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);

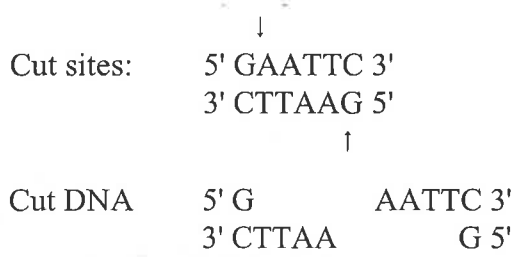
Introduction Restriction enzymes

Genetic engineering is possible because of special enzymes that cut DNA. These enzymes are called restriction enzymes 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'. or 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* (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, oducing 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?

Blunt or Sticky end?				Blunt or Sticky end?
_____	*Eco*RI	5' GAATTC 3' 3' CTTAAG 5'	*Hin*dIII 5' AAGCTT 3' 3' TTCGAA 5'	_____
_____	*Bam*HI	5' GGATCC 3' 3' CCTAGG 5'	*Alu*I 5' AGCT 3' 3' TCGA 5'	_____
_____	*Sma*I	5' CCCGGG 3' 3' GGGCCC 5'	*Hba*I 5' GCGC 3' 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 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:

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

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 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 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 ıds 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:

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 EcoRI or one fragment cut by EcoRI with one cut by HindIII? _____
Explain: