



# Isolation and Genomic Analysis of *Mycobacterium smegmatis* and *Rhodobacter capsulatus* bacteriophages

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## Introduction

The 2015-16 Illinois Wesleyan SEA-PHAGES class isolated a total of 16 novel phages that infect the host organism *Mycobacterium smegmatis* MC<sup>2</sup> 155 (*Ms*), and 6 novel phages which infect the host *Rhodobacter capsulatus* (*Rc*), strain YW1. Unlike *Ms*—an organism found in a wide variety of environments—*Rc* is primarily found in aqueous environments, and has rarely been used to study bacteriophages. Whereas the extensively-studied *Ms* phages now total over 1,000 isolates, only six phages that infect *Rc* had been isolated before our work. All phages were characterized and categorized based upon their plaque morphology, ability to form lysogens, susceptibility of these lysogens to infection by other phages, and, for the *Rc* phages, their ability to infect related host strains. Through such testing, three *Ms* phages (Daffodil, Erdmann and Yucca) and three *Rc* phages (Dormio, McDreamy, and Tiptonus) were sequenced and studied further. The genomes of each of these phages was then annotated using bioinformatics resources, and their relatedness was then determined using the sequencing results.

## Immunity Testing:

For each phage, 3 attempts were made to isolate an infection-resistant lysogenic bacterial strain. These strains were then challenged with each of the other captured phages to reveal possible relatedness. An example is depicted below. The ability of eight different phage isolates and the phage used to create the possible lysogen (center spot) to infect a candidate lysogen are compared to the original host used for the isolation:

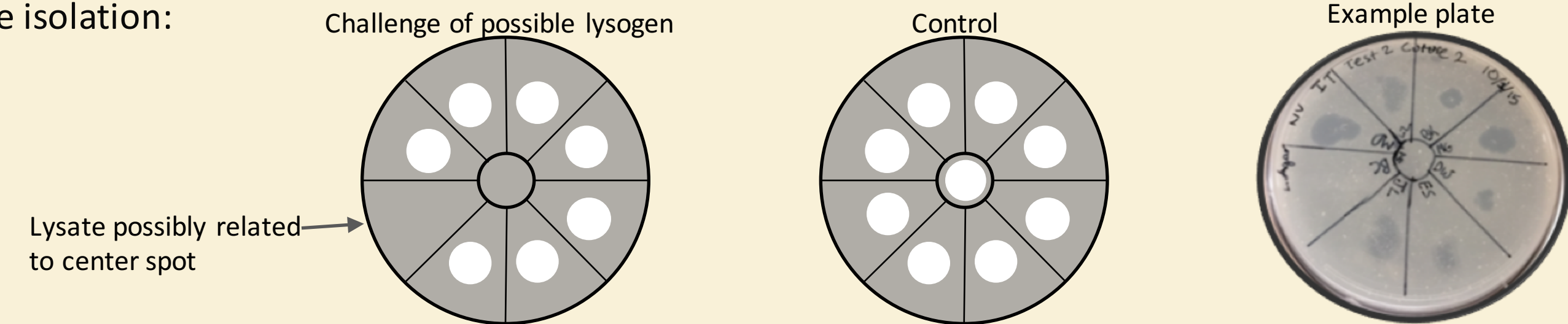


Table 1: Lysogens vs. Non-Lysogenic Phages

Lysogen	Non-Lysogenic Phages:					
	D	P	G	B	I	E
C	+	+	+	+	+	+
O	+	+	+	+	+	+
H	+	+	+	+	+	+
M	+	+	+	+	+	+
J	+	+	+	+	+	+
K	+	+	+	+	+	+
N	+	+	+	+	+	+
F	+	+	+	+	+	+
A	+	+	+	+	+	+

Table 2: Lysogens vs. Lysogenic Phages

Lysogen	Lysogenic Phages:									
	C	O	H	M	J	K	N	F	A	
C	-	+	+	+	-	+	+	+	-	
O	+	-	+	+	+	+	+	+	+	
H	+	+	-	+	+	+	+	-	+	
M	+	+	+	-	+	+	+	+	+	
J	+	+	+	+	-	+	+	+	-	
K	+	+	+	+	+	-	+	+	+	
N	+	+	+	+	+	+	-	+	+	
F	+	+	+	+	+	+	+	-	+	
A	-	+	+	+	+	+	+	+	-	

Key: A—Arcadian, B—Archibald, C—CheshireCat, D—Daffodil, E—Erdmann, F—Faffa, G—Flowers, H—KylieDog, I—Marco, J—Mnemosyne, K—Morganlafey, L—Prefontaine, M—Quadeluntina, N—Samantha, O—Tater, P—Yucca.

