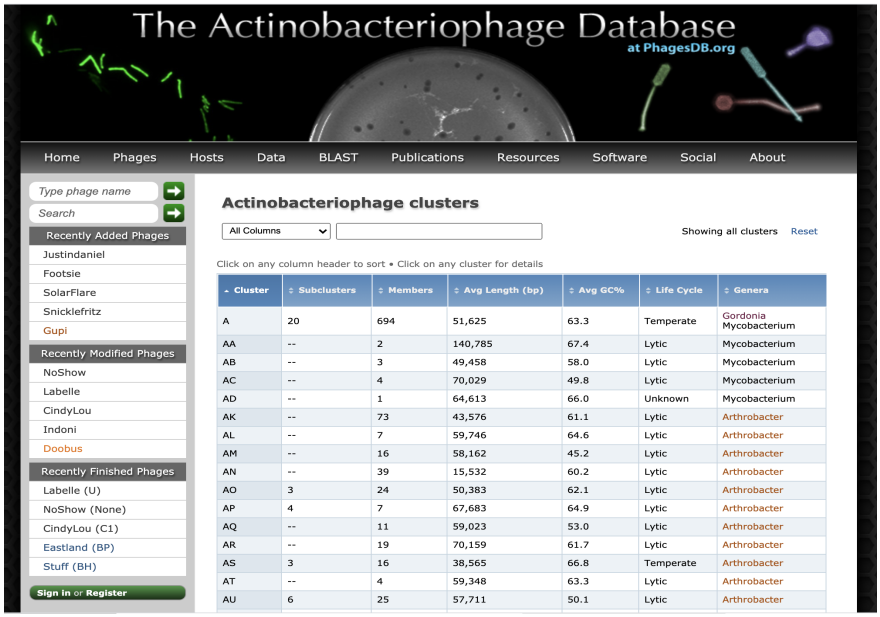
Activity 4: Virtual digest of Phage genomes

In order to examine whether the bacteriophages you and your classmates isolated during the semester are similar or different genetically, restriction enzyme analyses can be performed to prioritize which Phages should be selected for sequencing analysis. To be able to interpret and understand these experiments, first you will perform a virtual digest on a few of the previously sequenced Phages on Phages DB.

