

DNA Scissors: Introduction to Restriction Enzymes

Student Activity

Background Reading

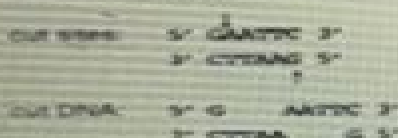
Genetic engineering is possible because of special enzymes that cut DNA. These enzymes are called *restriction enzymes*, or *restriction endonucleases*. Restriction enzymes are proteins produced by bacteria to prevent or restrict invasion by foreign DNA. They act as DNA scissors, cutting the foreign DNA into pieces so that it cannot function.

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'. For example,



is a DNA *palindrome*. To verify this, read the sequence of the top strand and the bottom strand from the 5' end to the 3' end. This sequence is also a restriction site for the restriction enzyme called *EcoRI*. Its name comes from the bacterium in which it was discovered: *Escherichia coli* strain (RY 13) (*EcoRI*, and "I" 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.

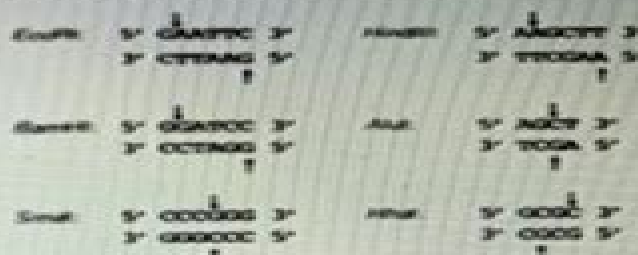


The *EcoRI* cut sites are not directly across from each other on the DNA molecule. When *EcoRI* cuts a DNA molecule, it therefore leaves single-stranded "tails" on the new ends (see above example). This type of end has been called a *sticky end* because it is easy to join it to complementary sticky ends. Not all restriction enzymes make sticky ends; some cut the two strands of DNA directly across from one another, producing a *blunt end*.

When scientists study a DNA molecule, one of the first things they do is to figure out where many restriction sites are. They then create a *restriction map*, showing the location of cleavage sites for many different enzymes.

These maps are used like road maps to the DNA molecule.

Below are the restriction sites of several different restriction enzymes, with the cut sites shown.



Which ones of these enzymes would leave blunt ends?

Which ones would leave sticky ends?

Refer to the above list of enzyme cut sites as you do the activity.

Exercises and Questions

Exercise 1

Take the figure with the DNA sequence strips and cut out the strips along the borders. These strips represent double-stranded DNA molecules. Each chain of letters represents the phosphodiester backbone, and the vertical lines between the 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 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 strips "SmaI" and keep the DNA fragments on your desk.

Restriction Enzymes Dna Scissors Answer Key

Camilla Rothe



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Table of Contents Restriction Enzymes Dna Scissors Answer Key

1. Understanding the eBook Restriction Enzymes Dna Scissors Answer Key
 - The Rise of Digital Reading Restriction Enzymes Dna Scissors Answer Key
 - Advantages of eBooks Over Traditional Books
2. Identifying Restriction Enzymes Dna Scissors Answer Key
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Restriction Enzymes Dna Scissors Answer Key
 - User-Friendly Interface
4. Exploring eBook Recommendations from Restriction Enzymes Dna Scissors Answer Key
 - Personalized Recommendations
 - Restriction Enzymes Dna Scissors Answer Key User Reviews and Ratings
 - Restriction Enzymes Dna Scissors Answer Key and Bestseller Lists
5. Accessing Restriction Enzymes Dna Scissors Answer Key Free and Paid eBooks
 - Restriction Enzymes Dna Scissors Answer Key Public Domain eBooks
 - Restriction Enzymes Dna Scissors Answer Key eBook Subscription Services
 - Restriction Enzymes Dna Scissors Answer Key Budget-Friendly Options

6. Navigating Restriction Enzymes Dna Scissors Answer Key eBook Formats
 - ePub, PDF, MOBI, and More
 - Restriction Enzymes Dna Scissors Answer Key Compatibility with Devices
 - Restriction Enzymes Dna Scissors Answer Key Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Restriction Enzymes Dna Scissors Answer Key
 - Highlighting and Note-Taking Restriction Enzymes Dna Scissors Answer Key
 - Interactive Elements Restriction Enzymes Dna Scissors Answer Key
8. Staying Engaged with Restriction Enzymes Dna Scissors Answer Key
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Restriction Enzymes Dna Scissors Answer Key
9. Balancing eBooks and Physical Books Restriction Enzymes Dna Scissors Answer Key
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Restriction Enzymes Dna Scissors Answer Key
10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine Restriction Enzymes Dna Scissors Answer Key
 - Setting Reading Goals Restriction Enzymes Dna Scissors Answer Key
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Restriction Enzymes Dna Scissors Answer Key
 - Fact-Checking eBook Content of Restriction Enzymes Dna Scissors Answer Key
 - Distinguishing Credible Sources
13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
14. Embracing eBook Trends
 - Integration of Multimedia Elements

- Interactive and Gamified eBooks

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