## Ms Phage Sequencing Results:

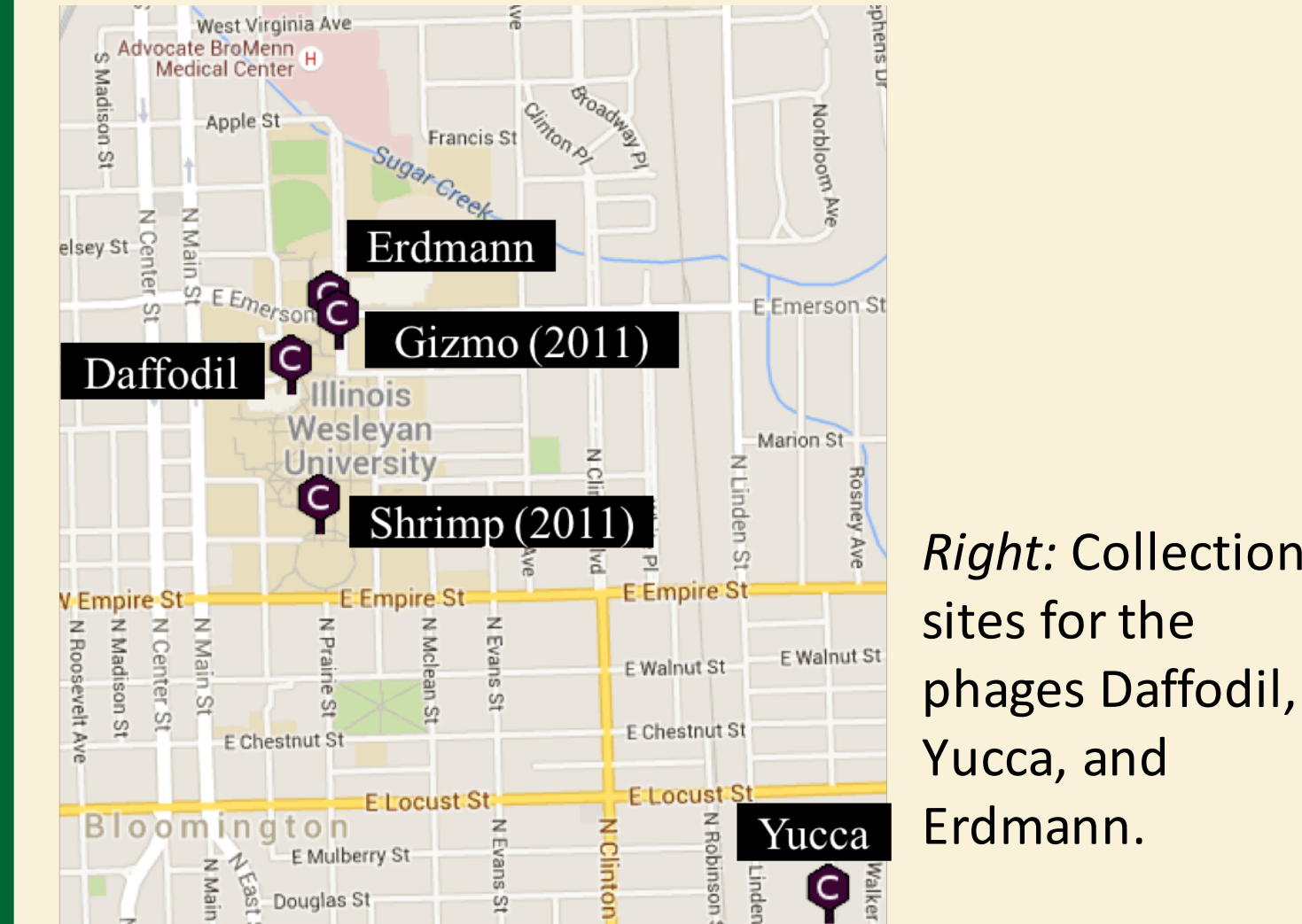
All three sequenced *Ms* phages were identified as cluster C1 phages. Table 3 (right) contains a brief comparison of the genomes of our isolated phages with that of the average C1 phage, the host *Ms* MC<sup>2</sup> 155, and the two C1 phages isolated by IWU in 2011.

Table 3: Comparison of Ms Genomes

	# of basepairs	GC Content	# of Genes	# of tRNAs
<i>Ms</i> MC <sup>2</sup> 155	6,988,209	67.4%	6,938	47
Avg. C1 phage	155,467	64.7%	229.9	32.5
Yucca	155,582	64.7%	243	35
Erdmann	155,565	64.7%	243	35
Daffodil	155,034	64.7%	240	35
Shrimp	157,482	64.6%	241	33
Gizmo	155,714	64.7%		Not given

## Ms Phage isolation sites:

Below: Map of *Ms* C1 phages found at IWU.

