

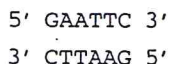
# DNA Scissors: Introduction to Restriction Enzymes

## Student Activity

### Background Reading

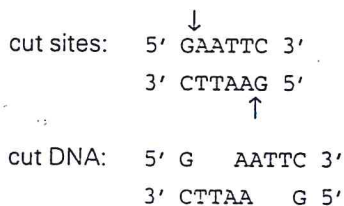
Genetic engineering is possible because of special enzymes that cut DNA. These enzymes are called *restriction enzymes*, or *restriction endonucleases*. Restriction enzymes are proteins produced by bacteria to prevent or restrict invasion by foreign DNA. They act as DNA scissors, cutting the foreign 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:



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.

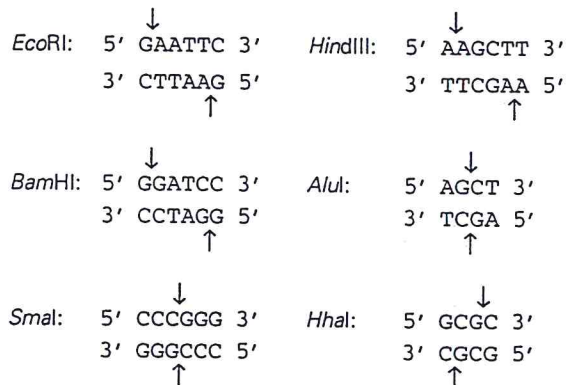


The *EcoRI* cut sites are not directly across from each other on the DNA molecule. When *EcoRI* cuts a DNA molecule, it therefore leaves single-stranded "tails" on the new ends (see above example). This type of end has been called a sticky end because it is easy to rejoin it to complementary sticky ends. 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.



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 1

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 *ApaI*?
3. What would be the products of a digestion with the three enzymes *HindIII*, *ApaI*, 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

