DNA Scissors: Introduction to Restriction Enzymes

Objectives

At the end of this activity, students should be able to

1 . Describe a typical restriction site as a 4- or 6-base- pair palindrome;
2. Describe what a restriction enzyme does (recognize and cut at its restriction site);
3. Use a restriction map to predict how many fragments will be produced in a given restriction digest.

Introduction

Restriction enzymes

Genetic engineering is possible because of special enzymes that cut DNA. These enzymes are called restriction enzymes or restriction endonucleases. Restriction enzymes are proteins produced by bacteria to prevent or restrict invasion by foreign DNA. They act as DNA scissors, cutting the foreign DNA into pieces so that it cannot function. A nuclease is any enzyme that cuts the phosphodiester bonds of the DNA backbone, and an endonuclease is an enzyme that cuts somewhere within a DNA molecule. In contrast, an exonuclease cuts phosphodiester bonds by starting from a free end of the DNA and working inward.

Restriction enzymes were originally discovered through their ability to break down, or restrict, foreign DNA. Restriction enzymes can distinguish between the DNA normally present in the cell and foreign DNA, such as infecting bacteriophage DNA. They defend the cell from invasion by cutting foreign DNA into pieces and thereby rendering it nonfunctional. Restriction enzymes appear to be made exclusively by prokaryotes.

Restriction enzymes recognize and cut at specific places along the DNA molecule called restriction sites. Each different restriction enzyme (and there are hundreds, made by many different bacteria) has its own type of site. In general, a restriction site is a 4- or 6-base-pair sequence that is a palindrome. A DNA palindrome is a sequence in which the "top" strand read from 5' to 3' is the same as the "bottom" strand read from 5' to 3'. For example,

5' GAATTC 3'
3' CTTAAG 5'

is a DNA palindrome. To verify this, read the sequences of the top strand and the bottom strand from the 5' ends to the 3' ends. This sequence is also a restriction site for the restriction enzyme called *Eco*RI. The name *Eco*RI comes from the bacterium in which it was discovered, *Escherichia coli* RY 13 (*Eco*R), and I, because it was the first restriction enzyme found in this organism.

*Eco*RI makes one cut between the G and A in each of the DNA strands (see below). After the cuts are made, the DNA is held together only by the hydrogen bonds between the four bases in the middle. Hydrogen bonds are weak, and the DNA comes apart.

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                ↓
Cut sites:  5' GAATTC 3'
            3' CTTAAG 5'
                    ↑
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Cut DNA 5' G AATTC 3'
3' CTTAA G 5'

The *Eco*RI cut sites are not directly across from each other on the DNA molecule. When *Eco*RI cuts a DNA molecule, it therefore leaves single stranded "tails" on the new ends (see the example just given). This type of end has been called a "sticky end" because it is easy to rejoin it to complementary sticky ends. Not all restriction enzymes make sticky ends; some cut the two strands of DNA directly across from one another, producing a blunt end.

The restriction enzymes commonly used in laboratories generally recognize specific DNA sequences of 4 or 6 base pairs. These recognition sites are palindromic in that the 5'-to-3' base sequence on each of the two strands is the same. Most of the enzymes make a cut in the phosphodiester backbone of DNA at a specific position within the recognition site, resulting in a break in the DNA. These recognition- cleavage sites are called restriction sites. Below are some examples of restriction enzymes (their names are combinations of italics and roman numerals) and their recognition sequences, with arrows indicating cut sites. Which ones of these enzymes would leave blunt ends? Which ones would leave sticky ends?

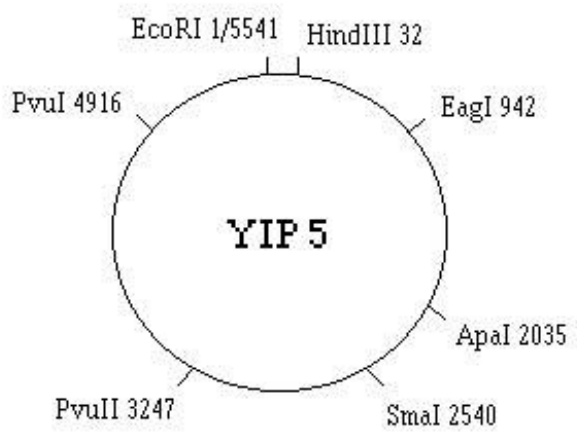
	↓		↓
*Eco*RI	5' GAATTC 3'	*Hin*dIII	5' AAGCTT 3'
	3' CTTAAG 5'		3' TTCGAA 5'
	↑		↑
	↓		↓
*Bam*HI	5' GGATCC 3'	*Alu*I	5' AGCT 3'
	3' CCTAGG 5'		3' TCGA 5'
	↑		↑
	↓		↓
*Sma*I	5' CCCGGG 3'	*Hba*I	5' GCGC 3'
	3' GGGCCC 5'		3' CGCG 5'
	↑		↑