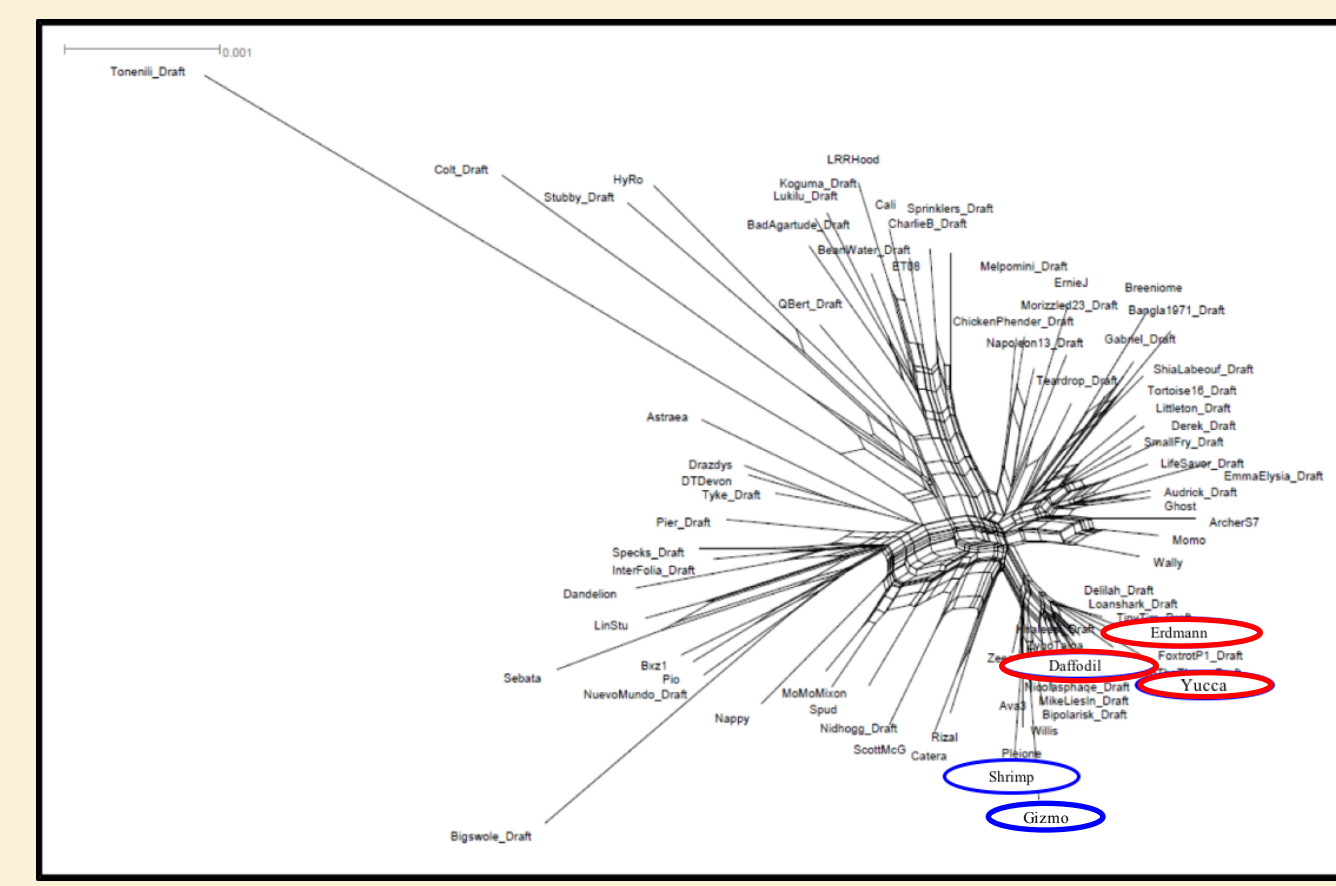


Right: Collection sites for the phages Daffodil, Yucca, and Erdmann.

Collection Sites for:



## Splitstree map of C1 phages:



Above: Relatedness diagram of all current cluster C1 phages. The variability in this cluster, is accounted for by the mosaicism of phage genomes. The circled phages are the 5 C1 phages found by IWU, two in 2011 (Shrimp and Gizmo), and other three this past year (Daffodil, Erdmann, Yucca).

