BHARATHIDASAN UNIVERSITY
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Programme: M.Sc., Biotechnology (Environment)

Course Title : Genetic Engineering
Course Code: CC 07

Unit-I
Restriction Enzymes

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Learning Objectives

1. What is a Restriction enzyme?
2. Restriction enzymes – Discovery, Biological Significance
3. Mode of Action
4. Types of Cuts
5. Applications

Definition

- A **restriction enzymes are** endonucleases that cleaves DNA sequence at specific sites known as restriction/ recognition sites.
- Example: EcoRI – 5`.....**G*AATTC.....3`**

  3`.....CTTAA*G.....5`
- It recognizes restriction sites within DNA or RNA molecule and cleave it by breaking the internal phosphodiester bonds.
- They are also called as '***Molecular Scissors***'

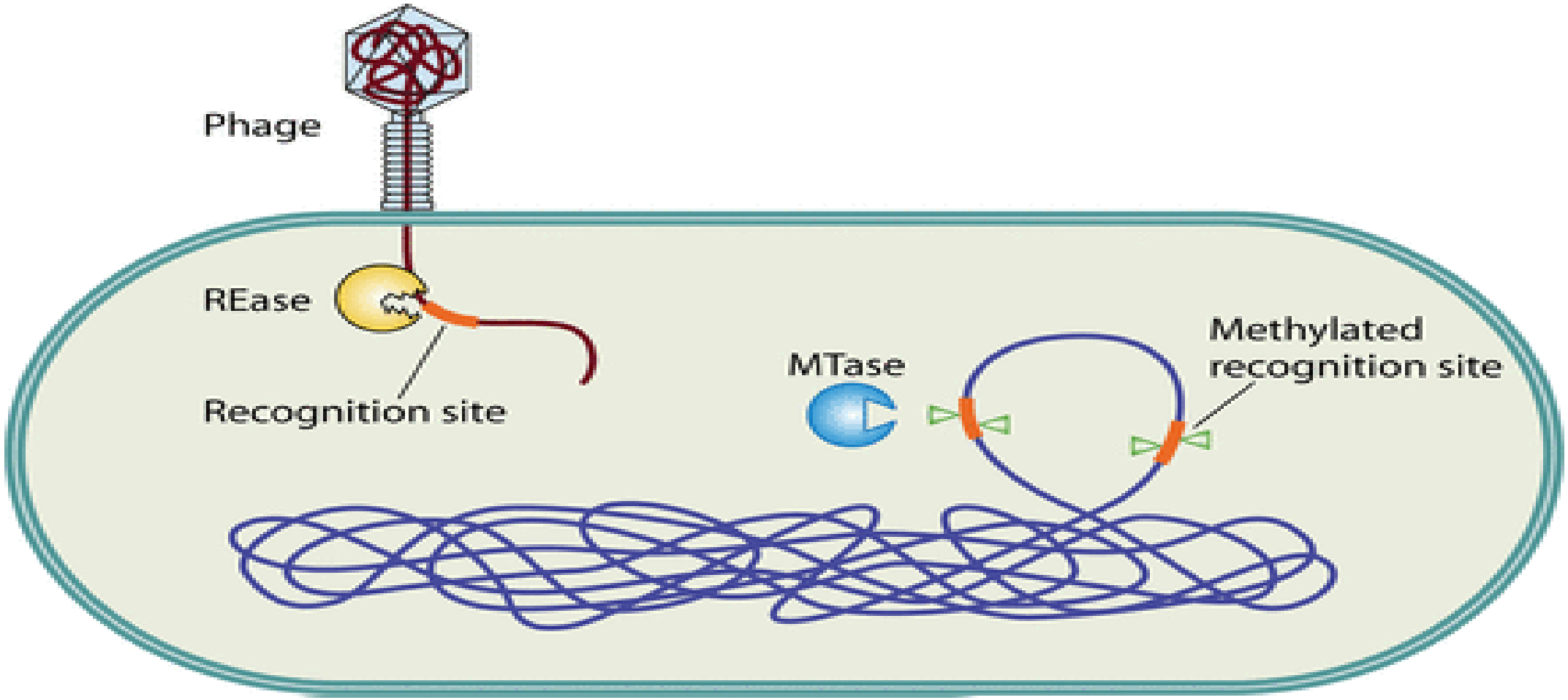
History

- The first restriction endonuclease enzyme ***Hind* II** was isolated in 1970.
- In 1978 Daniel Nathans, Werner Arber, and Hamilton O. Smith were awarded **Nobel Prize** (Medicine) for discovery and characterization of restriction endonucleases.

