

Overview and Purpose

While this standard has an asterisk, which indicates it is an optional standard, knowing how DNA is cut and reconnected is critical to student understanding of biotechnology. This understanding is critical to standard 5c on genetic engineering which is not optional.

Educational Standards Addressed:

5d.* Students know how basic DNA technology (restriction digestion by endonucleases, gel electrophoresis, ligation, and transformation) is used to construct recombinant DNA molecules.

Purpose:

To find out what restriction enzymes are and how they work in molecular biology to cut and reattach genes from one organism to another during genetic engineering.

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 the bacterium's 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 scissors" are routinely used to cut and piece together DNA to create transgenic organisms 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 and produce similarly sized fragments by a given restriction enzyme; forensic science relies on this fact to determine the guilt or innocence in criminal cases.

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Restriction Enzymes

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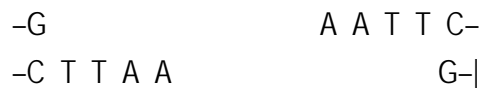
Subject: Biology
Grade Level: 9–12
Duration: One Class Period

Background (Cont'd)

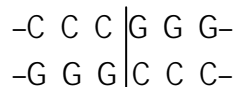
The keys to understanding these enzymes is to study the sequence of bases that are recognized by the enzymes and the kind of cut made in the DNA once that sequence is found. One of the most commonly used restriction enzymes is EcoRI from the bacterium *E. Coli*; its recognition sequence is:



The line indicates where each strand of the DNA molecule will be cut. After the cut, the DNA looks this:



We say that the above DNA has been cut with “sticky ends” because it has short single-stranded regions. If two different pieces of DNA are cut with the same enzyme they 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 enzyme that makes blunt cuts; here is its recognition sequence:



The line indicates where each strand of the DNA molecule will be cut. After the cut, the DNA looks like this:



As you can see, the two ends of the DNA are the same shape 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 search both strands of the DNA.

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Procedure

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 going forward and backward, write a pencil mark for each of the cuts and label each cut as blunt or sticky ends. Write down the number of cuts made on each DNA sequence.

1. Use *Thal* to cut the following DNA:

AATTCCTTTACCGCGCCGGATCGATAGCTAGTATATTAACGCGATTTAATCGAT
TTAAGGAAATGGCGCGGCCTAGCTATCGATCATATAATTGCGCTAAATTAGCTA

2. Use *Bam*HI to cut the following DNA:

GGGATCCGCGTGTTCAGCTGGCGCGCAAATATCGCGCTGCTATCGTAAGGGCTGCA
CCCTAGGCGCACAGTCGACCGCGCGTTTATAGCGCGACGATAGCATTCCCGACGT

3. Use *Sma*I to cut the following DNA:

TTGTATGGGCCACCCGGGATAGCTGCGCGCTAGCTGATGCGCGCGCGGGCCCGCTGAT
AACATACCCGGGTGGGCCCTATCGACGCGCGATCGACTACGCGCGCGCGCCCGGGCGACTA

4. Use *Taq*I to cut the following DNA:

ATTATTAGTGTGCGCGCCCGTAGATGCTGACTGACTGACGATTTATATTGCCTTAATTATT
TAATAATCACAGCGCGGGCATCTACGACTGACTGACTGCTAAATATAACGGAATTAATAA

5. Use *Taq*I to cut the following DNA:

AATTCCTTTACCGCGCCGGATCGATAGCTAGTATATTAACGCGATTTAATCGAT
TTAAGGAAATGGCGCGGCCTAGCTATCGATCATATAATTGCGCTAAATTAGCTA

6. Use BOTH *Ball* and *Bgl*III to cut this DNA (use different colors to indicate each enzymes cuts):

GGGATCTAGACGCGTGTTCAGCTGGCGCGCAAATATCGCGCTGCTACCGGTATCGTAAGGGCTGA
CCCTAGATCTGCGCACAGTCGACCGCGCGTTTATAGCGCGACGATGGCCATAGCATTCCCGACT

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Conclusions/Questions

1. How has restriction technology been employed by law enforcement, site cases where its use was critical?
2. Why is a result of zero cuts just as important and 2, 3 or 5 cuts? Explain using examples.
3. Companies who sell restriction enzymes are constantly interested in newly found bacteria, explain their interest.

Common Restriction Enzymes and their Recognition Sequences

Microorganism	Sticky or Blunt Ends	Name of Enzyme	Recognition Sequence
<i>E. coli</i>	Sticky	EcoRI	G AATTC CTTAA G
<i>Bacillus amyloliquefaciens</i> H	Sticky	BamHI	G GATCC CCTAG G
<i>B. globigii</i>	Sticky	BglII	A GATCT TCTAG A
<i>Haemophilus aegyptius</i>	Blunt	HaeIII	PuGCGC Py PyCGCG Pu
<i>Haemophilus influenza</i>	Sticky	HindIII	A AGCTT TTCGA A
<i>Providencia stuarti</i>	Sticky	PstI	CTGCA G G ACGTC
<i>Streptococcus albus</i> G	Sticky	SalI	G TCGAC CAGCT G
<i>Thermus aquaticus</i>	Sticky	TaqI	T CGA AGC T
<i>Brevibacterium albidum</i>	Blunt	BalI	TGG CCA ACC GGT
<i>Serratia marcescens</i>	Blunt	SmaI	CCC GGG GGG CCC
<i>Thermoplasma acidophilum</i>	Blunt	ThaI	CG CG GC GC
<i>Proteus vulgaris</i>	Blunt	PvuII	CAG CTG GTC GAC

(Pu and Py refer to any purine or pyrimidine, respectively.)

Extensions Beyond the Classroom

Research companies that sell restriction enzymes on the web. Give examples of the number of different enzymes a company sells and the costs for a couple of examples. Remember that that price quoted on their website represents fractions of a single drop: (100 – 300 l).

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