## Rc Phages Background:

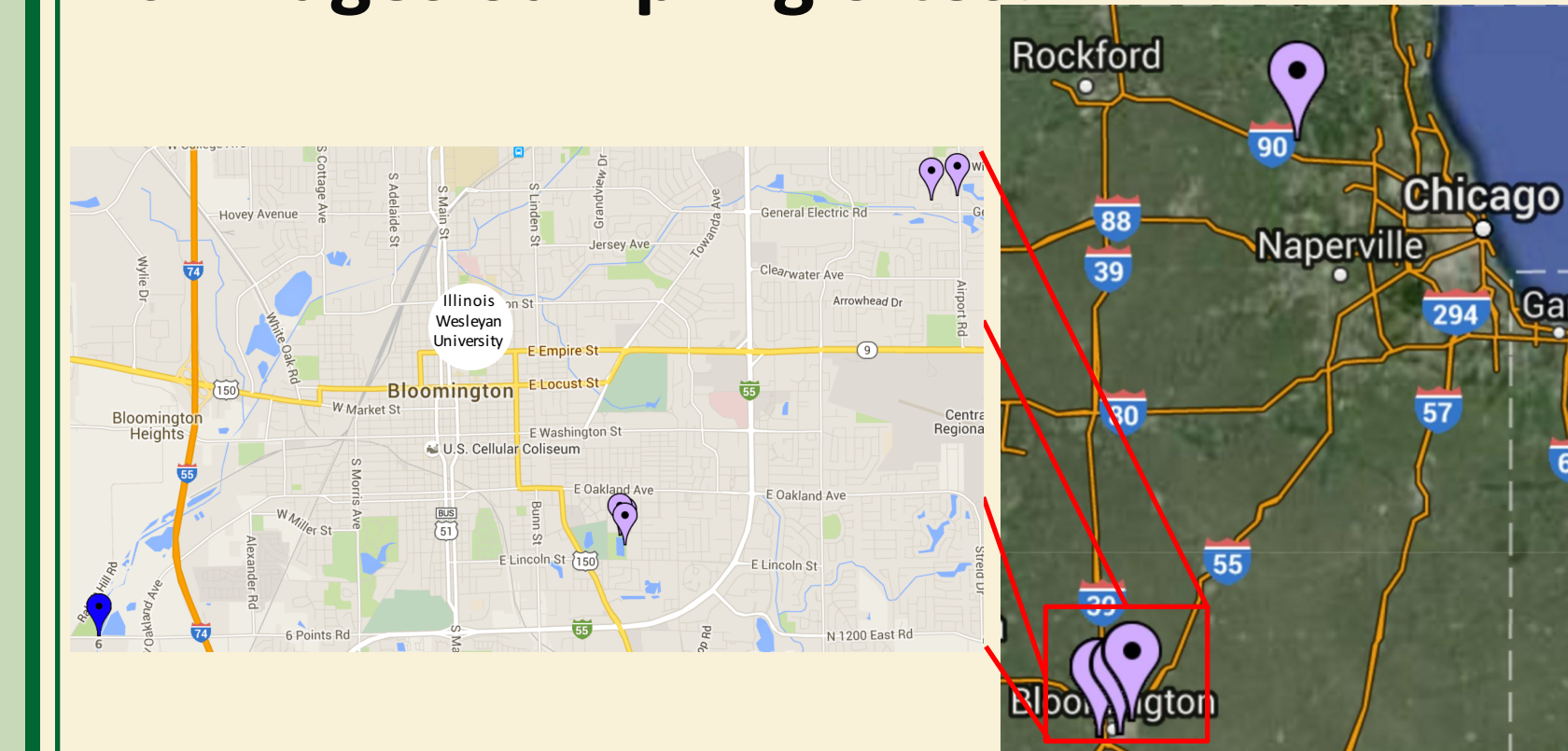
Table 4: Rc Phage Clusters Before

Cluster A	Rhea	Cronus	Saxon
Cluster B	Titan	Spartan	
Singletons	Oceanus		

Rc Phage Genome Comparison Before

	# of Genes	GC Content	# of Basepairs
Avg. Rc Phage	54.56	62.531%	39,072
Rc Strain YW1	3,481	66.6%	3,642,667

## Rc Phages Sampling Sites:



Right: Image of the successful *Rc* sampling sites. Above: Close up map of successful and failed *Rc* sampling sites in Bloomington/Normal. The blue pin represents where all the previous 6 phages were found and the purple represents where the new phages were found.

