ENZYMES IN GENETIC ENGINEERING
RESTRICTION ENZYMES

BY

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INTORDUCTION:

- A Restriction enzyme is a nuclease enzyme that cleave DNA sequence at a random or specific site known as restriction sites.
- In bacteria, restriction enzymes form a combined system (restriction + modification system) with ,modification enzymes that methylate the bacterial cell.
- Methylation of bacterial DNA at recognition sequence typically protects the own DNA of the bacteria from being cleaved by restriction enzymes.

HISTORY:

- In 1970 the first restriction endonuclease enzyme Hind III was isolated for discovery and characterization numerous other enzymes.
- In 1978 Daniel Nathans, Werner Arber and Hamilton O.Smith awarded Noble prize for physiology.
- Since then, restriction enzymes hve been used as an essential tool in recombinant DNA tecq.

Restriction Endonucleases Nomenclature:

- R.E are named accordingly to the org in which they are discovered, using a system of numbers and letters.
- E.g, Hind III pronounced (hindee-three) was discovered in Haemophilus influenza(strain d).
- The roman numericals are used to identify specific enzymes from bacteria that contain multiple restriction enzymes indicating the order in which R.E were discovered in a particular strain.

Hind III

1 st letter of genes name (Haemophilus) and 1st two letters of species(influenza)

“d” is the stain type

“III” is for 3rd enzyme discovered in that org.

Types of Restriction Enzymes

Type I:

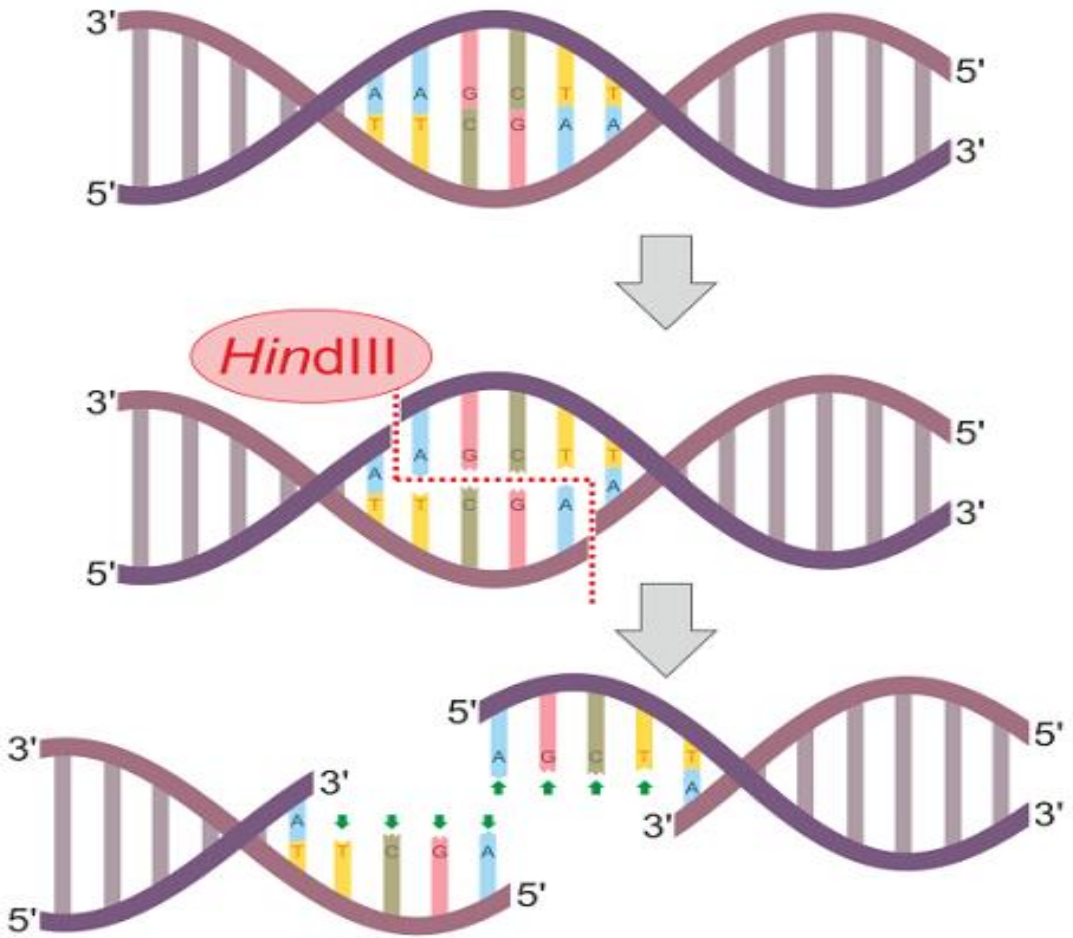
- These restriction enzymes cut the DNA far from the recognition sequences.
- However, they do not produce discrete restriction fragments, hence, are of not much practical value.
- These are complex, multi-subunit restriction and modification enzymes.
- They were initially thought to be rare, but through genomic analysis, they are found to be common and are of considerable biochemical interest.

