Restriction Enzymes

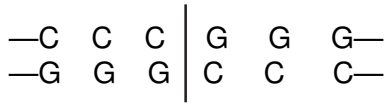
Purpose: To find out what restriction enzymes are and how they work in molecular biology.

Background: Recombinant DNA technology was partially made possible by the discovery of restriction endonucleases (also known as restriction enzymes) by W. Arber in 1960. He discovered that bacteria had special enzymes used to cut up foreign DNA entering their cells before it could become incorporated into its own DNA. These enzymes act as very specific biological scissors that cut the DNA at a particular sequence of bases. Since their initial discovery by Arber, scientists have discovered hundreds of different restriction enzymes. These "DNA tools" are routinely used to cut and piece together DNA in the laboratory and to identify if two pieces of DNA are the same. If two pieces of DNA have the same sequence, they will be cut the same number of times by at given restriction enzyme; more on this later.

The keys to understanding these enzymes is to study the sequence of bases that are recognized by the enzymes and the kind of cut made in the DNA once that sequence is found. One of the most commonly used restriction enzymes is EcoRI from the bacterium E. coli; its recognition sequence is:

The arrows indicate where each strand of the DNA molecule will be cut. After the cut, the DNA would look this:

We say that the above DNA has been cut with "sticky ends" because it has short single-stranded portions of a known sequence so that if other DNA is cut with the same enzyme it can be linked together easily. Other restriction enzymes cut and yield "blunt ends" that do not have single-stranded regions only double-stranded ends. These enzymes require other treatments before the cut DNA can be attached to other DNA pieces. SmaI (from the bacterium Serratia marcescens) is an example of an enzymes that makes blunt cuts; here is its recognition sequence:



The arrows indicate where each strand of the DNA molecule will be cut. After the cut, the DNA would look this:

As you can see, the two ends of the DNA are the same with only double-stranded DNA left after the cut. Note that the recognition sequence is found going in one direction on the top strand of DNA and in the opposite direction on the bottom strand. For this reason when you wish to know if a recognition sequence is present you need to look at both strands of the DNA.

Table 1. Some restriction endonucleases and their recognition sequences.

Microorganism	Sticky or Blunt Ends	Name of Enzyme	Recognition Sequence
E. coli	Sticky	EcoRI	G|A A T T C C T T A A|G
Bacillus amyloliquefaciens H	Sticky	BamHI	G|G A T C C C C T A G|G
Bacillius globigii	Sticky	BglII	A|G A T C T A C T A G|A
Haemophilus aegyptius	Blunt	HaeIII	PuGCGC | Py PyCGCG | PU
Haemophilus influenza	Sticky	HindIII	A|A G C T T T T C G A|A
Providencia stuarti	Sticky	PstI	C T G C A|G G|A C G T C
Streptococcus albus G	Sticky	SalI	G|T C G A C C A G C T|G
Thermus aquaticus	Sticky	TaqI	T|C G A A G C|T
Brevibacterium albidum	Blunt	BalI	T G G|C C A A C C|G G T
Serratia marcescens	Blunt	SmaI	C C C | G G G G G G | C C C
Thermoplasma acidophilum	Blunt	ThaI	C G|C G G C|G C
Proteus vulgaris	Blunt	PvuII	C A G|C T G G T C|G A C

(Pu and Py refer to any purine {guanine or adenine} or pyrimidine {thymine or cytosine}, respectively.)

Practice for you:

Use the following sequences of DNA and use the particular restriction endonuclease to cut the DNA. In each case you will need to search for recognition sites on both strands of DNA, forward and backward for the restriction sequence. The entire sequence of the restriction enzyme (all the letters) and in that exact order are both needed to make a cut. There are four parts to your assignment for each of the 6 practice problems below. When you find the recognition sequence - (1) draw a box around it precisely. Now, use a colored pen to (2) mark the cut(s) on the DNA; be sure to exactly follow the pattern shown on the previous page for each restriction enzyme. Label each as blunt cut or sticky end cut (3). Finally, count up the number of cuts (4) you found and write it to the right of the DNA with blunt or sticky**.** An answer of no cuts is just as good as many cuts! ***Remember to go in both directions (forward and backward) on both strands.***

Sample problem: Use HindIII to cut the following DNA

GGGATCTAGACGCGAAGCTTTGTCAGCTGGCGCGCAAATATCGCGCTGCTACAAGCTTCGGTATCG
CCCTAGATCTGCGCTTCGAAACAGTCGACCGCGCGTTTATAGCGCGACGATGTTCGAAGCCATAGC 2 cuts, sticky

sticky cut sticky cut

Practice Problems

1. Use ThaI to cut the following DNA:

AATTCCTTTACCGCGCCGGATCGATAGCTAGTATATTAACGCGATTTAATCGAT
TTAAGGAAATGGCGCGGCCTAGCTATCGATCATATAATTGCGCTAAATTAGCTA

2. Use BamHI to cut the following DNA:

GGGATCCGCGTGTCAGCTGGCGCGCAAATATCGCGGATCCGCTGTAAGGGCTGCA
CCCTAGGCGCACAGTCGACCGCGCGTTTATAGCGCCTAGGCGACATTCCCGACGT

3. Use SmaI to cut the following DNA:

TTGTATGGGCCCACCCGGGATAGCTGCGCGCTAGCTGATGCGCGCGCGCGGGCCCGCTGAT
AACATACCCGGGTGGGCCCTATCGACGCGCGATCGACTACGCGCGCGCGCCCGGGCGACTA

4. Use TaqI to cut the following DNA:

ATTATTAGTGTCGCGCCCGTAGATGCTGACTGACTGACGATTTATATTGCCTTAATTATT
TAATAATCACAGCGCGGGCATCTACGACTGACTGACTGCTAAATATAACGGAATTAATAA

5. Use TaqI to cut the following DNA:

AATTCCTTTACCGCGCCGGATCGATAGCTAGTATATTAACGCGATTTAATCGAT
TTAAGGAAATGGCGCGGCCTAGCTATCGATCATATAATTGCGCTAAATTAGCTA

6. Use BOTH BalI and BglII to cut this DNA:

GGGATCTAGACGCGTGTCAGCTGGCGCGCAAATATCGCGCTGCTACCGGTATCGTAAGGGCTGGCCACA
CCCTAGATCTGCGCACAGTCGACCGCGCGTTTATAGCGCGACGATGGCCATAGCATTCCCGACCGGTGT