Table 5: Plaque Morphology

Phage Name	Plaque Sizes	Plaque Features
Bellator	Large	Clear
Dormio	Medium	Cloudy
DrBanner	Medium	Cloudy
FrancesLouise	Medium	Cloudy
McDreamy	Very Small	Clear
Tiptonus	Tiny	Clear



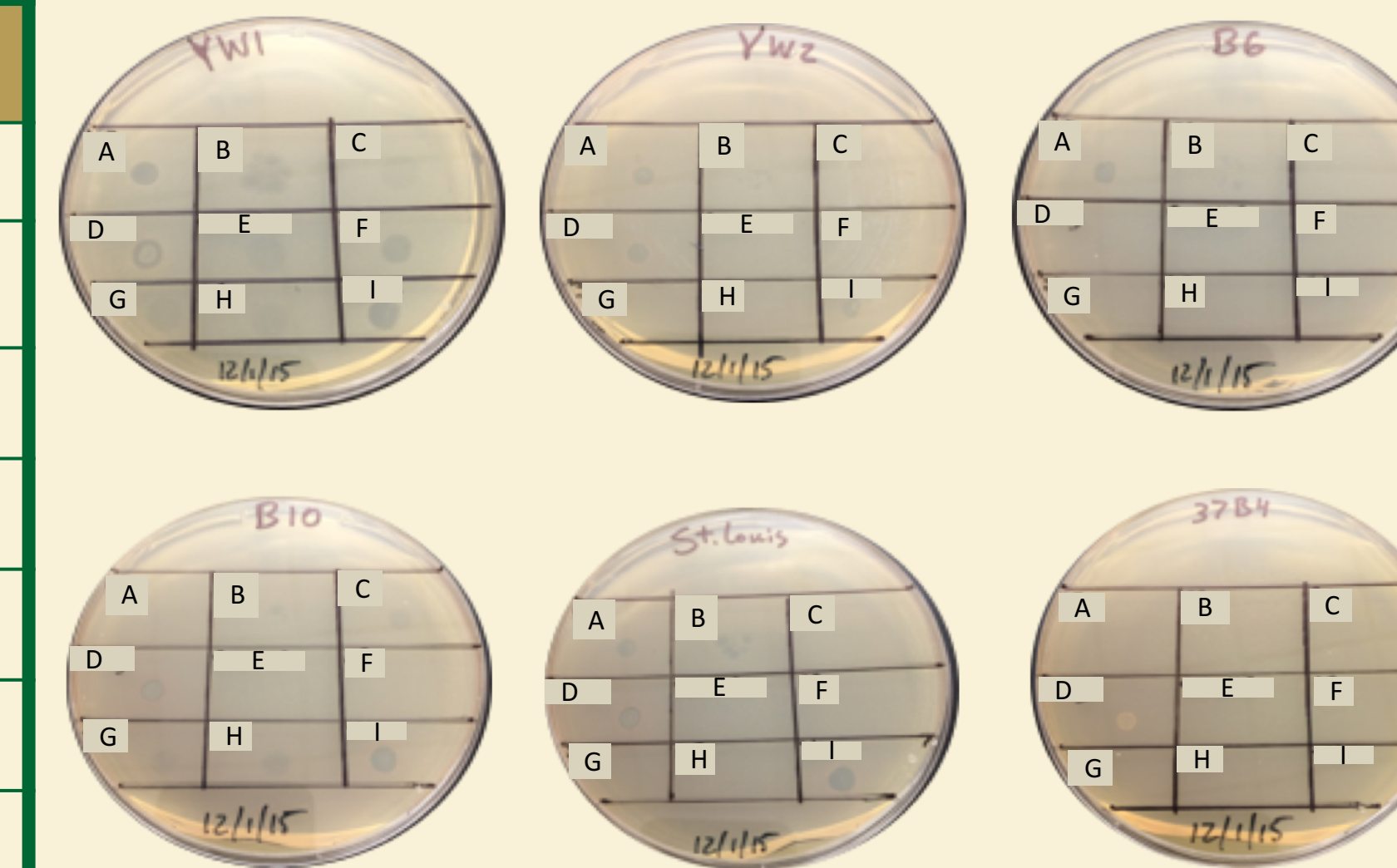
Above: Table describing the plaque morphology of the six *Rc* phages. Right: Plaque photos of the phages McDreamy, Dormio, and Bellator.

## Host Range Testing

Host range testing was conducted to analyze the effectiveness of nine *Rc* phages in infecting 6 different strains of their host. The procedure was the same for each host. First a plate with a specific bacterial strain was made, then aliquots of each *Rc* phage were spotted on each of the plates. The images and table below provide the results that were obtained upon using the six different host bacterial strains.

