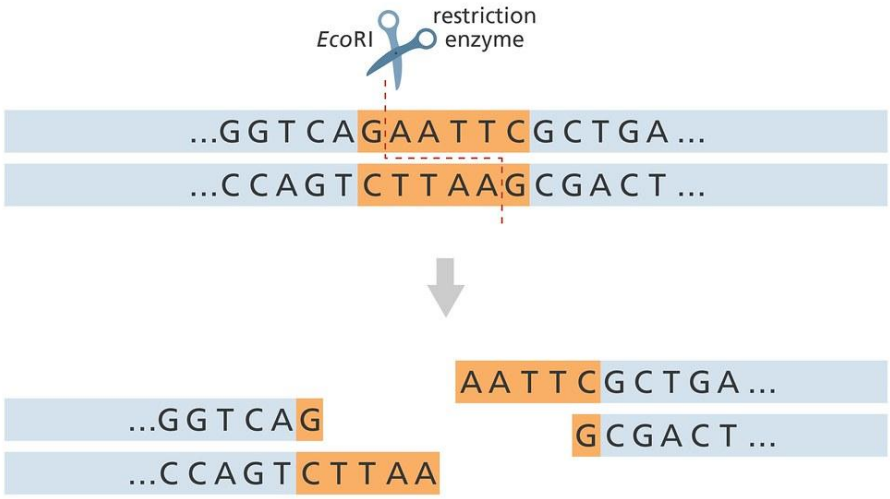
Activity 3: Virtual Lamda-HindIII Digest

A step in characterizing your phage DNA to determine if its genome is similar to or different from other phages is to cut it with restriction enzymes and then separate the DNA fragments by size using gel electrophoresis (1). To determine the size of each DNA fragment, or band, as it appears on the gel, a DNA ladder containing DNA fragments of known size is also run on the gel. This ladder can be used to estimate the size of each band from your sample. A common DNA ladder that you might use is the lambda DNA/*Hind*III marker.

Restriction enzymes are proteins produced by bacteria that specifically recognize short DNA sequences (4-8 bp in length) and can break the phosphodiester backbone of each strand within this sequence to cut or digest the DNA. The recognition sequences, or sites, are usually palindromic in that both strands of DNA will have the same sequence when reading 5' to 3'.



First isolated by Esther Lederberg in 1951 and sequenced by Fred Sanger in 1982, bacteriophage lambda (λ) infects *Escherichia coli* and has a DNA genome that is 48,502 base pairs (bp) in length (2, 3). Recognition sites for several restriction enzymes are distributed throughout the lambda genome, so a digest of lambda DNA with a particular enzyme will yield a characteristic collection of fragments, each with a distinct size, that produces a banding pattern.

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Exercise 3.1

On the New England Biolabs (NEB) REBsites webpage http://tools.neb.com/NEBeta/REBsites/index.php, choose Lambda from the “Select a standard sequence” drop-down menu and click the “Submit” button.

On the results page, you will be presented with virtual restriction enzyme digests of lambda phage DNA shown in alphabetical order of the restriction enzyme name, with *Acl*1 digest run in the last lane (Fig. 1).

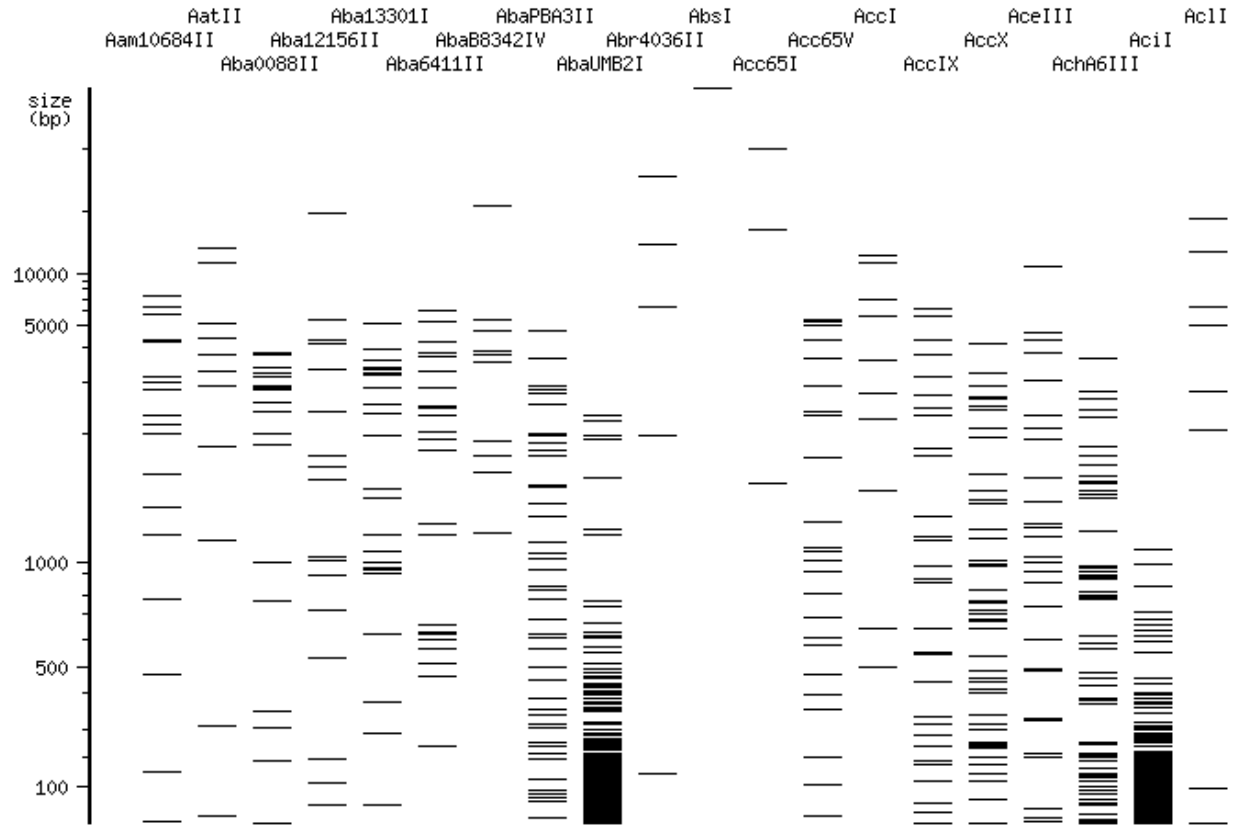

Figure 1: Virtual restriction enzyme digest of lambda DNA using restriction enzyme *Aat*II (lane 2) and *Acl*1 (lane 20) resolved in a 0.7% agarose gel. The fragment size in base pairs (bp) is indicated on the left.

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The second lane is a virtual digest using the restriction enzyme *Aat*II, which recognizes the DNA sequence 5'-GACGTC-3' and forms a four-base 3' overhang. *Aat*II is a six-base cutter restriction enzyme isolated from *Acetobacter aceti* IFO 3281 (4). The cleavage positions are indicated by arrows.

Click on the restriction enzyme name and a popup window will display a map of the restriction enzyme cut sites and a table that has information about the location of the cut sites and the size of the fragment produced. The location of the *Aat*II cut site closest to the 5' end of the lambda genome is between nucleotides 5,109 and 5,110 and produces a fragment that is 5,109 base pairs (bp) in size. The next cut site is between nucleotides 9,398 and 9,399 and produces a fragment that is 4,289 bp in length. Altogether there are 10 *Aat*II cut sites in the lambda genome that when digested with the restriction enzyme will produce 11 DNA fragments that range from 29 bp to 14,062 bp in size (Fig. 1).

Question Set 1:

3.1.1. Using the information on the map and table for *Aat*II digest of the lambda genome, circle the band in Fig. 1 that corresponds to the fragment produced between the second and third cut sites from the 5' end.

3.1.2. Using only the information shown in the virtual digest in Fig. 1:
- 3.1.2.1. How many fragments are generated when lambda DNA is digested by *Acl*1 (lane 20, the last lane)?
- 3.1.2.2. How many *Acl*1 recognition sites are there in the lambda genome?

3.1.3. In the agarose gel % drop-down menu, select each of the percent agarose gels (0.7%, 1%, 1.2%, 1.4%, 2%, and 3%) and analyze the banding patterns of *Aat*II and *Acl*I.
- 3.1.3.1. Describe any changes that you observe.
- 3.1.3.2. Based on your observation, why might different percent agarose gels be used?
- 3.1.3.3. Explain how a restriction enzyme digest using *Aat*II and lambda DNA could have a different-looking banding pattern if performed by two different researchers.

3.1.4. In the "order by" drop-down menu, select "number of fragments" and browse the results of the first five pages using the "[Next>>]" button. From this data, name a restriction enzyme that you think could be used to make a useful DNA ladder and explain your reasoning for choosing that restriction enzyme.

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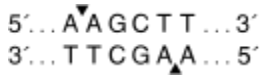
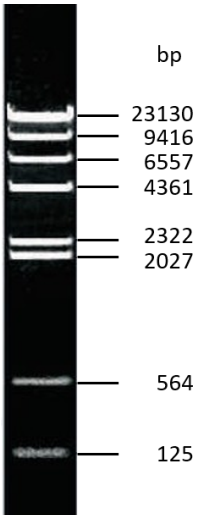
Exercise 3.2

The first site-specific restriction enzyme was isolated from *Haemophilus influenzae* bacteria in 1972 by Hamilton Smith, who later shared the Nobel Prize in Medicine in 1978. The discovery came by chance when Smith incubated bacteria and phage together and he happened to notice that the phage DNA degraded over time.

*Hind*III (pronounced "Hin D Three") recognizes and cuts the palindromic DNA sequence 5'-AAGCTT-3' (5) (Reviewed in Excercise 1).



The *Hind*III digest of lambda DNA yields 8 fragments suitable for use as molecular weight markers for linear double-stranded large DNA fragments in agarose gel electrophoresis.



1. Go to the NEB DNA Sequences and Maps Tool webpage, https://www.neb.com/tools-and-resources/interactive-tools/dna-sequences-and-maps-tool.
2. On the row for Lambda, open the Fasta file from the Nucleotide Sequences column, https://www.neb.com/-/media/nebus/page-images/tools-and-resources/interactive-tools/dna-sequences-and-maps/text-documents/lambdafsa.txt?rev=c0c6669b9bd340ddb674ebfd9d55c691&hash=0FA7153BEE1D9D85A249FDDB91BF53BF.
   You can then either:
   a. Download as a .txt or .fasta file or
   b. Copy the entire text
3. Open a second browser tab and direct it to the NEB REBsites webpage http://tools.neb.com/NEBeta/REBsites/index.php
   and either:
   a. Upload the Lambda_NEB .txt or .fasta file or
   b. Paste the entire text into the DNA sequence text field
   and select "The pasted sequence is:" "Linear".
4. For "Enzymes to use:" select "These oligonucleotide sequences:"

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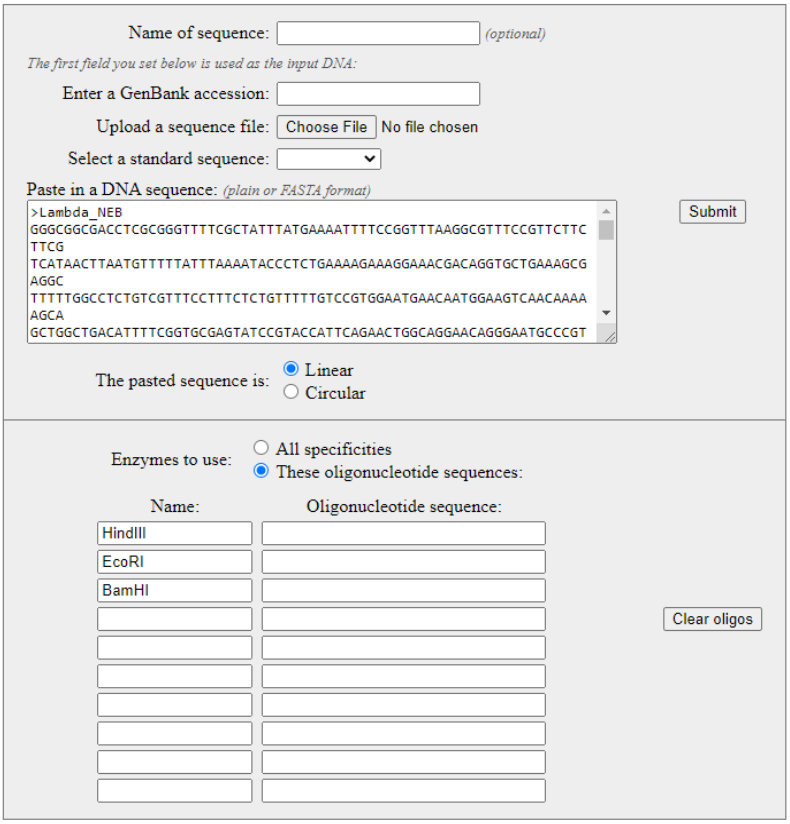
5. In the “Name” text fields, enter “HindIII”, “EcoRI”, and “BamHI”.



6. In additional browser tabs, open up the webpages for the following restriction enzymes:
   a. *Hind*III https://www.neb.com/products/r0104-hindiii
   b. *Eco*RI https://www.neb.com/products/r0101-ecori
   c. *Bam*HI https://www.neb.com/products/r0136-bamhi

   and find within the webpage the recognition sequence for each restriction enzyme.
7. In the “Oligonucleotide sequence:” text field, enter the restriction site sequence for each listed restriction enzyme.
8. Click the “Submit” button to generate the results page.

Question Set 2:

3.2.1. Does your *Hind*III virtual digest appear similar to the photo of the gel shown above? If not, what are some reasons your results are different?

3.2.2. How many *Hind*III, *Eco*RI, and *Bam*HI sequences are there within the lambda genome?

3.2.3. What are the sizes of the fragments produced by *Hind*III, *Eco*RI, and *Bam*HI?

3.2.4. Do you think that “better” DNA ladders can be made by cutting lambda DNA with more than one restriction enzyme? Explain the reasoning behind your answer.

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. Lederberg EM, Lederberg J. 1953. Genetic Studies of Lysogenicity in Escherichia Coli. Genetics 38:51–64.

3. Sanger F, Coulson AR, Hong GF, Hill DF, Petersen GB. 1982. Nucleotide sequence of bacteriophage λ DNA. J Mol Biol 162:729–773.

4. Sugisaki H, Maekawa Y, Kanazawa S, Takanami M. 1982. New restriction endonucleases from Acetobacter aceti and Bacillus aneurinolyticus. Nucleic Acids Res 10:5747–5752.

5. Smith HO, Nathans D. 1973. Letter: A suggested nomenclature for bacterial host modification and restriction systems and their enzymes. J Mol Biol 81:419–423.