Instructions

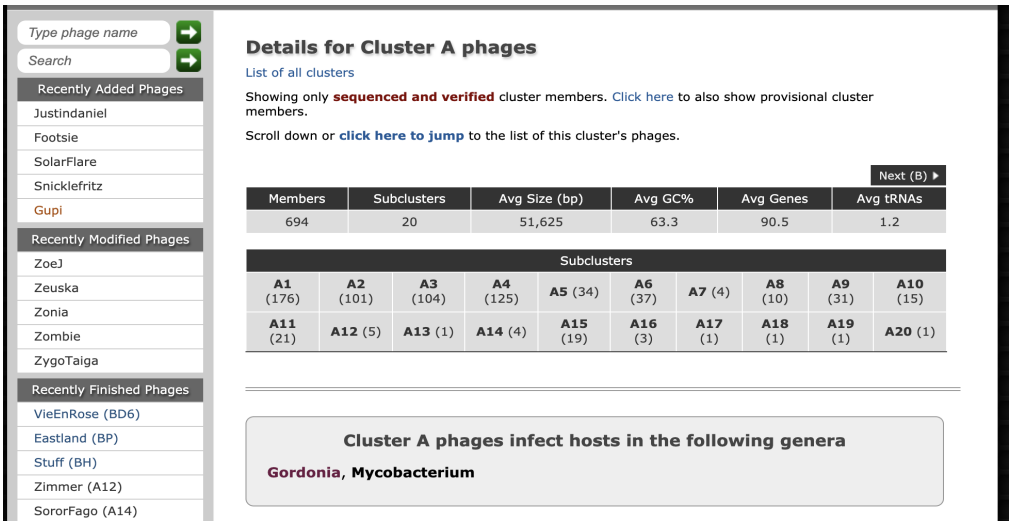
Step 1) Getting Phage Genome sequences

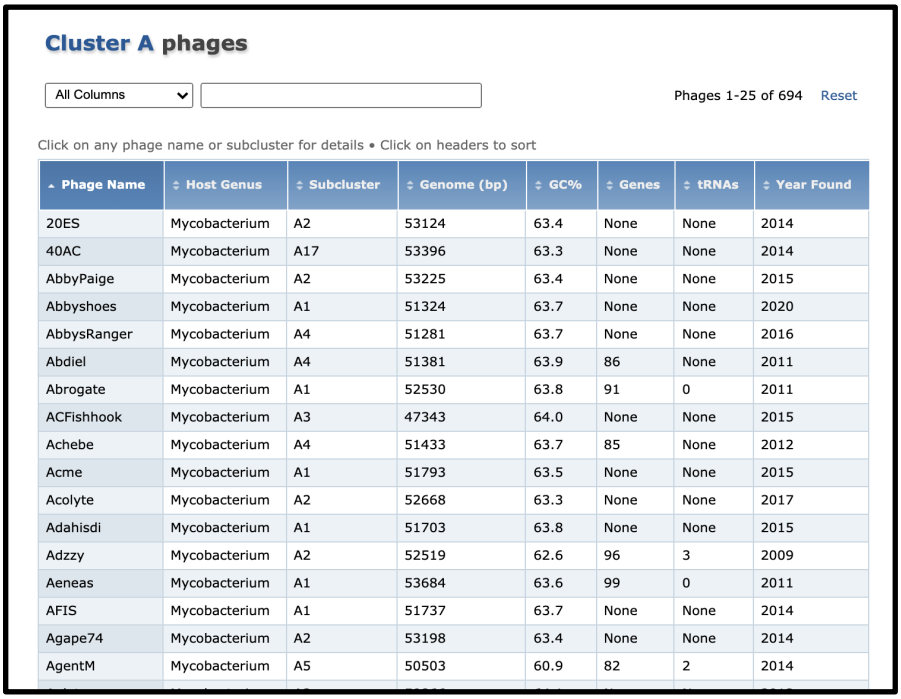
1.1 Go to Phages DB cluster page by navigating to https://phagesdb.org/clusters/



Cluster	Subclusters	Members	Avg Length (bp)	Avg GC%	Life Cycle	Genera
A	20	694	51,625	63.3	Temperate	Gordonia Mycobacterium
AA	--	2	140,785	67.4	Lytic	Mycobacterium
AB	--	3	49,458	58.0	Lytic	Mycobacterium
AC	--	4	70,029	49.8	Lytic	Mycobacterium
AD	--	1	64,613	66.0	Unknown	Mycobacterium
AK	--	73	43,576	61.1	Lytic	Arthrobacter
AL	--	7	59,746	64.6	Lytic	Arthrobacter
AM	--	16	58,162	45.2	Lytic	Arthrobacter
AN	--	39	15,532	60.2	Lytic	Arthrobacter
AO	3	24	50,383	62.1	Lytic	Arthrobacter
AP	4	7	67,683	64.9	Lytic	Arthrobacter
AQ	--	11	59,023	53.0	Lytic	Arthrobacter
AR	--	19	70,159	61.7	Lytic	Arthrobacter
AS	3	16	38,565	66.8	Temperate	Arthrobacter
AT	--	4	59,348	63.3	Lytic	Arthrobacter
AU	6	25	57,711	50.1	Lytic	Arthrobacter

1.2 Click on your favorite cluster. It doesn't matter which cluster you pick, however if you are interested in looking at Phages from a cluster that belongs to the host you are working in the lab, take a note of the host listed on the last column before selecting a specific cluster. The Cluster page will display known details about the cluster, eg what kind of Phages are usually found within the cluster, any important publications etc. Scroll to the bottom of the page and it will show a list of the Bacteriophages in the cluster.



