

# DNA Scissors: Introduction to Restriction Enzymes

Dry Lab Student Activity

An accompaniment to Restriction Enzymes and DNA Kit,  
catalog #21-1185 and 21-1187.



Adapted by Carolina Biological Supply Company, from *Recombinant DNA and Biotechnology: A Guide for Teachers*, by Helen Kreuzer and Adrianne Massey, 1996, American Society for Microbiology Press.

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## Teacher Information

In this activity, students are introduced to restriction enzymes and simulate the activity of restriction enzymes with scissors. They are also introduced to restriction maps and asked to make simple predictions based on a map.

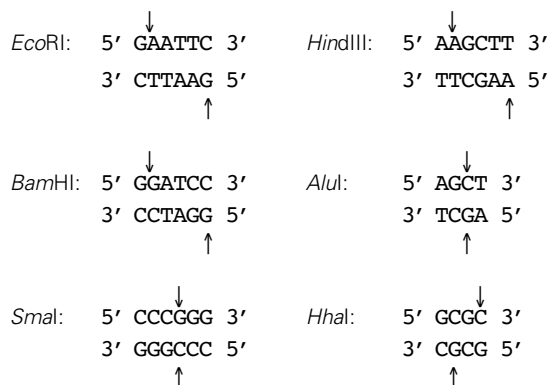
*Class periods required: 1½–1*

### Introduction

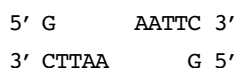
#### Restriction Enzymes

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.

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 (names in a combination of italics and Roman numerals) and their recognition sequences, with arrows indicating cut sites:



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 *SmaI* that cut both strands at the same place are said to produce “blunt” ends. Enzymes like *EcoRI* leave two identical DNA ends with single-stranded protrusions:



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 DNA (approximately 40,000 base pairs) contains no *EcoRI* sites. The action of restriction enzymes is introduced and modeled in *DNA Scissors*.

Restriction enzymes, or *restriction endonucleases*, are proteins that recognize and bind to specific DNA sequences and cut the DNA at or near the recognition site. A nuclease is any enzyme that cuts the phosphodiester bonds of the DNA backbone, and an endonuclease is one that cuts somewhere within a DNA molecule. In contrast, an exonuclease cuts the phosphodiester bonds starting from a free end and working inward.

#### Restriction Enzymes and Genetic Engineering

We often read that the discovery of restriction enzymes made genetic engineering possible. Why is that so? It is so because restriction enzymes first made it possible to work with small, defined pieces of DNA. Chromosomes are huge molecules usually containing 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 it.

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 be rapidly degraded by cellular nucleases. Cloning usually requires a vector to introduce and maintain 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 small, specific regions of chromosomes, clone segments of DNA into plasmids and viruses, and otherwise manipulate specific pieces of DNA. The science of molecular biology literally exploded with new information. And genetic engineering, which is essentially the directed manipulation of specific pieces of DNA, became possible.

### Separating Restriction Fragments

After restriction digestion, the fragments of DNA can be separated by gel electrophoresis.

### 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.

### Materials and Preparation

Photocopy the appropriate number of Student Activity Sheets, including the sheet of DNA sequence strips and the restriction map of YIP5 DNA. Supply students with scissors.

### Tips

As students use the paper models, remind them that real DNA is three-dimensional and has no “back” and “front” sides, nor does it matter if the letters representing the bases are upside down.

### Answers to Exercise Questions

The numbers correspond to the item numbers under *Exercises and Questions* in the Student Activity. Since two of the items, Numbers 1 and 5, contain only instructions and no questions, they are not represented below.

