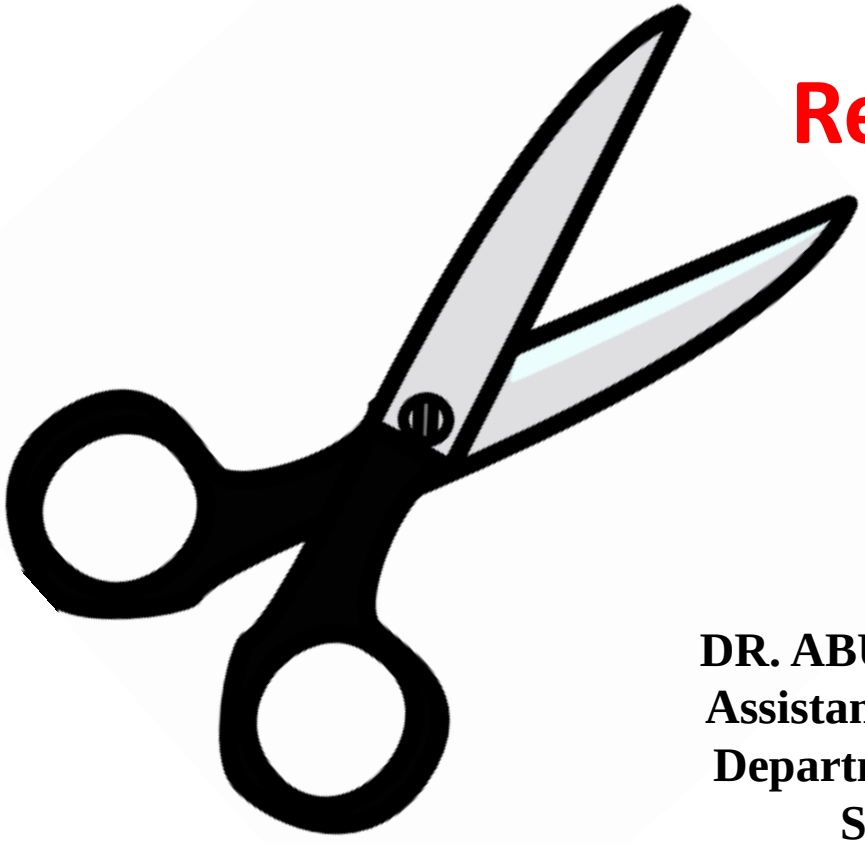
Sarsuna College
4/HB/A, Ho-Chi-Minh Sarani, Sarsuna,
Upanagari, Kolkata - 700061, West Bengal

Molecular Scissors



Restriction enzymes are molecular scissors

DR. ABUL HASAN SARDAR
Assistant Professor and Head
Department of Microbiology
Sarsuna College

RESTRICTION ENZYMES

- **A restriction enzyme (or restriction endonuclease) is an enzyme that cuts double-stranded or single stranded DNA at specific recognition nucleotide sequences known as restriction sites.**

Property of restriction enzymes

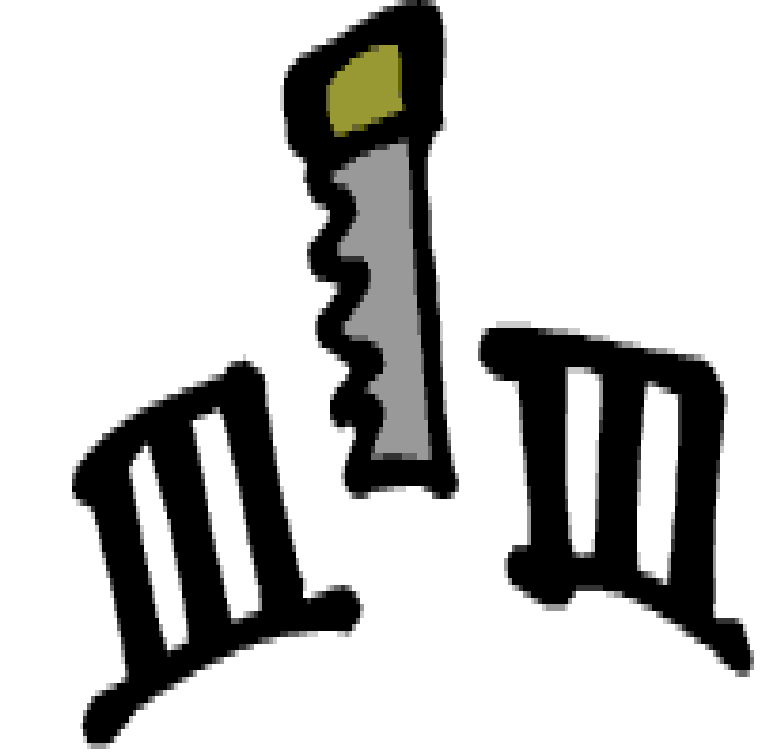
- **They break the phosphodiester bonds that link adjacent nucleotides in DNA molecules.**

HOW RESTRICTION ENZYMES WORKS?

- Restriction enzymes **recognize a specific sequence of nucleotides**, and produce a double-stranded cut in the DNA, these cuts are of two types:

- **BLUNT ENDS**.

- **STICKY ENDS.**



Blunt end

Sticky end

BLUNT ENDS

- These blunt ended fragments can be joined to any other DNA fragment with blunt ends.

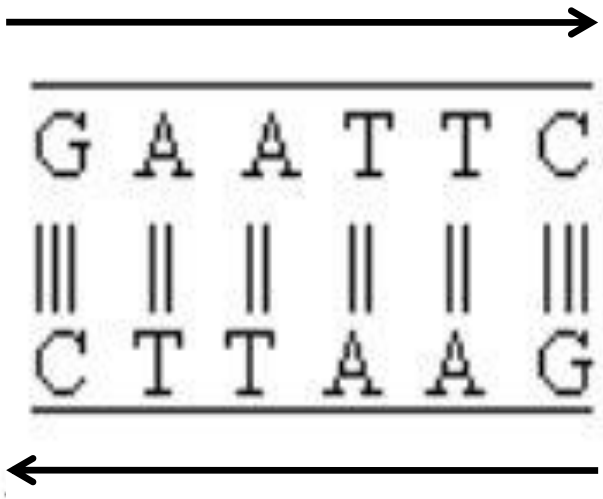
- Enzymes useful for certain types of DNA cloning experiments

“STICKY ENDS” ARE USEFUL

DNA fragments with complimentary sticky ends can be combined to create new molecules which allows the **creation and manipulation of DNA sequences from different sources.**

- **While recognition sequences vary widely , with lengths between 4 and 8 nucleotides, many of them are palindromic.**

PALINDROMES IN DNA SEQUENCES



Genetic palindromes are similar to verbal palindromes. A palindromic sequence in **DNA is one in which the 5' to 3' base pair sequence is identical** on both strands (the 5' and 3' ends refers to the chemical structure of the DNA).

PALINDROME SEQUENCES

- **The mirror like palindrome in which the same forward and backwards are on a single strand of DNA strand, as in GTAATG**
- **The Inverted repeat palindromes is also a sequence that reads the same forward and backwards, but the forward and backward sequences are found in complementary DNA strands (GTATAC being complementary to CATATG)**
- **Inverted repeat palindromes are more common and have greater biological importance than mirror-like palindromes.**

STAR EFFECT

- **Optimum conditions are necessary for the expected result.**

- **Under extreme conditions such as elevated pH or low ionic strength, RE are capable of cleaving sequences which are similar but not identical to their recognition sequence.**

NOMENCLATURE OF RESTRICTION ENZYME

- Each enzyme is named after the bacterium from which it was isolated using a naming system based on **bacterial genus, species and strain.**

For e.g **EcoRI**

Derivation of the EcoRI name		
Abbreviation	**Meaning**	**Description**
E	*Escherichia*	genus
co	*coli*	species
R	RY13	strain
I	First identified	order of identification in the bacterium

TYPES OF RESTRICTION ENZYMES

- Restriction endonucleases are categorized into three general groups.

- **Type I**
- **Type II**
- **Type III**

TYPES OF RESTRICTION ENZYMES

Type I

Type II

Type III

Type IV

Artificial restriction enzymes

CONTINUE.....

These types are categorization based on:

- **Their composition.**
- **Enzyme co-factor requirement.**
- **The nature of their target sequence.**
- **Position of their DNA cleavage site relative to the target sequence.**

TYPE I

- Capable of both restriction and modification activities

- The co factors S-Adenosyl Methionine(AdoMet), ATP, and mg++are required for their full activity

- Contain:
  - two R(restriction) subunits
  - two M(methylation) subunits
  - one S(specifity) subunits
- Cleave DNA at random length from recognition sites

TYPE II

- These are the most commonly available and used restriction enzymes
- They are composed of only one subunit.
- Their recognition sites are usually undivided and palindromic and 4-8 nucleotides in length,
- They recognize and cleave DNA at the same site.
- They do not use ATP for their activity
- They usually require only mg2+ as a cofactor.

TYPE III

- Type III restriction enzymes cut DNA about 20-30 base pairs after the recognition site.
- These enzymes contain more than one subunit.
- And require AdoMet and ATP cofactors for their roles in DNA methylation and restriction

TYPE IV

- Cleave only normal and modified DNA (methylated, hydroxymethylated and glucosyl-hydroxymethylated bases).
- Recognition sequences have not been well defined
- Cleavage takes place ~30 bp away from one of the sites

ARTIFICIAL RESTRICTION ENZYMES

- Generated by fusing a natural or engineered DNA binding domain to a nuclease domain

- can target large DNA sites (up to 36 bp)

- can be engineered to bind to desired DNA sequences

Examples of Type II restriction enzymes

***Eco*RI**

E = genus *Escherichia*

co = species *coli*

R = strain RY13

I= first endonuclease isolated

***Bam*HI**

B = genus Bacillus

am = species *amyloliquefaciens*

H = strain H

I = first endonuclease isolated

***Hin*dIII**

H = genus *Haemophilus*

in = species *influenzae*

d = strain Rd

III = third endonuclease isolated

Isoschizomer

- Restriction enzymes specific to the **same recognition sequence**. For example, **SphI** (**CGTAC/G**) and **BbuI** (**CGTAC/G**) are **isoschizomers** of each other.

Neoschizomer

- Enzyme that recognizes the **same sequence but cuts it differently** is a **neoschizomer.**

- For example, **SmaI(CCC/GGG)** and **XmaI (C/CCGGG)** are **neoschizomers** of each other.

APPLICATIONS

They are used in gene cloning and protein expression experiments

Restriction enzymes are most widely used in recombinant DNA technology.

Detection of RFLPs

DNA Mapping

Genotype a DNA sample by SNP