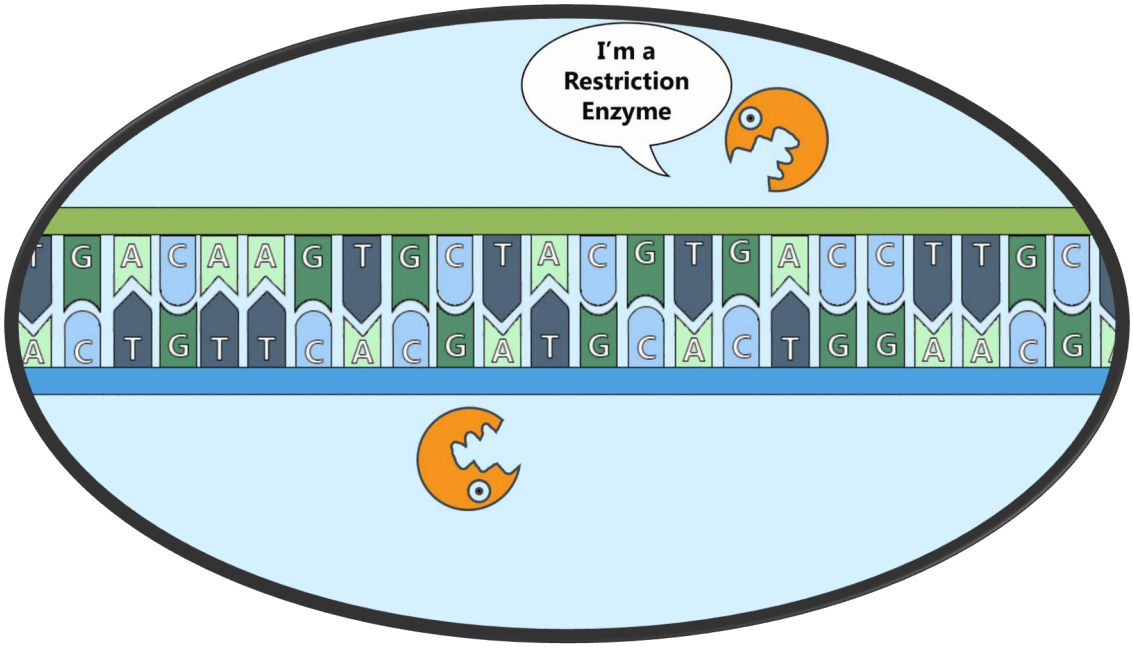
Restriction Enzyme

Introduction / Definition

Restriction Enzymes or Restriction endonucleases was unknown until 1968 , In 1968 it was discovered by Matthew Meselson and Robert Yuan , they reported that they had identified an enzyme in the bacterium Escherichia coli, strain K12, that appeared to be able to recognize and digest foreign DNAs , it's cleave the sugar-phosphate backbone of DNA strands by recognize a specific DNA base sequence and cleave both strands of a double-stranded DNA molecule at or near the recognition site.



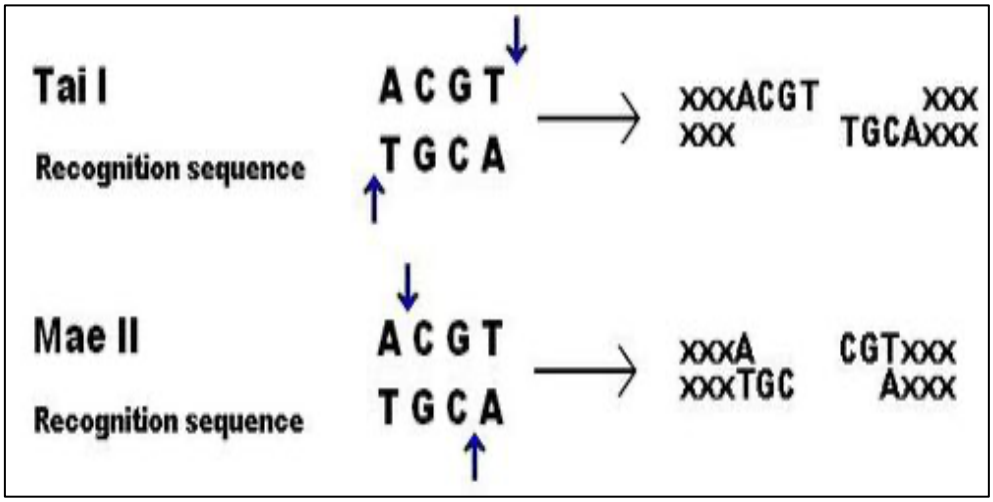
Properties of Restriction Enzymes

- Due to the rapidly growing numbers of each new enzyme the naming would be convey both the genus and the species of the bacterium from which it was isolated, the strain number, and the order in series in which the enzyme was found.