2. The DNA should be cut so that 5' AATT protrudes from each end. The ends are *sticky*.

3. The DNA should be cut straight across between the C and G in the middle of the *Sma*I site. The ends are blunt.
4. The DNA should be cut so that 5' AGCT protrudes from each end. The ends are *sticky*.
6. The two tails are (*Eco*RI) 5' AATT 3' and (*Hind*III) 5' AGCT 3'. They are not complementary.
7. Each single-stranded tail has the sequence 5' AATT 3'. They are complementary. Remember that to look for complementarity, you compare the 5' to 3' sequence of one strand with the 3' to 5' sequence on the other strand.
8. If the fragments were generated in an *Eco*RI digest, all of them will have the single-stranded 5' AATT 3' extensions on the ends. The ends of all of the fragments will be complementary.
9. Answers may vary. It is easier for DNA ligase to form a phosphodiester bond between two *Eco*RI fragments because of the complementary single-stranded tails. Hybridization between the bases in the tails brings the backbones into just the right position for resealing. With the noncomplementary tails (*Hind*III and *Eco*RI), the non-complementary base pairs keep the nucleotide backbones from coming into proper position for bond formation. It is very difficult to get two fragments with noncomplementary sticky ends to reseat with DNA ligase. Two fragments with blunt ends of any sequence can be connected by DNA ligase. It is more difficult to get blunt-ended fragments to connect than fragments with complementary sticky ends, but much easier than fragments with noncomplementary sticky ends (which hardly ever happens). In a sense, sticky ends is a poor name for the ends with single-stranded tails, because these ends are only sticky with respect to complementary sticky ends and very unsticky with respect to noncomplementary sticky ends.
10. Two linear fragments of 942 and 4599 base pairs (5541-942 = 4599).
11. Two linear fragments of 2003 (2035-32) and 3538 (5541-2003) base pairs.
12. Three linear fragments of 2003, 2881 (4916-2035), and 657 [5541-(2003+2881)] base pairs.
13. The 942-base pair fragment contains no *Pvu*II sites and would not be cut. The 4599-base pair fragment would be cleaved into two fragments of 2305 (3247-942) and 2294 (4599-2305) base pairs, giving three total fragments.

# DNA Scissors: Introduction to Restriction Enzymes

## Student Activity

### Background Reading

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.

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:

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5' GAATTC 3'
3' CTTAAG 5'
  
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is a DNA palindrome. To verify this, read the sequence of the top strand and the bottom strand from the 5' end to the 3' end. This sequence is also a restriction site for the restriction enzyme called *EcoRI*. Its name comes from the bacterium in which it was discovered: *Escherichia coli* strain RY 13 (*EcoR*), and "I" because it was the first restriction enzyme found in this organism.

*EcoRI* 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'
           ↑
cut DNA:    5' G   AATTC 3'
           3' CTTAA   G 5'
  
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The *EcoRI* cut sites are not directly across from each other on the DNA molecule. When *EcoRI* cuts a DNA molecule, it therefore leaves single-stranded "tails" on the new ends (see above example). 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.

When scientists study a DNA molecule, one of the first things they do is to figure out where many restriction sites are. They then create a *restriction map*, showing the location of cleavage sites for many different enzymes.

These maps are used like road maps to the DNA molecule.

Below are the restriction sites of several different restriction enzymes, with the cut sites shown.

<i>EcoRI</i> : 5' GAATTC 3' 3' CTTAAG 5' ↑	<i>HindIII</i> : 5' AAGCTT 3' 3' TTCGAA 5' ↑
<i>BamHI</i> : 5' GGATCC 3' 3' CCTAGG 5' ↑	<i>AluI</i> : 5' AGCT 3' 3' TCGA 5' ↑
<i>SmaI</i> : 5' CCCGGG 3' 3' GGGCCC 5' ↑	<i>HhaI</i> : 5' GCGC 3' 3' CGCG 5' ↑

Which ones of these enzymes would leave blunt ends?

Which ones would leave sticky ends?

Refer to the above list of enzyme cut sites as you do the activity.

### Exercises and Questions

#### Exercise I

Take the figure with the DNA sequence strips and cut out the strips along the borders. These strips represent double-stranded DNA molecules. Each chain of letters represents the phosphodiester backbone, and the vertical lines between the base pairs represent hydrogen bonds between the bases.

1. You will now simulate the activity of *EcoRI*. Scan along the DNA sequence of Strip 1 until you find the *EcoRI* site (refer to the list above for the sequence). Make cuts through the phosphodiester backbone by cutting just between the G and first A of the restriction site on both strands. Do not cut all the way through the strip. Remember that *EcoRI* 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 *EcoRI*. Are they sticky or blunt? Write "*EcoRI*" on the cut ends. Keep the cut fragments on your desk.
3. Repeat the procedure with Strip 2, this time simulating the activity of *SmaI*. Find the *SmaI* 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 "*SmaI*" and keep the DNA fragments on your desk.