Notice that the "top" and "bottom" strands read the same from 5' to 3'; this characteristic defines a DNA palindrome. Also notice that some of the enzymes introduce two staggered cuts in the DNA, while others cut each strand at the same place. Enzymes like *Sma*I that cut both strands at the same place are said to produce blunt ends. Enzymes like *Eco*RI leave two identical DNA ends with single stranded protrusions:

5' G AATTC 3'
3' CTTAA G 5'

Under appropriate conditions (salt concentration, pH, and temperature), a given restriction enzyme will cleave a piece of DNA into a series of fragments. The number and sizes of the fragments depend on the number and location of restriction sites for that enzyme in the given DNA. A specific combination of 4 bases will occur at random only once every few hundred bases, while a specific sequence of 6 will occur randomly only once every few thousand bases. It is possible that a DNA molecule will contain no restriction site for a given enzyme. For

example, bacteriophage T7 (approximately 40,000 base pairs) contains no *Eco*RI sites.



When scientists study a DNA molecule, one of the first things they do is figure out where many restriction sites are. They then create a restriction map, showing the locations of cleavage sites for many different enzymes. These maps are used like road maps to the DNA molecule. A restriction map of a plasmid is shown in Fig. 1.

Figure 1 Restriction map of Y1P5, a 5,541 base pair plasmid. The number after each restriction enzyme name indicates at which base pair the DNA is cut by that enzyme.

Rejoining restriction fragments

DNA fragments generated by restriction digestion can be put back together with the enzyme DNA ligase, which forms phosphodiester bonds between the 5' and 3' ends of nucleotides. As you might expect, any blunt ended DNA can be ligated to any other blunt ended DNA without regard to the sequence of the two molecules. Restriction fragments with single-stranded protrusions, as the *Eco*RI products shown above, are pickier. For efficient ligation, the single stranded regions must he able to hybridize to a complementary single stranded region. The idea of rejoining restriction fragments and the need for complementarity in the single stranded 'tails' is introduced in this activity.

This requirement for complementarity may sound limiting, but an examination of the *Eco*RI digestion products shown above reveals that two *Eco*RI ends are perfectly complementary. Any two DNA fragments produced by *Eco*RI digestion can he ligated together, because their single stranded protrusions are complementary. In fact, fragments with complementary single stranded protrusions can be ligated much more readily than blunt ended fragments, presumably because hybridization between the single stranded regions holds the fragments together in the proper position for ligation. Because these single stranded protrusions actually facilitate the joining of DNA segments with matching protrusions, they are often called sticky ends.

Restriction enzymes and DNA ligase play starring roles in DNA cloning. To a molecular biologist, cloning a piece of DNA means adding that piece to a plasmid or other vector and then putting the plasmid (or other vector) back into a host cell. One of the simplest methods of cloning is to ligate a restriction fragment into a plasmid that has been cut once with the same restriction enzyme(s). The restriction fragment becomes part of the plasmid when DNA ligase forms phosphodiester bonds between the two formerly separate DNA molecules.

Restriction enzymes and genetic engineering

The discovery of restriction enzymes made genetic engineering possible. Why is that so? Because restriction enzymes first made it possible to work with small, defined pieces of DNA. Chromosomes are huge molecules that usually contain many genes. Before restriction enzymes were discovered, a scientist might be able to tell that a chromosome contained a gene for an enzyme required to ferment lactose because he knew that the bacterium could ferment lactose and he could purify the protein from bacterial cells. He could use genetic analysis to tell what other genes were close to "his" gene. But he could neither physically locate the gene on the chromosome nor manipulate that gene.

The scientist could purify the chromosome from the bacterium, but then he had a huge piece of DNA containing thousands of genes. The only way to break the chromosome into smaller segments was to use physical force and break it randomly. Then what would he have? A tube full of random fragments. Could they be cloned? Not by themselves. If you introduce a simple linear fragment of DNA (like those produced by shearing) into most bacteria, it will rapidly he degraded by cellular nucleases. Cloning usually requires a vector to introduce and niaintain the new DNA. Could our scientist use a vector such as a virus or plasmid to clone his DNA fragments? No. In order to clone DNA into a vector, you have to cut the vector DNA to insert the new piece. Could he simply study the random fragments? No. Every single chromosome from each bacterial cell would give different fragments, preventing systematic analysis. So for many years, physical manipulation of DNA was virtually impossible.