Table 6: Host Range Testing Results

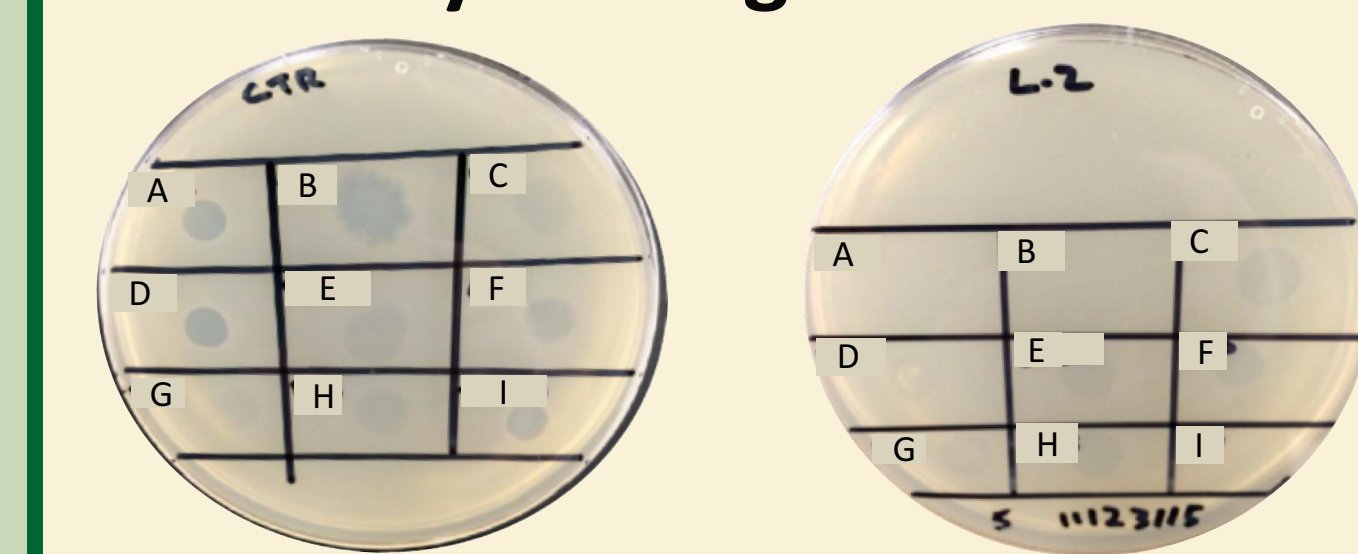
Phage Name:	<i>Rhodobacter capsulatus</i> strain					
	YW1	YW2	B6	B10	St. Louis	37B4
Viking (D)	+	+	+	+	+	-
Titan (I)	+	+	-	+	+	-
Oceanus (E)	+	-	-	-	-	-
Tiptonus (A)	+	+	+	+	+	-
Bellator (B)	+	+	+	+	+	-
McDreamy (F)	+	-	-	+	-	-
DrBanner (H)	+	-	-	+	-	-
Dormio (C)	+	-	-	+	-	-
FrancesLouise (G)	+	-	-	+	-	-



Above: Images showing the abilities of the 9 phages in table 6 to infect a range of 6 different strains of *Rhodobacter capsulatus*.

Table 6: Analysis of the ability of various *R. capsulatus* bacteriophages to form plaques by infecting different strains of *R. capsulatus*.

## Immunity Testing:



Above: YW1 Control Above: Tiptonus Lysogen

Using Immunity Testing procedures for the *Rc* phage Tiptonus, a lysogen resistant to Tiptonus was created and then spot-tested against the phages listed in the "Phages Key" below. Of those phages, all save for Bellator and Titan formed a plaque on the Tiptonus resistant lysogen.

## Rc Phage Sequencing Results:

Table 7: Rc Phage Clusters After

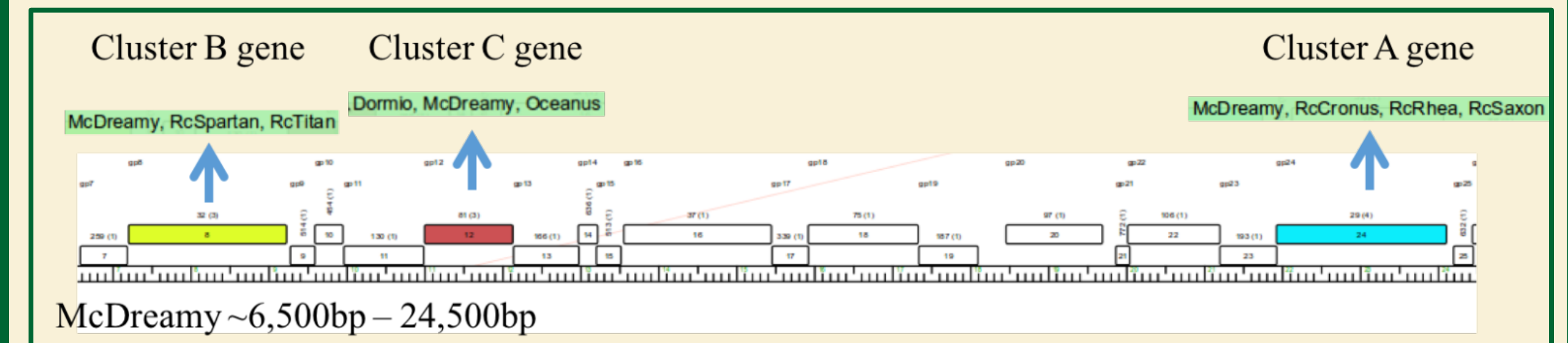
Cluster A	Rhea	Cronus	Saxon
Cluster B	Titan	Spartan	
Cluster C	Oceanus	Dormio	
Singletons	McDreamy	Tiptonus	

Rc Phage Genome Comparison After

	# of Genes	GC Content	# of Basepairs
Dormio	73	64.083%	41,656
McDreamy	104	59.964%	68,244
Tiptonus	148	57.988%	95,520
Avg. Rc Phage (After)	67.92	62.068%	45,278

After sequencing the three *Rc* phages Dormio, McDreamy and Tiptonus, several things were discovered:

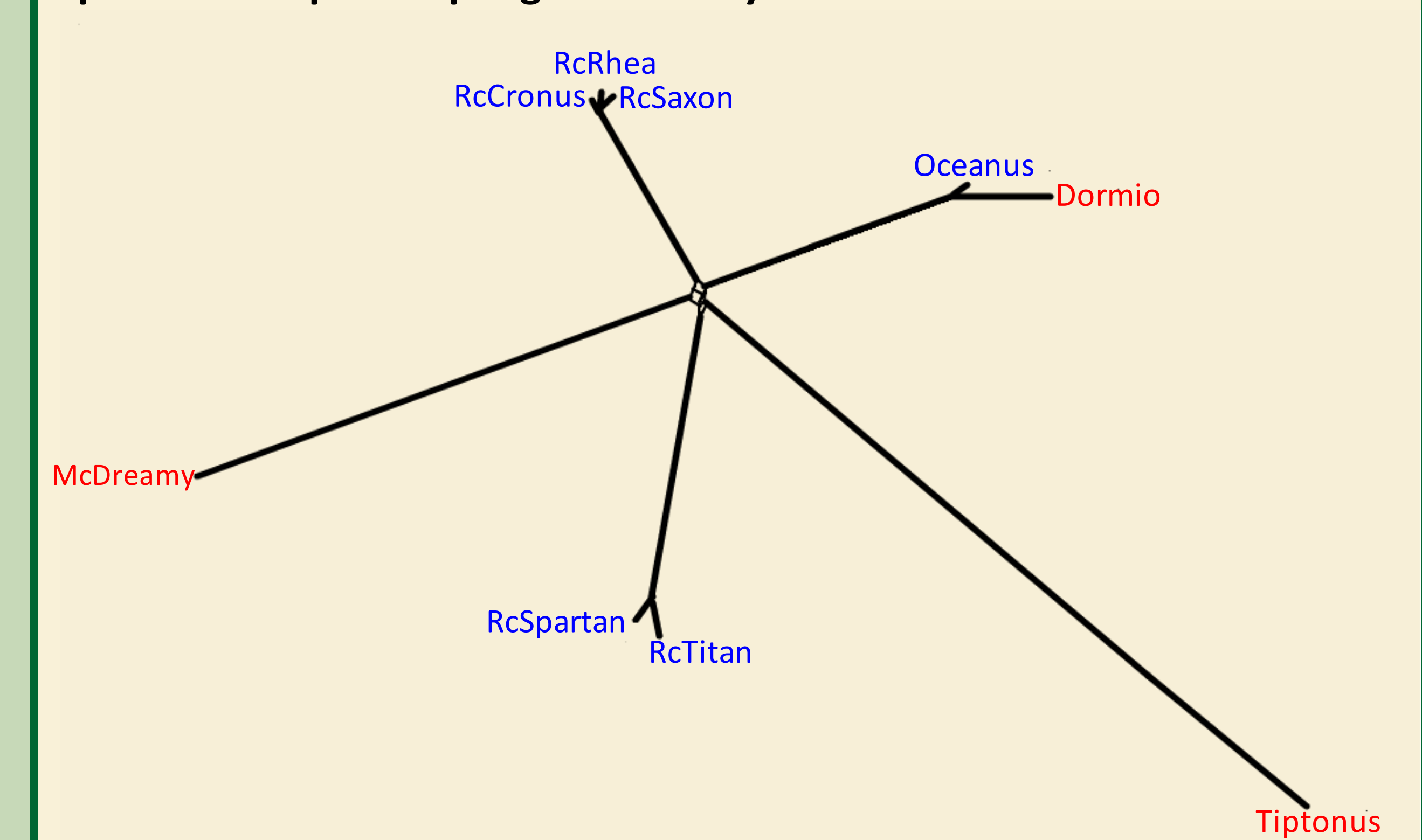
1. Dormio is very similar to Oceanus, and thus allowed the creation of the third cluster, Cluster C.
2. McDreamy currently has the second largest *Rc* phage genome, and though it contains many orphans, it also demonstrates mosaicism as it shares genes with phages from all clusters (see the image below).



