Restriction digests on paper

Instructor copy

Learning objectives

- Describe how restriction enzymes cut DNA at specific sites
- Compare and contrast different types of cutters in terms of length of recognition sequence and shape of end product
- Explain why restriction enzymes are useful tools in biotechnology

Notes: the last two pages contain

- a reusable gel figure and
- all of the cut-outs for each exercise on one page to make this resource better suited for double-sided printing

Exercise 1: Parts of a gel

The example gel that you will work with is on the last page of the handout. Using the background information and other resources provided, label the following parts of the gel:

- ❑ Sample wells
- ❑ Negative electrode
- ❑ Positive electrode
- ❑ Direction of DNA movement (draw an arrow)
- ❑ Size standard (sometimes called a “ladder” or “gene ruler”)

Exercise 2: separation based on size

The following rectangles represent fragments of DNA that are different lengths, meaning they have different numbers of total nucleotides. When different DNA fragments are in a solution with each other, we can't tell the difference between them directly. Instead, we use gel electrophoresis, which separates fragments of DNA based on their size. Smaller fragments can travel faster than larger fragments (review the background information).

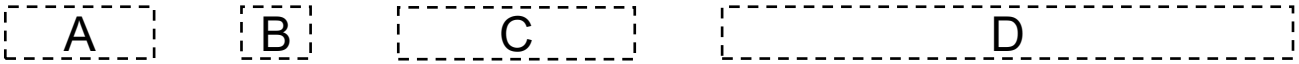
Cut out the following rectangles, then place them on the gel in the expected order based on size assuming the gel has completed running. The length of the rectangle corresponds to the length of the DNA fragment. Do not worry about the well size in this example. Have your order checked by a classmate or instructor as directed.

1. What is the order of fragments from smallest to largest?

   B, A, C, D

2. Which fragment will be closest to the negative electrode after the gel has run? Why?

   D, because it is the longest fragment so it will move the least. The longer fragments get "stuck" in the agarose matrix more than the smaller fragments.



Exercise 3.1: Cutting DNA with precision

When we work with genomic DNA (the total DNA purified from an organism), we typically need to modify it in specific ways to be able to measure, visualize, and characterize the properties of that DNA sample. There are many different methods that can be used, and restrictions enzymes are one of our best tools to make predictable changes to DNA.

Restriction enzymes are naturally occurring enzymes made by bacteria to protect them from invading viruses (phages!). These enzymes are able to bind to DNA and cut the DNA backbone, which inhibits the viral functions. This is very interesting on its own, but the really valuable feature of restriction enzymes is that they are "restricted" to cutting only the specific sequence that the enzyme recognizes, and each enzyme can only read one nucleotide word. Some restriction enzymes read and recognize a 4-letter word, like ACCA, while others recognize 6, 8 or other length words. This specificity means that we can learn something about our input DNA sequence depending on whether it is cut by a specific enzyme.

For example, the enzyme EcoRI recognizes the sequence (from 5' to 3') "G A A T T C" and cuts the DNA backbone between the G and A. When we write out the two complementary strands of DNA we find something interesting:

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5′-GAATTC-3′
3′-CTTAAG-5′
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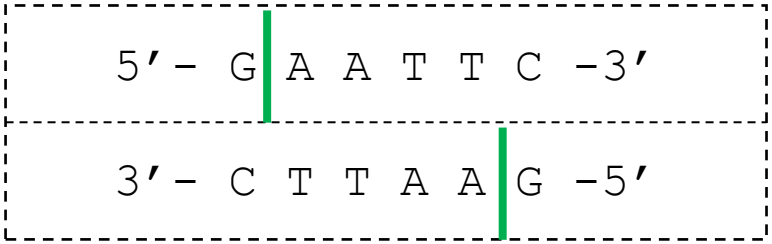
The sequences are the same reading 5' to 3', and the enzyme will recognize the sequence and cut site on each strand. The enzyme HaeIII recognizes the sequence "GGCC" and cuts between the second G and first C. Again, on the other strand, reading 5' to 3', the sequence will still be GGCC.