Restriction & Modification System

- Restriction-modification system as a defense mechanism of bacteria.
- Recognition sites present on bacterial DNA are methylated by methylases thus recognised as self and not acted upon by its own restriction endonucleases .
- The phage genome lacking methylation of restriction sites is identified as non-self and thus cleaved by Restriction Endonucleases.

Biological Significance



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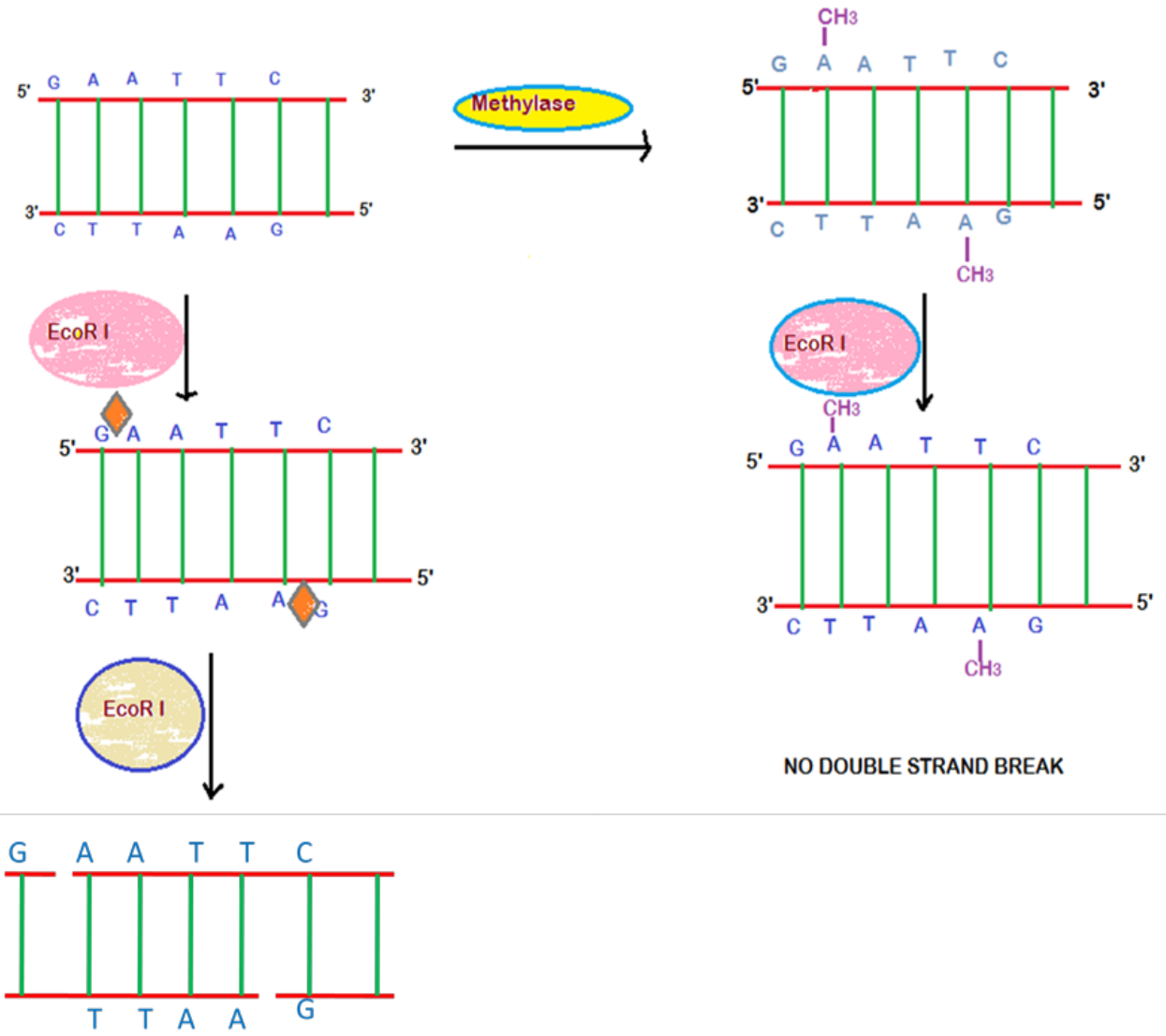
Activity of Restriction and Methylase Enzymes