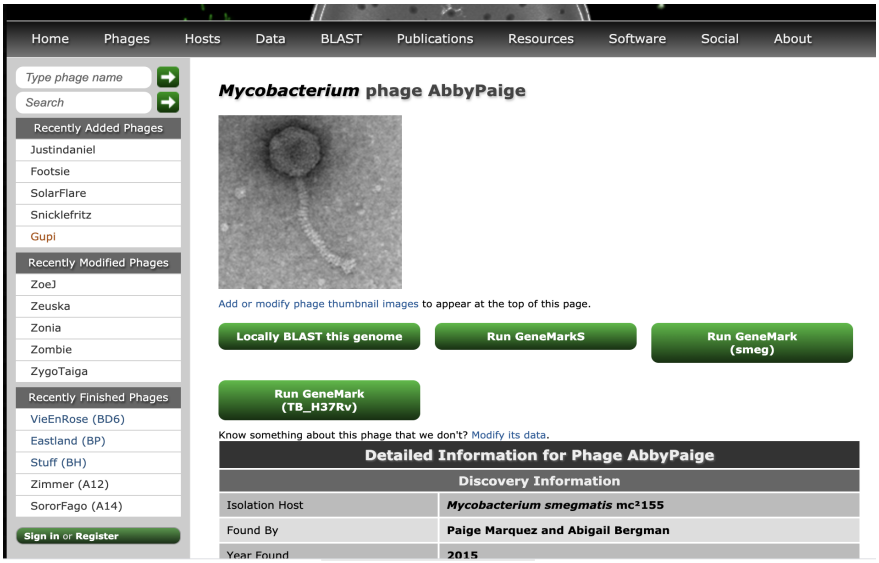


Phage Name	Host Genus	Subcluster	Genome (bp)	GC%	Genes	tRNAs	Year Found
20ES	Mycobacterium	A2	53124	63.4	None	None	2014
40AC	Mycobacterium	A17	53396	63.3	None	None	2014
AbbyPaige	Mycobacterium	A2	53225	63.4	None	None	2015
Abbyshoes	Mycobacterium	A1	51324	63.7	None	None	2020
AbbysRanger	Mycobacterium	A4	51281	63.7	None	None	2016
Abdiel	Mycobacterium	A4	51381	63.9	86	None	2011
Abrogate	Mycobacterium	A1	52530	63.8	91	0	2011
ACFishhook	Mycobacterium	A3	47343	64.0	None	None	2015
Achebe	Mycobacterium	A4	51433	63.7	85	None	2012
Acme	Mycobacterium	A1	51793	63.5	None	None	2015
Acolyte	Mycobacterium	A2	52668	63.3	None	None	2017
Adahisdi	Mycobacterium	A1	51703	63.8	None	None	2015
Adzzy	Mycobacterium	A2	52519	62.6	96	3	2009
Aeneas	Mycobacterium	A1	53684	63.6	99	0	2011
AFIS	Mycobacterium	A1	51737	63.7	None	None	2014
Agape74	Mycobacterium	A2	53198	63.4	None	None	2014
AgentM	Mycobacterium	A5	50503	60.9	82	2	2014

Note there might be sub-clusters in a specific Cluster. For the first exercise, you have to compare three phages from the same Cluster. If the Cluster is a really large one then it is better to pick Phages from the same sub-cluster.

1.3 Once you have made your selection, click on the Phage name and you will be taken to the page where Phage information is displayed. As an example, https://phagesdb.org/phages/AbbyPaige/ shows all the information known about bacteriophage AbbyPaige. Navigate to the link for Fasta file on the page. This is

the genome of the Phage. Make a folder on your computer, download and save the FASTA file with the Phage name. You will need this later.



1.4 Repeat steps 1.1-1.3, for two other Phages from the same cluster and again for two from other Clusters.

Step 2 Analyzing the genome by creating restriction enzyme maps

Once you have obtained the FASTA file for all Phages, you are ready to analyze them using a virtual DNA digest tool from NEB. This tool allows you to visualize differences or similarities in the genome of phages based on restriction enzyme recognition sequences. The most commonly used restriction enzymes used by SEA-PHAGES are BamHI, ClaI, EcoRI, HaeI, HindIII. See Activity 1 for how restrictions enzymes are named and how they recognize specific DNA sequences. You will need to pick any three enzymes from the above list.