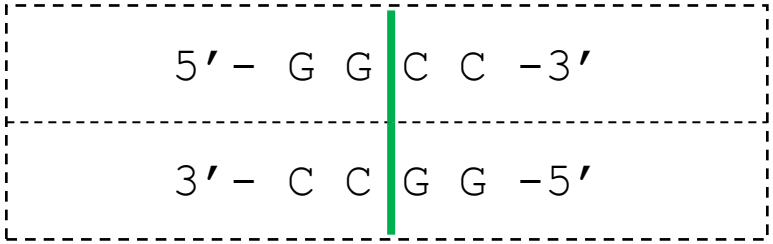
Cut out the following rectangles, cut the center line to separate the strands of DNA, and then cut the backbone of each strand with the indicated enzyme.

1. Where will the DNA backbone be cut in each strand? Shown below
2. How are the resulting ends of the sequences different between these two cuts? EcoRI leaves overlapping, "sticky" ends. HaeIII leave blunt ends.

Cut with EcoRI



Cut with HaeIII



Exercise 3.2: Cutting DNA with precision

We can learn some important information from restriction enzyme digests due to the specificity of each enzyme for a particular DNA sequence. Different DNA sequences will be cut differently with a restriction enzyme depending on their specific sequence.

Cut out the following rectangles with DNA sequences (*note that only one strand is being shown for simplicity*).

These sequences represent two different biological samples (e.g., two different phages). Their total length is equal, so if we only visualize the genomic DNA on a gel, they will look identical. What will they look like on a gel if cut with different enzymes?

Cut the sequences in each place where the specific enzyme will recognize. Then, place the fragments on the gel to observe your results.

Different samples, because they have different cut sites which will give different fragment sizes and patterns on a gel

1. From the restriction digest and gel visualization, would you conclude that these samples are from the same or different biological samples? Why?
2. How is this information helpful to us in learning about the genomes from these samples? Specifically for bacteriophage research, why is this a valuable method to complete before selecting which phage genome to sequence out of a large collection of phages? Keep in mind that phage genomes are typically small (40-200kb)

Digest 1: Cut with EcoR1 - recognition sequence 5′-GAATTC-3′

Sample 1 –5′ C A C A C A A T T G | A A T T C G T G C C A C A G T A T G | A A T T C A A G T A C A

Sample 2 –5′ C A C A C A A T T G A C T T C G G G C C A C A G T A T G | A A T T C A A G T A C A

Digest 2: Cut with HaeIII - recognition sequence 5′-GGCC-3′

Sample 1 –5′ C A C A C A A T T G A A T T C G T G C C A C A G T A T G A A T T C A A G T A C A

Sample 2 –5′ C A C A C A A T T G A C T T C G G G | C C A C A G T A T G A A T T C A A G T A C A

3.2

Question1:
Different samples, because they have different cut sites which will give different fragment sizes and patterns on a gel.

Question 2:
Phage genomes are small enough that we can often see distinct patterns of DNA fragments after restriction digests.
This is useful, because we may encounter the same phage in two different samples. So, if we see the exact same banding pattern on a gel, we might have the same phage in two different samples. In that case, we can select a greater variety of phages to sequence for full genome analysis.

Exercise 4: Applying restriction enzymes to a known phage genome

The bacteriophage lambda is the best studied phage and has been key to discoveries of basic molecular biology along with phage biology. The genome has been sequenced many times over and is often used as a control when preparing other sequencing experiments. Additionally, the lambda genome restriction map has been very well-documented. A restriction map involves finding and marking the locations of all possible cuts that can be made by a known restriction enzyme.