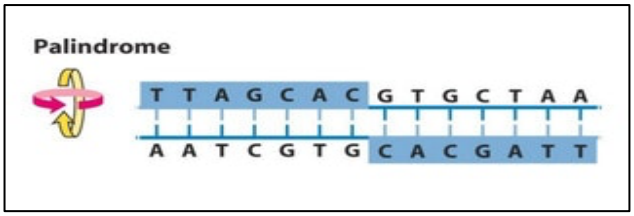
Derivation of the EcoRI name

Abbreviation	Meaning	Description
E	*Escherichia*	Genus
co	*Coli*	species
R	RY13	Strain
I	First identified	order of identification

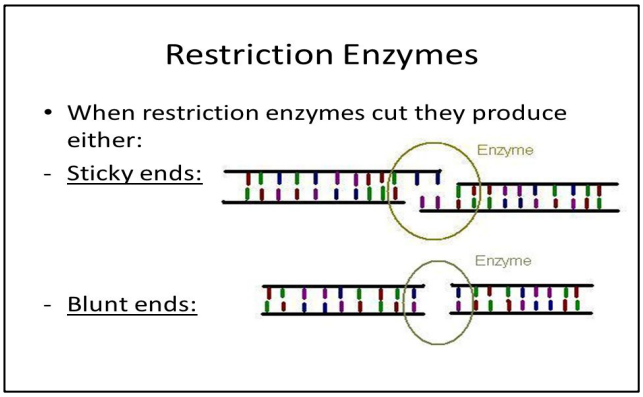
- In some cases, it was found that more than one enzyme could recognize the same sequence. that's enzymes called (same cutter) that recognized the same DNA sequence.
- Additional diversity was found among the isoschizomers. For example, the enzymes Sma I and Xma I both recognize the six base sequence CCCGGG but give different fragments with the former cutting CCC|GGG and the latter cutting C|CCGGG. Similarly, the isoschizomeric pair Hha I and Hin PI both recognize the sequence GCGC but the former cuts GCG|C and the latter G|CGC.



- Most restriction enzyme recognition sequences are from four to eight bases long and most are palindromic



- most restriction enzymes recognize CG-rich DNA sequences
- most restriction enzymes will cleave the DNA inside the recognition site but there are several that do not.
- Some enzymes cut produce “sticky ends” , while the other produce “blunt ends”.



Type of Restriction Enzyme

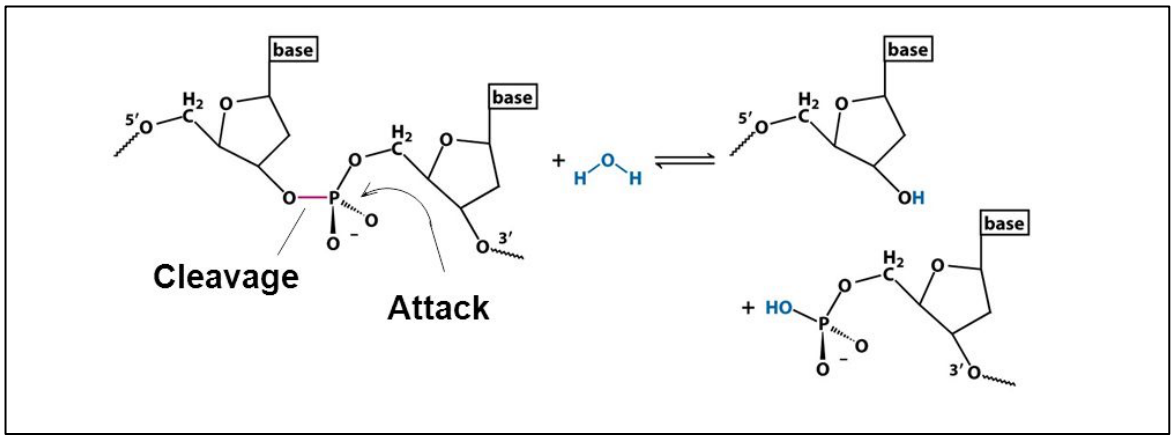
Naturally restriction enzymes are categorized into three groups (Types I, II, and III)

All types of enzymes recognize specific short DNA sequences and carry out the endo nucleolytic cleavage of DNA to give specific fragments with terminal 5'-phosphates, but they different from each other based on their composition, the nature of their target sequence, enzyme cofactor requirements, and the position of their DNA cleavage site relative to the target sequence.

	Characteristics	Mode of activity	Example
Type I	Multi subunit complex	Cuts DNA random, away from recognition site	- EcoK I - EcoA I - CfrA I
Type II	Group of unrelated protein & commonly used in molecular biology.	Cut at defined sequence at or near recognition site	- EcoR I - BamH I - Hind III
Type III	Restriction and modification enzyme.	Cut outside restriction site & require 2 restriction site in opposite orientation	- EcoP I - HinF III - EcoP15 I

Mechanism of Action

The restriction enzyme will scan and recognized a specific sequence on the length of DNA, then bind to it and make one cut in each of the sugar phosphate backbones of the double strand by hydrolyzing the phosphodiester bond; the bond between 3' O atom and the Phosphate atom will broken.



Applications

Isolated restriction enzymes are used for different scientific applications like;

- DNA sequencing
- DNA storage – libraries
- Transformation
- Large scale analysis – gene chips
- They are used in gene cloning and protein expression experiments.
- Restriction enzymes are used in biotechnology to cut DNA into smaller strands in order to study fragment length differences among individuals (Restriction Fragment Length Polymorphism – RFLP)