Name_____ Per_____ Date _____

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);

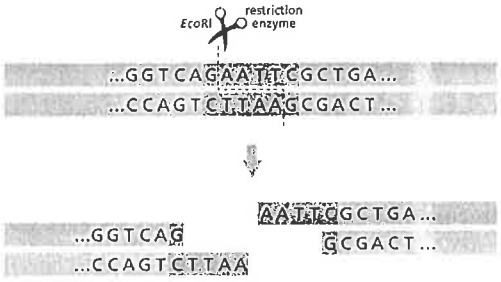
Introduction Restriction enzymes

Genetic engineering is possible because of special enzymes that cut DNA. These enzymes are called restriction enzymes. 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 specific 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* (Eco), and RI, 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 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.

Below are some examples of restriction enzymes and their recognition sequences, with arrows indicating cut sites. Which ones of these enzymes would leave blunt ends? Which ones would leave sticky ends?

Blunt or Sticky end?					Blunt or Sticky end?
_____	*Eco*RI	5' G↓AATTC 3' 3' CTTAA↑G 5'	*Hin*dIII	5' A↓AGCTT 3' 3' TTCGA↑A 5'	_____
_____	*Bam*HI	5' G↓GATCC 3' 3' CCTAG↑G 5'	*Alu*I	5' AG↓CT 3' 3' TC↑GA 5'	_____
_____	*Sma*I	5' CCC↓GGG 3' 3' GGG↑CCC 5'	*Hba*I	5' GC↓GC 3' 3' CG↑CG 5'	_____

Exercise 1

Look at the sheet of DNA sequences. Cut the DNA sequence strips along their borders. These strips represent double stranded DNA nucleotides. Each chain of letters represents the DNA bases (the sugar and phosphate molecules are omitted), and the vertical lines between 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 the 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 bases 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 sequences 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 (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 have 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? _____ Explain:

An enzyme called DNA ligase reforms 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? _____
Explain: