

DNA Scissors

10

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,

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 *EcoRI*. The name *EcoRI* comes from the bacterium in which it was discovered, *Escherichia coli* 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.

↓
Cut sites: 5’ GAATTC 3’
 3’ CTTAAG 5’
 ↑

Cut DNA: 5’ G AATTC 3’
 3’ CTAA G 5’

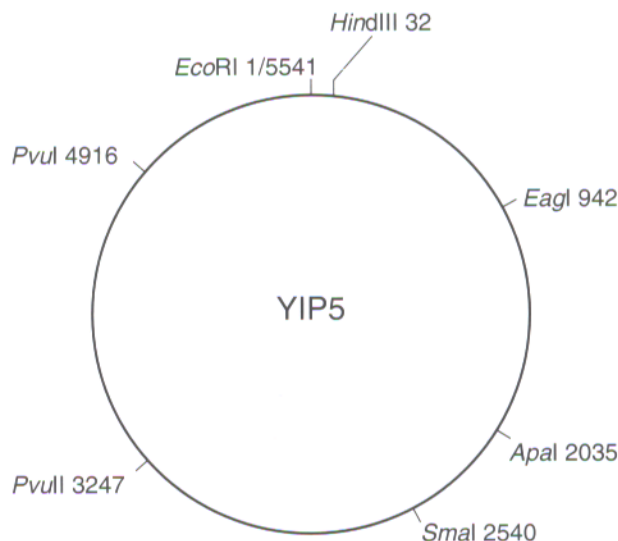


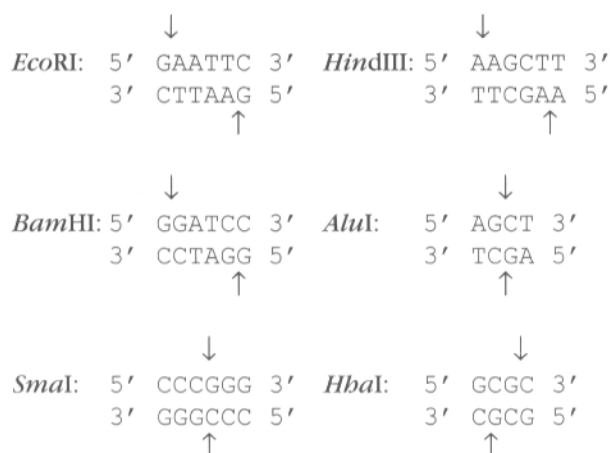
Figure 10.1 Restriction map of YIP5, a 5,541-base-pair plasmid. The number after each restriction enzyme name indicates at which base pair the DNA is cut by that enzyme.

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 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.

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. 10.1.

The restriction sites of several different restriction enzymes, with their cut sites, are shown on the next page.





Which ones of these enzymes would leave blunt ends? Which ones would leave sticky ends? Refer to this list of enzyme cut sites as you do the activity.

Exercises and Questions

Exercise 1

Cut the DNA sequence strips (Appendix A) along their borders. These strips represent double-stranded DNA molecules. 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 *Hind*III with strip 3. Are these ends sticky or blunt? Label the new ends *Hind*III, 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" *Hind*III 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 *Hind*III. Label the 5' and 3' ends. Are the base sequences of the *Hind*III and *Eco*RI tails complementary?
7. Put down the *Hind*III 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** 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 *Eco*RI or one fragment cut by *Eco*RI with one cut by *Hind*III? What is your reason?

Exercise 2

Figure 10.1 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 YIP5 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 *Hind*III and *Apa*I?
12. What would be the products of a digestion with the three enzymes *Hind*III, *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?

DNA sequence strips for *DNA Scissors*