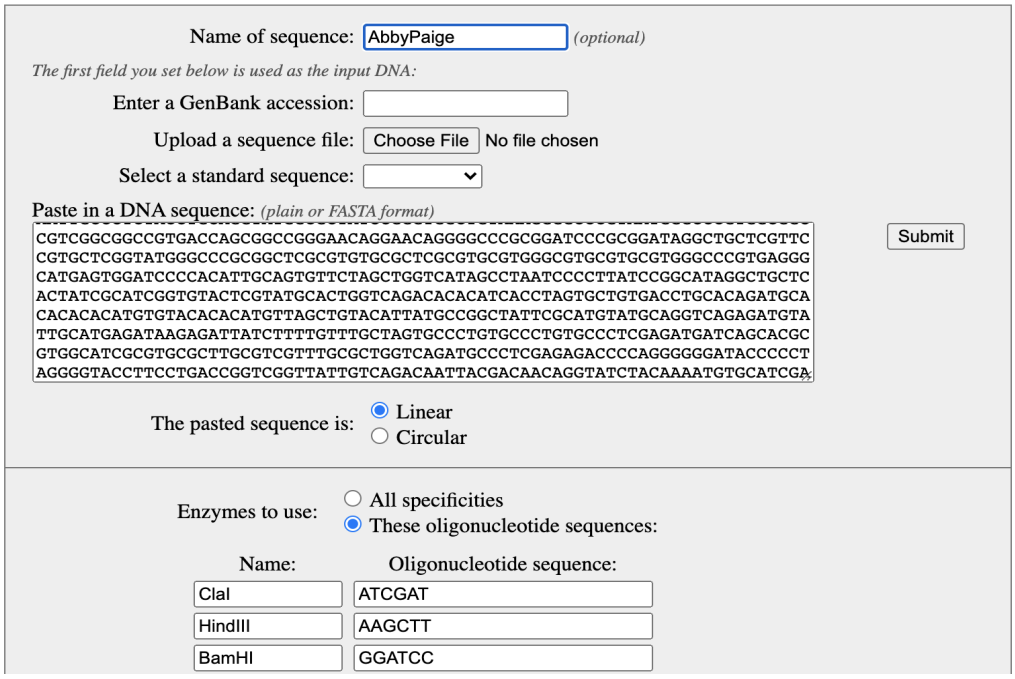
2.1 Open a browser tab and direct it to the NEB REBsites webpage
http://tools.neb.com/NEBeta/REBsites/index.php
Upload the first Phage .fasta file or Paste the entire text into the DNA sequence text field and select “The pasted sequence is:” “Linear”.

2.2 For “Enzymes to use:” select “These oligonucleotide sequences:”

a. In the “Name” text fields, enter the names of your chosen enzymes eg “HindIII”, “EcoRI”, and “BamHI”.

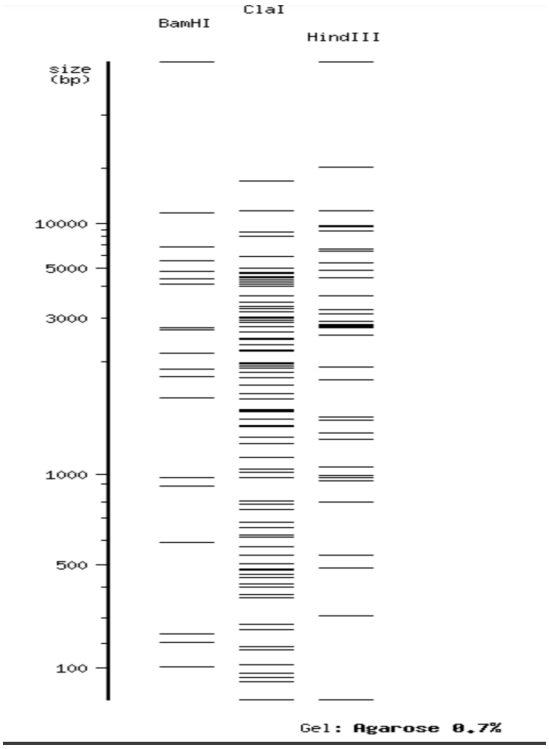
b. In an additional browser tabs, open up the webpages for the individual restriction enzymes (as you did for Activity 3):
Eg., HindIII https://www.neb.com/products/r0104-hindiii and find within the webpage the recognition sequence for each restriction enzyme.