Restriction enzyme *EcoR*I cleaves within the recognition sequence if the DNA is unmethylated. On methylation by methylases, the restriction enzyme *EcoR*I is inhibited from cleaving within the restriction site. Methylation normally occurs on cytosine (C) residue in DNA sequence



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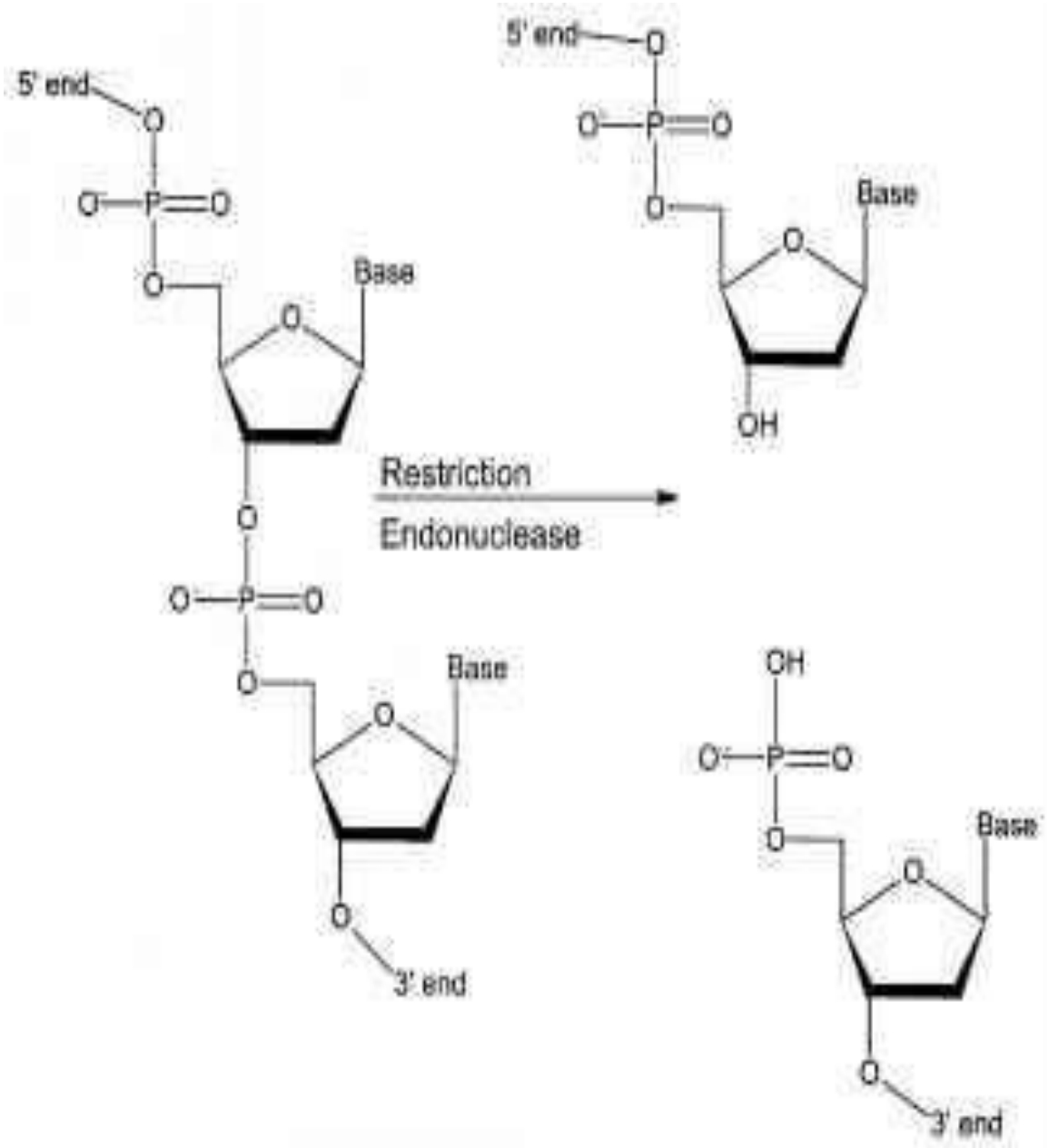
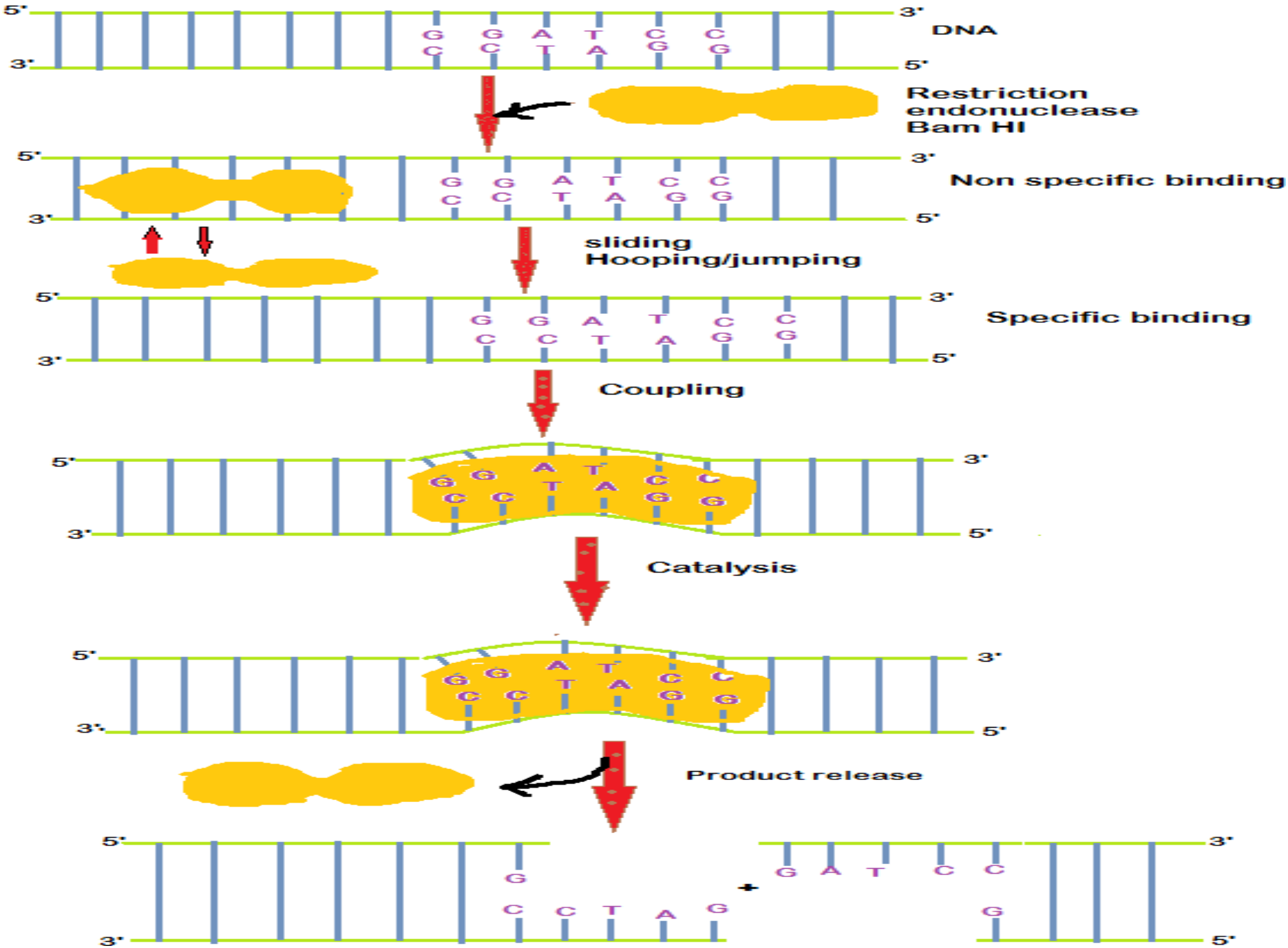
Mode of Action

Restriction Enzyme recognizes specific sites and makes one cut in each of the sugar phosphate backbones of the double helix by hydrolyzing the phosphodiester bond , in particular it breaks the bond between the 3`O atom and the P atom.





Source: https://nptel.ac.in/courses

Staggered & Blunt Cuts

EcoRI - Staggered Cuts- Sticky Ends

5`...G
3`...CTTAA...5`
AA TTC...3`
G...5`

EcoRV -Blunt Cuts- Flushy ends

5'...GAT
3`...CTA
ATC...3`
TAG...5`

Sticky Ends

- In a staggered cut, two strands of a dsDNA are not cut at the same position, but at a position 2 to 3 bases away from each other.
- This causes the formation of a small single stranded overhangs called ‘5' overhangs’ or ‘3' overhangs’.
- These ends are called as ‘sticky ends’ or ‘cohesive ends’ as they are complementary to each other & stick back via transient base pairing

5` and 3`Overhangs

- 5` overhangs- EcoRI – 5`....G* AATTC....3`

  3`....CTTAA *G....5`

  5` AATTC....3`

  G....5`

  5`....G

  3`....CTTAA 5`

- 3` overhangs- PvuI – 5`....CGAT* CG....3`

  3`....GC* TAGC....5`

  5`....CGAT 3`

  3`....GC

  CG....3`

  3` TAGC....5`

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Blunt Ends

- In a blunt cut, two strands of a dsDNA are cut at the same position to produce flushy ends
- Blunt-end ligation is covalently joining two double-stranded DNA fragments with flush ends by the enzyme DNA Ligase .

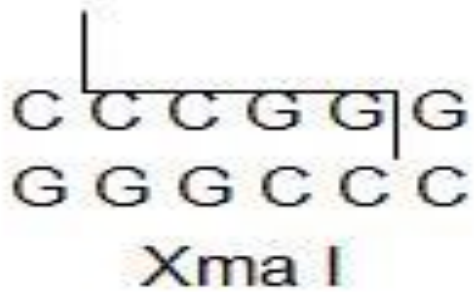
Types of Restriction Enzymes

	Cleavage site	Location of methylase	Examples
Type I	Random Around 1000bp away from recognition site	Endonuclease and methylase located on a single protein molecule	EcoK I EcoA I CfrA I
Type II	Specific Within the recognition site	Endonuclease and methylase are separate entities	EcoR I BamH I Hind III
Type III	Random 24-26 bp away from recognition site	Endonuclease and methylase located on a single protein molecule	EcoP I Hinf III EcoP15 I

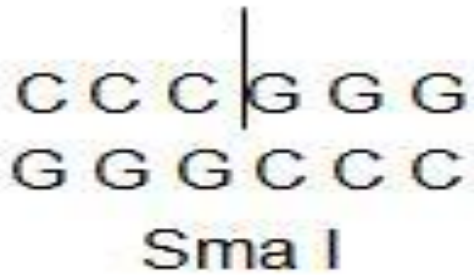
Isoschizomers and Neochischizomers

- Restriction enzymes that have the same recognition sequence as well as the same cleavage site are Isoschizomers.
- SphI (CGTAC/G) and BbuI (CGTAC/G)

- Restriction enzymes that have the same recognition sequence but cleave the DNA at a different site within that sequence are Neochizomers. Eg:SmaI and XmaI



Xma I



Sma I

Applications

- They are used in the process of insertion of genes into plasmid vectors during gene cloning and protein expression experiments.
- RFLP analysis (Restriction Fragment Length Polymorphism) for identifying individuals or strains of a particular species
- DNA sequencing Experiments
- Construction of Genomic DNA and cDNA libraries