Rita
 G Primers + Rita
 F1 Primers + Rita
 F1 Control
 1 KB Ladder

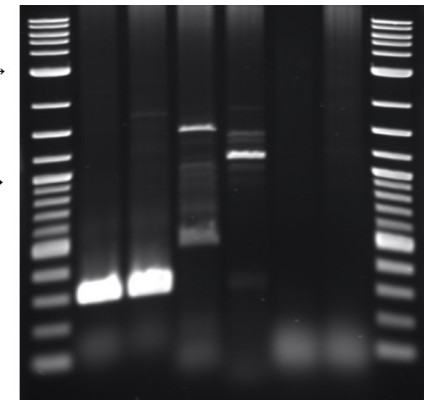


Figure 6: Annotation

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.

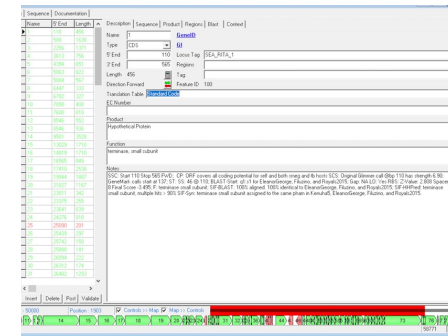


Figure 7: Phamerator Map

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

