3-D Molecular Designs
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Biotechnology Kit®

Restriction Enzymes: Nature's Molecular Scissors

Enzymes, as you have learned, are "one trick ponies" - in that they can do one job very well. A **restriction enzyme** (or **restriction endonuclease**) is a type of enzyme that cleaves DNA into fragments at a specific sequence of DNA bases. The discovery of these enzymes and their application has proven to be essential for scientists.

Before you make a single cut with our scissors: In the space below, write down everything you remember about the structure of DNA, both in the monomer and polymer form.

Goal: To model the way that restriction enzymes make cuts in dsDNA and understand the applications of these enzymes in the biotechnology industry.

Introduction

You DNA is made up of 3 billion base pairs and is about 6 feet long! If you tried to run DNA electrophoresis or conduct any analysis on a segment of DNA that was that long, you would likely encounter several problems along the way. The way that you deal with this is to "hone in" on a specific segment or gene of interest and cut it out of the rest of the genome. Even though your genome is 6 feet long, the average length of a gene segment is approximately 1,000 base pairs, or 0.0000139 inches! If you wanted to just investigate a single gene, blocking out all of the rest of the genome can be very helpful. But how can you cut out this segment? Like so many times before, scientists turned to prokaryotes for a way to solve this dilemma. In both bacteria and archaea, restriction enzymes provide a defence mechanism against invading viruses by cutting foreign DNA through a process called restriction digestion.

Restriction enzymes (usually) cut at sequences that are **palindromic**. Here is an example of a cut that can be made in DNA using a specific restriction enzyme, **EcoRI**.

5' - T A T G C A G A A T T C C G - **3'**
3' - A T A C G T C T T A A G G C - **5'**

EcoRI →

5' - T A T G C A **G** **A A T T C** C G - **3'**
3' - A T A C G T **C T T A A** **G** G C - **5'**

- Using the above picture, define what a palindrome is? Provide an example of a common word or name that is palindromic in nature.

  _____

  _____

- Why do you think restriction enzymes would be apt to cleave at a sequence that is palindromic?

  _____

  _____

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Discovering the Endonucleases

There are several different types of restriction enzymes that come from a variety of prokaryotes, each one having their own sequence of DNA that the enzyme searches for. At the endonuclease domain of the enzyme, the site where the cut occurs, there are two types of ends that can form: sticky ends and blunt ends. Using the pictures below, can you define the type of resulting cut?:

- **Sticky Ends:**

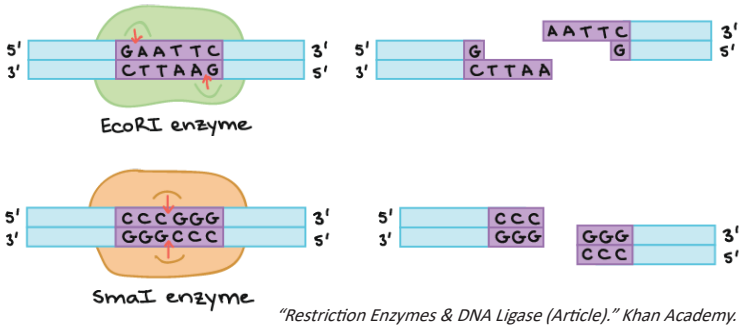
_____

_____

- **Blunt Ends:**

_____

_____



"Restriction Enzymes & DNA Ligase (Article)." Khan Academy.

Now that you know a little bit about restriction enzymes, consider the following questions.

- What type of bonds is the restriction enzyme breaking?

_____

- What do you think governs which restriction enzyme a researcher would use? What information would researchers consider when coming to their decision?

_____

_____

- What would happen if a digest is completed using multiple restriction enzymes?

_____

_____

Types of Restriction Enzymes

Restriction Enzyme Name	Recognition Sequence	Restriction Enzyme Cleavage Site
BamHI		
ClaI		
EcoRI		
EcoRV		
HaeIII		
HindIII		

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Using Restriction Enzymes

Within the biotechnology industry restriction enzymes are used to produce restriction digests of a sample of DNA to break it up into smaller pieces.

- Using the multi-colored foam deoxyribonucleotides, construct a model of a double-stranded DNA with the following nucleotide sequence:

5' - C G T A C C T A G G A G C T T A A G C C T C C T A C C G G A C C - 3'
3' - G C A T G G A T C C T C G A A T T C G G A G G A T G G C C T G G - 5'

Using the restriction enzyme provided to you by your teacher, perform a restriction digest using the enzyme provided to your group on the distributed card.

After performing the restriction digest, answer the following questions:

- How many fragments did your digest produce? How could you describe your fragments *(long, short, etc.)*

- Using the space below, construct how your digest would appear on an agarose gel. Defend how you described the movement of the gel in the space to the bottom right.

- Now, compare your digest with others in your class. What similarities and/or differences do you see? What causes these changes?

- Using the space below, construct how the class restriction digest would appear on an agarose gel. Given the ladder that your teacher provides you, how would each group's digest appear when modeled on a larger gel? Label the lane with the group number and draw how the gel would appear.

Ladder					