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* 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 1: Purification

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

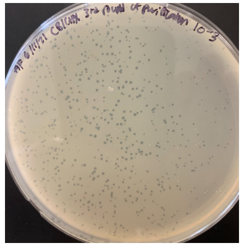


Figure 2: Electron Microscopy

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

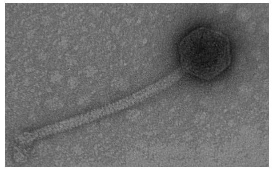
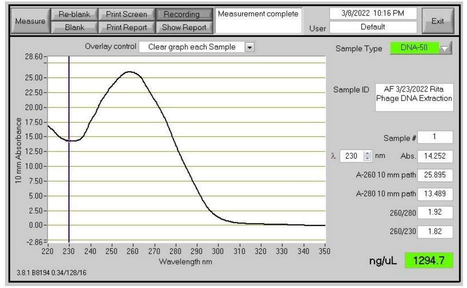


Figure 3: Isolated DNA

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



Measurement complete
3/8/2022 10:16 PM
User Default
Overlay control: Clear graph each Sample
Sample Type: DNA-50
Sample ID: AF 3/23/2022 Rita Phage DNA Extraction
Sample #: 1
λ 230 nm Abs. 14.252
A-260 10 mm path: 25.895
A-280 10 mm path: 13.489
260/280: 1.92
260/230: 1.82
ng/uL: 1294.7
3.8.1 B8194 0.34/128/16

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.

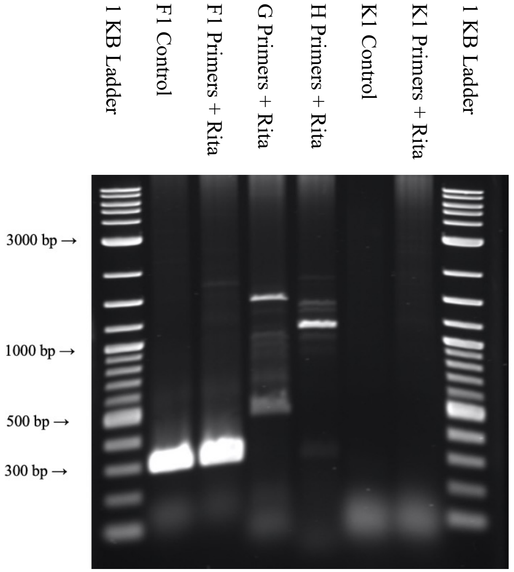
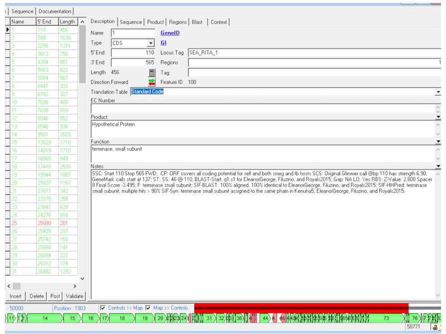


Figure 6: Annotation

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.



Name	5' End	Length
1	110	456
2	588	1638
3	2256	1371
4	3613	756
5	4394	651
6	5063	822
7	5884	567
8	6447	333
9	6782	327
10	7098	408
11	7600	810
12	8546	552
13	8546	936
14	9501	3528
15	13029	1710
16	14819	1710
17	16565	849
18	17410	2538
19	19944	1887
20	21827	1167
21	23011	342
22	23375	255
23	23641	639
24	24276	810
25	25090	201
26	25439	297
27	25742	150
28	25888	141
29	26094	222
30	26312	174
31	26482	1293

Name: 1
Type: CDS
5' End: 110
3' End: 565
Length: 456
Direction Forward
Locus Tag: SEA_RITA_1
Feature ID: 100
Translation Table: Standard Code
EC Number:
Product: Hypothetical Protein
Function: terminase, small subunit
Notes: SSC: Start 110 Stop 565 FWD; CP: ORF covers all coding potential for self and both smeg and tb hosts SCS: Original Glimmer call @bp 110 has strength 6.90; GeneMark calls start at 137; ST: SS: 46 @ 110; BLAST-Start: q1:s1 for EleanorGeorge, Filuzino, and Royals2015; Gap: NA LO: Yes RBS: Z-Value: 2.808 Spacer 8 Final Score -3.495; F: terminase small subunit; SIF-BLAST: 100% aligned, 100% identical to EleanorGeorge, Filuzino, and Royals2015; SIF-HHPred: terminase small subunit, multiple hits > 90% SIF-Syn: terminase small subunit assigned to the same pham in Kenuha5, EleanorGeorge, Filuzino, and Royals2015.
50000 Position : 1903
58771

Figure 7: Phamerator Map

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