Arguably, one of the most commonly used features of lambda DNA and restriction enzymes is the lambda + HindIII size standard (ladder). This enzyme (HindIII) cuts the lambda genome at the following nucleotide positions:

23,130
25,157
27,479
36,895
37,459
37,584
44,141

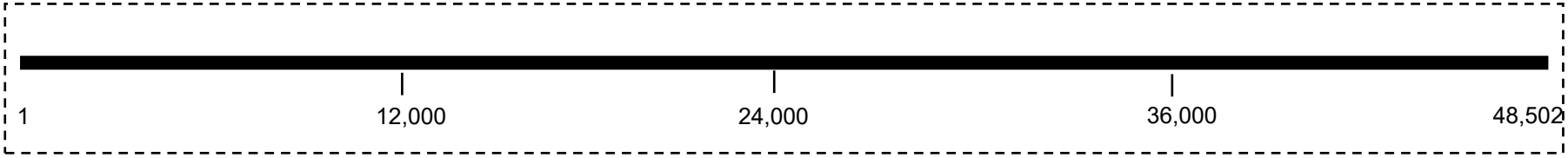
In the diagram below, a line is used to represent the lambda genome, which is 48,502 bp long. We wouldn't be able to read 48,000+ letters on one page, so we're using the line to represent the total DNA of this genome. The numbers below the line give position indicators with the very first base of the genome on the left marked as "1" and the last base of the genome on the right. Approximate midpoint and quarters are shown.

Label each of the cut sites on the map, then cut out the genome and cut at each site. Place the fragments on the gel in order of size.

How many basepairs long is the longest fragment? How long is the shortest fragment?

Longest fragment is 23,130 bp long. Shortest fragment is 125 bp long.

Cut with HindIII

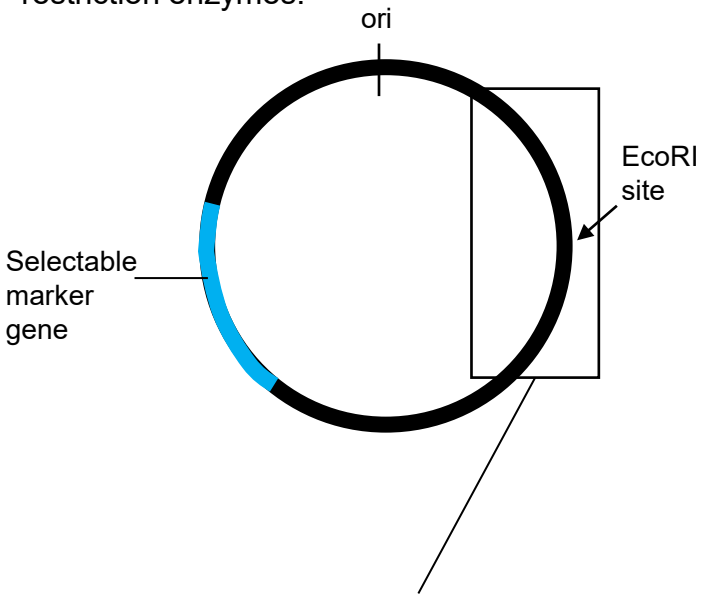


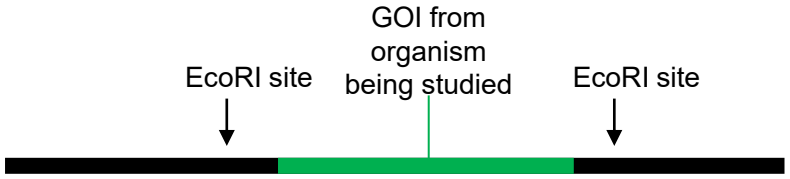
Exercise 5: Applying restriction enzymes to circular DNA

A common tool of biotechnology is another naturally occurring feature of bacteria, plasmid DNA. Plasmids are small, circular pieces of DNA that are separate from the main bacterial chromosome and usually carry a handful of genes that allow the bacteria to perform functions that they may not have the ability to do without the plasmid. For example, many antibiotic resistance genes are carried on plasmids, and these plasmids can be passed along to other bacteria in the same environment.