4. Simulate the activity of *HindIII* with Strip 3. Are these ends sticky or blunt? Label the new ends "*HindIII*" and keep the fragments.
5. Repeat the procedure once more with Strip 4, again simulating *EcoRI*.
6. Pick up the "front end" DNA fragment from Strip 4 (an *EcoRI* fragment) and the "back end" *HindIII* fragment from Strip 3. Both fragments have single-stranded tails of 4 bases. Write down the base sequence of the two tails and label them "*EcoRI*" and "*HindIII*." Label the 5' and 3' ends. Are the base sequences of the *HindIII* and *EcoRI* "tails" complementary?
7. Put down the *HindIII* fragment and pick up the back end DNA fragment from Strip 1 (Strip 1 cut with *EcoRI*). Compare the single-stranded tails of the *EcoRI* fragment from Strip 1 and the *EcoRI* 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 cut a completely unknown DNA fragment with *EcoRI*. 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. There is an enzyme called DNA ligase that re-forms 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 *EcoRI* or one fragment cut by *EcoRI* with one cut by *HindIII*? What is your reason?

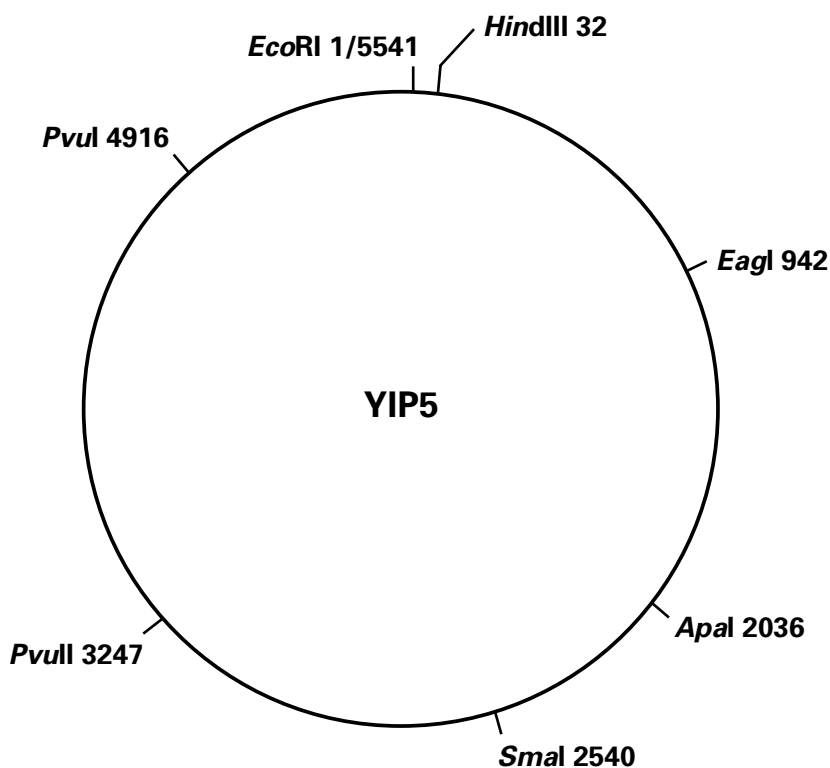
### Exercise II

Examine the figure that is the restriction map of the circular plasmid YIP5. This plasmid contains 5,541 base pairs. There is an *EcoRI* site at base pair 1. The locations of other restriction sites are shown on the map. The numbers after the enzyme names tell at what base pair that enzyme cleaves the DNA. If you digest YIP5 with *EcoRI*, you will get a linear piece of DNA that is 5,541 base pairs in length.

1. What would be the products of a digestion with the two enzymes *EcoRI* and *EagI*?
2. What would be the products of a digestion with the two enzymes *HindIII* and *Apal*?
3. What would be the products of a digestion with the three enzymes *HindIII*, *Apal*, and *PvuI*?
4. If you took the digestion products from question #1 and digested them with *PvuI*, what would the products be?

## Restriction Map of YIP5 DNA

5541 base pairs



DNA Sequence Strips for *DNA Scissors*