3. Tiptonus contains the longest known *Rc* phage genome, and is currently classified as a singleton. Tiptonus shares few genes with the other isolated *Rc* phages.

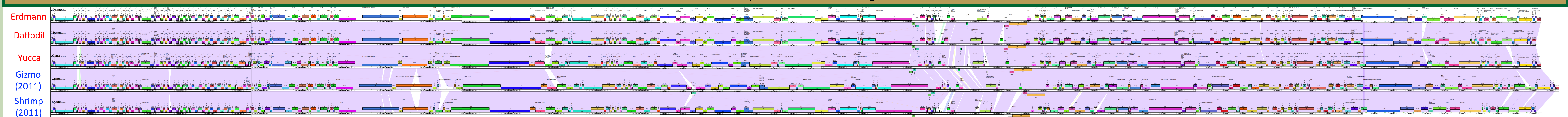
See Phamerator map showing a comparison of all currently isolated and sequenced *Rc* phages.

## Splitstree map of Rc phages found by IWU:

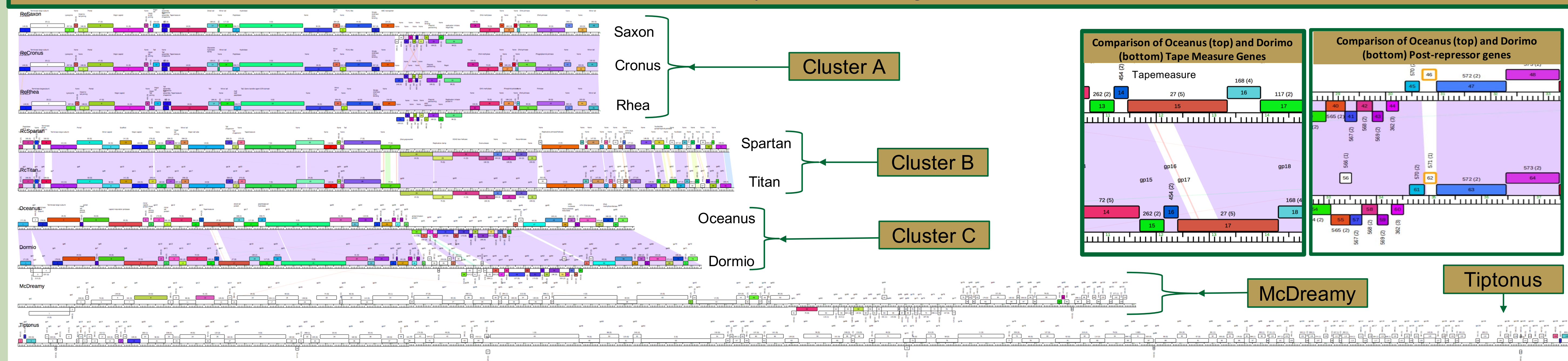


Above: Relatedness diagram of the 9 *Rc* phages known to have been isolated on the host strain YW1.

## Genome Comparison of IWU C1 Ms Phages



## Genome Comparison of IWU Rc Phages



## Future Directions Ms phages:

1. Determine functions of the many genes with no known function.
2. The remaining 13 phages that were not sequenced could be sequenced in order to determine better understand them.

## Future Directions Rc phages:

1. The three phages that were found and not sequenced could be sequenced, once purified DNA is obtained.
2. Determine how related Tiptonus is to Bellator and Titan.
3. Determine why Dormio is unable to infect the *Rc* strain B10, whether this lies in the Tape Measure Protein or in the post-repressor Gene.

## Acknowledgements:

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