2.3 In the “Oligonucleotide sequence:” text field, enter the restriction site sequence for each of the listed restriction enzymes.



Note: Always double check that the option “These oligonucleotide sequence” is selected under Enzymes to use.

2.4 When you click “Submit” button the following results showing a virtual gel should appear. Get a screen shot of the gel and save it with the Phage name and enzyme names.



2.5 Repeat these Steps 2.1-2.4 and generate similar screen shots for each of your selected Phages.

Step 3 Generating final assignment figures

For the final assignment you will submit two completed figures. One figure will have the side by side comparison of Phages from the same cluster. This figure should have 3 panels in total one for each of the Phages displayed side by side. The second figure should have same format but these Phages will be from different clusters.

Assessment:

1. Upload Fig 1 : picture of the virtual digest gel of same cluster phages
2. Upload Fig 2 : picture of the virtual digest gel of different cluster phages
3. Briefly comment on your main observations from inferences that can be drawn from each figure comparing the restriction enzyme profiles of the Phages. What similarities or differences did you find between the phages based on the above criteria?
    a. For Phages that have a similar restriction digest profiles is it the same across all enzymes?
    b. For Phages that have distinct restriction digest profile which enzymes work best in identifying the differences?
4. What might be a reason that there are major biological differences such a host specificity in two phages that have identical restriction enzyme profile?

5. Compare these phages using these other criteria:
    - Morphotype
    - GC content
    - Location they were isolated
    - Host
    - Plaque type (lytic/ temperate)

Note for the instructors:
Delivery Mode: This exercise can be assigned as a pre-lab or an activity that accompanies the restriction digest labs. It can also be used as an online stand alone lab. This exercise could work well in groups or individually. Group activity will encourage discussion and critical thinking where members of a group could each work on one Phage /enzyme set and the group comes together to analyze and compare the restriction enzyme profiles. If implemented during lab, the activity would work well when students are waiting for digests to incubate or gels to finish running.

Assessment : Using some-kind of learning based system such as Canvas could be a place for collecting student responses to the questions.

Time for implementation: Depending on how familiar students are with Phages Db , this exercise can take 30-45 minutes. As mentioned earlier, group work will allow discussion and critical thinking and will reduce the time spent on the activity.

Additional tools:
There are some additional tools available for performing virtual digests of Phage DNA that the instructors can have students explore if they want to expand this activity or explore using a different tool.

Virtual gels on Phages DB. On PhagesDB, some phages already have virtual gels associated with them. To see all virtual gels from a particular cluster or subcluster, go to http://phagesdb.org/compare/ and select “Virtual Digest” from the “Picture Type” dropdown menu. Creating your own virtual digests can be done in several ways.

Case It was developed at the University of Wisconsin, River Falls (a SEAPHAGES institution) and allows users to run gels for different times or with different agarose concentrations, to better match real student gels. There are enzymes built into the software, but you can also upload any enzyme site. Case It can be found at http://www.caseitproject.org/ Phage Enzyme Tools

The Phage Enzyme Tools were developed at Louisiana, Monroe, and are a great way to do analysis of digest results. You can use them to quite easily predict your unknown phage’s cluster from its digest pattern. Go to phageenzymetools.com to give them a try, and watch a video tutorial on seaphages.org to learn how they can be used

References

1. Poxleitner M, Pope W, Jacobs-Sera D, Sivanathan V, Hatfull G. 2018. Phage discovery guide. Howard Hughes Medical Institute, Chevy Chase, MD.

2. Spencer D. Heringa, JinKyung Kim, Xiuping Jiang, M. P. Doyle, and M. C. Erickson 2010. Use of a Mixture of Bacteriophages for Biological Control of Salmonella enterica Strains in Compost, Applied and Environmental Microbiology.

3. Chris R. Gissendanner, Allison M. D. Wiedemeier, Paul D. Wiedemeier, Russell L. Minton, Swapan Bhuiyan, Jeremy S. Harmson and Ann M. Findley 2013. A web-based restriction endonuclease tool for mycobacteriophage cluster prediction, Journal of basic Microbiology.