



What is a restriction enzyme?

Restriction enzyme is a protein produced by bacteria that cleaves DNA at specific sites along the molecule. In the bacterial cell, restriction enzymes cleave foreign DNA, thus eliminating infecting organisms. Restriction enzymes can be isolated from bacterial cells and used in the laboratory to manipulate fragments of DNA, such as those that contain genes; for this reason they are indispensable tools of recombinant DNA technology (genetic engineering).

A bacterium uses a restriction enzyme to defend against bacterial viruses called bacteriophages, or phages. When a phage infects a bacterium, it inserts its DNA into the bacterial cell so that it might be replicated. The restriction enzyme prevents replication of the phage DNA by cutting it into many pieces. **Restriction enzymes** were named for their ability to restrict, or limit, the number of strains of bacteriophage that can infect a bacterium. Each restriction enzyme recognizes a short, specific sequence of nucleotide bases (the four basic chemical subunits of the linear double-stranded DNA molecule-**adenine**, **cytosine**, **thymine**, and **guanine**). These regions are called recognition sequences, or recognition sites, and are randomly distributed throughout the DNA. Different bacterial species make restriction enzymes that recognize different nucleotide sequences.

Identity of Restriction Enzymes

Restriction enzymes are named for the organism from which they were first isolated.

For example

- *EcoRI* is isolated from *E. coli* strain RY13.
 - *Eco* refers to the genus and species (1st letter of genus; 1st two letters of species).
 - R is the strain of *E. coli*
 - I (Roman numeral) indicate it was the first enzyme of that type isolated from *E. coli* RY13.
 - *BamHI* is isolated from *Bacillus amyloliquefaciens* strain H
 - *Sau3A* is isolated from *Staphylococcus aureus* strain 3A.
 - And so on.
- ✓ Some restriction enzymes also cut DNA to form "blunt" ends (without single-stranded tails), which also can be inserted into target DNA via the action of DNA ligase.
- ✓ DNA ligase isn't picky: it can't tell the difference between foreign and host DNA (who'd figure it would ever have to?), and this enables the creation of chimeric DNA--DNA from two separate sources.
- ✓ Each enzyme recognizes and cuts specific DNA sequences. For example, *BamHI* recognizes the double stranded sequence to form "sticky ends".



Notes:

- Most restriction enzymes are specific to a single restriction site.
- Restriction sites are recognized no matter where the DNA came from.
- The number of cuts in an organism's DNA made by a particular restriction enzyme is determined by the number of restriction sites specific to that enzyme in that organism's DNA.
- A fragment of DNA produced by a pair of adjacent cuts is called a **restriction fragment**.
- A particular restriction enzyme will typically cut an organism's DNA in to many pieces, from several thousand to more than a million!
- There is a great deal of variation in restriction sites even within a species.
- Although these variations do not have phenotypic expression beyond the base sequences themselves, the variants can be considered molecular "**alleles**," and they can be detected with sequencing techniques.

As such, they can be used in mapping studies similar to the way true genes with known phenotypic effects can be used, but skipping the breeding steps and going straight to the molecules.