The discovery of restriction enzymes gave scientists a way to cut DNA into defined pieces. Every time a given piece of DNA was cut with a given enzyme, the same **fragments were** produced. These defined pieces could be put back together in new ways. A new phrase was coined to describe a DNA molecule that had been assembled from different starting molecules: recombinant DNA.

The seemingly simple achievement of cutting DNA molecules in a reproducible way opened a whole new world of experimental possibilities. Now scientists could study specific small regions of chromosomes, clone segments of DNA into plasrnids and viruses, and otherwise manipulate specific pieces of DNA. The science of molecular biology literally exploded with the new information that became available. And genetic engineering, which essentially is the directed manipulation of specific pieces of DNA, became possible.

Separating restriction fragments

After restriction digestion, the fragments of DNA are often separated by gel electrophoresis.

Exercise 1

Cut the DNA sequence strips along their borders. These strips represent double stranded DNA nucleotides. Each chain of letters represents the phosphodiester backbone, and the vertical lines between base pairs represent hydrogen bonds between the bases.

1. You will now simulate the activity of *Eco*RI. Scan along the DNA sequence of strip 1 until

you find the *Eco*RI site (refer to the list above for the sequence). Make cuts through the phosphodiester backbone by cutting just between the G and the first A of the restriction site on both strands. Do not cut all the way through the strip. Remember that *Eco*RI cuts the backbone of each DNA strand separately.
2. Now separate the hydrogen bonds between the cut sites by cutting through the vertical lines. Separate the two pieces of DNA. Look at the new DNA ends produced by *Eco*RI. Are they sticky or blunt? Write *Eco*RI on the cut ends. Keep the cut fragments on your desk.
3. Repeat the procedure with strip 2, this time simulating the activity of *Sma*I. Find the *Sma*I site, and cut through the phosphodiester backbones at the cut sites indicated above. Are there any hydrogen bonds between the cut sites? Are the new ends sticky or blunt? Label the new ends *Sma*I, and keep the DNA fragments on your desk.
4. Simulate the activity of *Hin*dIII with strip 3. Are these ends sticky or blunt? Label the new ends *Hin*dIII, and keep the fragments.
5. Repeat the procedure once more with strip 4 again simulating *Eco*RI.
6. Pick up the 'front-end' DNA fragment from strip 4 (an *Eco*RI fragment) and the "back end" *Hin*dIII fragment from strip 3. Both fragments have single stranded tails of 4 bases. Write down the base sequences of the two tails, and label them *Eco*RI and *Hin*dIII. Label the 5' and 3' ends. Are the base sequences of the *Hin*dIII and *Eco*RI tails complementary?
7. Put down the *Hin*dIII fragment, and pick up the back end DNA fragment from strip 1 (cut with *Eco*RI). Compare the single-stranded tails of the *Eco*RI fragment from strip 1 and the *Eco*RI fragment from strip 4. Write down the base sequences of the single stranded tails, and label the 3' and 5' ends. Are they complementary?
8. Imagine that you have cut a completely unknown DNA fragment with *Eco*RI. Do you think that the single stranded tails of these fragments would be complementary to the single stranded tails of the fragments from strip 1 and strip 4?
9. An enzyme called DNA ligase reforms phosphodiester bonds between nucleotides. For DNA ligase to work, two nucleotides must come close together in the proper orientation for a bond (the 5' side of one must be next to the 3' side of the other). Do you think it would be easier for DNA ligase to reconnect two fragments cut by *Eco*RI or one fragment cut by *Eco*RI with one cut by *Hin*dIII? What is your reason?

Exercise 2

Figure 1 is a restriction map of the circular plasmid YIP5. This plasmid contains 5,541 base pairs. There is an *Eco*RI site at base pair 1. The locations of other restriction sites are shown on the map. The numbers after the enzyme names tell at which base pair that enzyme cleaves the DNA. If you digest Y1P5 with *Eco*RI, you will get a linear piece of DNA that is 5,541 base pairs long.
10. What would be the products of a digestion with the two enzymes *Eco*RI and *Eag*I*?*
11. What would be the products of a digestion with the two enzymes *Hin*dIII and *Apa*I*?*
12. What would be the products of a digestion with the three enzymes *Hin*dIII*, Apa*I*, and Pvu*I*?*
13. If you took the digestion products from question 10 and digested them with *Pvu*II*,* what would the products be?

1.

5' -TAGACTGAATTCAAGTCA- 3'
3' -ATCTGACTTAAGTTCAGT- 5'

2.

5' -ATACGCCCGGGTTCTAAA- 3'
3' -TATGCGGGCCCAAGATTT- 5'

3.

5' -CAGGATCGAAGCTTATGC- 3'
3' -GTCCTAGCTTCGAATACG- 5'

4.

5' -AATAGAATTCCGATCCGA- 3'
3' -TTATCTTAAGGCTAGGCT- 5'