**Restriction Enzymes and Recombinant DNA**

Restriction enzymes (or restriction endonucleases) are enzymes that cut DNA at specific sequences. They are enzymes produced by bacteria to prevent or restrict invasion by foreign DNA, by cutting the foreign DNA into pieces so that it cannot function. A nuclease is any enzyme that cuts the sugar-phosphate bonds of the DNA backbone, and an endonuclease is an enzyme that cuts somewhere within a DNA molecule. (Contrast that with an exonuclease that cuts sugar-phosphate bonds by starting from a free end of the DNA and working inward.)

The specific sequence the restriction enzyme recognizes is called the restriction site and each different restriction enzyme (there are hundreds known) has its own restriction site. Restriction sites are four or six base pair sequences that are palindromic. This means the sequence on one strand read from 5' to 3' is the same as the other strand read from 5' to 3'. For example,

5' GAATTC 3'
3' CTTAAG 5'

This sequence is also the restriction site for the restriction enzyme called *Eco*RI. The name *Eco*RI comes from the bacterium in which it was discovered, *Escherichia coli* RY 13 (*Eco*R), and I, because it was the first restriction enzyme found in that organism. *Eco*RI makes one cut between the G and A in each of the DNA strands, as shown 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.

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

Ends of DNA fragments   5' G          AATTC 3'
                        3' CTTAA          G 5'
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As you can see, the *Eco*RI cut sites are not directly across from each other on the DNA molecule. When the DNA is cut, the ends of the strands are not equal lengths. These are called sticky ends because they are complementary to one another and can stick back together. Some restriction enzymes cut the two strands of DNA directly across from one another, producing blunt ends.

Q1. [SP 2] For each of the restriction enzymes, indicate whether sticky or blunt ends would be produced.

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a) HindIII      ↓
                5' AAGCTT 3'
                3' TTCGAA 5'
                      ↑

                  ↓
b) BamHI        5' GGATCC 3'
                3' CCTAGG 5'
                      ↑

                    ↓
c) AluI         5' AGCT 3'
                3' TCGA 5'
                  ↑
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DNA fragments generated by restriction digestion can be put back together with the enzyme DNA ligase. Any blunt ended fragment can be ligated to any other blunt ended fragment but sticky ends must be able to hybridize to a complementary sticky end.

Q2. [SP 1] Fragments with sticky ends join only to fragments with complementary sticky ends. Describe the usefulness of this specificity in genetic engineering.

When a piece of DNA is cut by a given restriction enzyme, the same fragments are produced. These pieces can be put back together, or recombined, in new ways and the new DNA molecule is called recombinant DNA. Recombinant DNA provides a way of introducing a gene into an organism.

Q3. [SP 1] Explain the importance of cutting the gene of interest and the plasmid with the same restriction enzyme.

Producing a recombinant DNA plasmid.
1. The first DNA strip represents a sequence of DNA containing the insulin gene. The sequences upstream and downstream of the gene are given. Using what you know about *Eco*RI, cut the DNA strip to produce the fragments you would expect to obtain from the enzyme. You should also separate the hydrogen bonds by cutting between the complementary bases on the two strands.
2. Now cut the plasmid to produce the fragments you expect to obtain using *Eco*RI.

Q4. [SP 2] Identify the ends produced from cutting the genomic DNA and the plasmid DNA as sticky or blunt. Identify them as complementary or not.

3. Attach the DNA fragment containing the insulin gene to the plasmid DNA.

Q5. [SP 1] Propose a reason a molecular biologist might insert a piece of DNA (such as the insulin gene) into a bacterial plasmid.

Q6. [SP 6] Imagine that you cut a completely unknown piece of DNA with *Eco*RI. Predict whether the sticky ends of the fragments produced would be complementary to those produced in the insulin example. Justify your prediction.

Q7. [SP 1, SP 6] This method of introducing a plasmid to bacterial cells is called transformation. The success rate is very low so very few cells actually take the plasmid. Molecular biologists often include a gene for antibiotic resistance in the plasmid. Propose a reason for doing so.

Q8. [SP 1, SP 6] This technique works very well for producing proteins in bacterial cells but there are applications which require the production of a protein in human cells. Propose a method for introducing a gene into a human cell using a virus.

DNA containing the insulin gene.

5' -TAACTGAATTCAA------gene sequence-------GTGATTCAAGGGAACTGAATTCCA- 3'
3' -ATTGACTTAAGTT ------gene sequence-------CAGTAAGTTCCCTTGACTTAAGGT- 5'

Segment of plasmid DNA

5' ----rest of plasmid-AATAGAATTCCGATCCGA----rest of plasmid-3'
3' ----rest of plasmid-TTATCTTAAGGCTAGGCT----rest of plasmid-5'