Biotechnologists in the 1970s had figured out how to combine plasmid DNA with restriction enzymes to shuffle genes in and out of bacteria for easier study or expression in a model organism. Likely the best known example of this was the cloning of the human insulin gene into bacteria to produce more consistent and less expensive insulin for diabetic treatments.

In order to get a "gene of interest" (GOI) into a plasmid and make more copies of it, every plasmid needs a few features such as an origin of replication (for bacteria to make more plasmid), a selectable marker gene, and recognition sites for restriction enzymes.





Cut out the following sequences (one from plasmid, the other with GOI). Then, cut these sequences with EcoRI and put the cut pieces together so that the GOI is in the plasmid. Tape your pieces together to make them more secure. In cells an enzyme called DNA ligase facilitates the phosphodiester bond between the separate segments, sealing the backbone back into a seamless circle again, but now containing our GOI..

Zoomed in to sequence level on boxed section of plasmid

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5′-CATCCAGAATTCGTGCCACAGTATCAAGTACA-3′

3′-GTAGGTCTTAAGCACGGTGTCATAGTTCATGT-5′
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Zoomed in to sequence level on GOI

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5′-GCCATGAATTCGCATCCATAAGAATTCAAGTACA-3′

3′-CGGTACTTAAGCCGTAGGTAACTTAAGTTCATGT-5′
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Agarose gel

This paper gel will be used for several different exercises. While directly writing on the gel is not needed for these exercises, the page could be laminated or put in a protective plastic sleeve and used with appropriate dry or wet-erase markers.

Cutouts for exercise 2

A

B

C

D

Cutouts for exercises 3.1

Cut with EcoRI

5′- G A A T T C -3′

3′- C T T A A G -5′

Cut with HaeIII

5′- G G C C -3′

3′- C C G G -5′

Cutouts for exercises 3.2

Digest 1: Cut with EcoR1 - recognition sequence 5′-GAATTC-3′

Sample 1 -5′ C A C A C A A T T G A A T T C G T G C C A C A G T A T G A A T T C A A G T A C A

Sample 2 -5′ C A C A C A A T T G A C T T C G G G C C A C A G T A T G A A T T C A A G T A C A

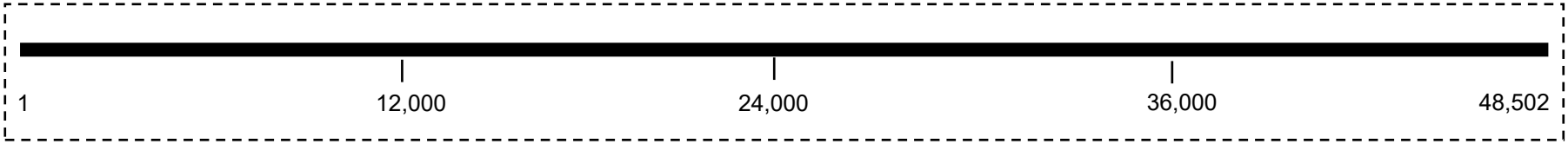
Digest 2: Cut with HaeIII - recognition sequence 5′-GGCC-3′

Sample 1 -5′ C A C A C A A T T G A A T T C G T G C C A C A G T A T G A A T T C A A G T A C A

Sample 2 -5′ C A C A C A A T T G A C T T C G G G C C A C A G T A T G A A T T C A A G T A C A

Cutout for exercise 4

Cut with HindIII



Cutouts for exercise 5

Zoomed in to sequence level on boxed section of plasmid

5′-CATCCAGAATTCGTGCCACAGTATCAAGTACA-3′

3′-GTAGGTCTTAAGCACGGTGTCATAGTTCATGT-5′

Zoomed in to sequence level on GOI

5′-GCCATGAATTCGCATCCATAAGAATTCAAGTACA-3′

3′-CGGTACTTAAGCCGTAGGTAACTTAAGTTCATGT-5′