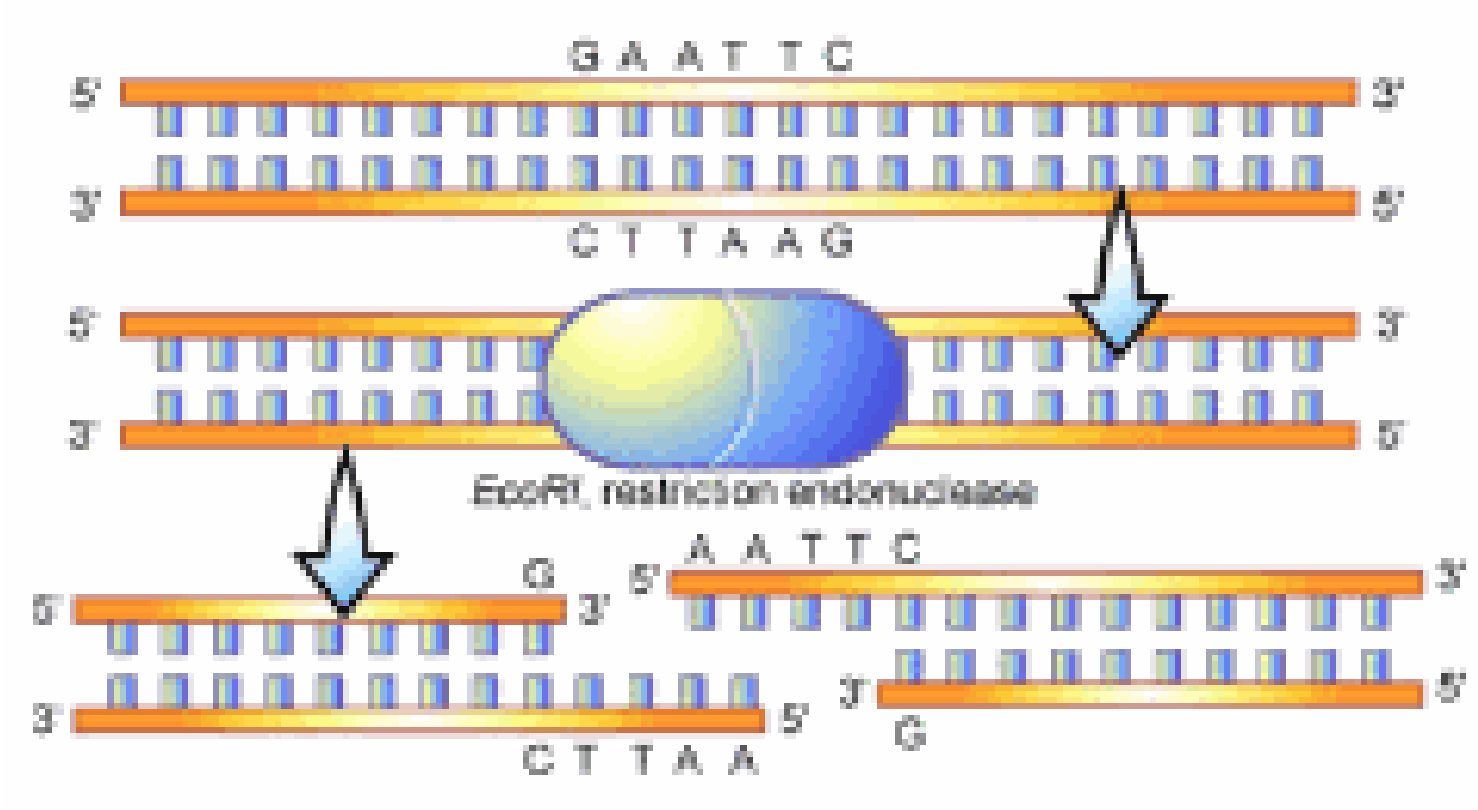
Type II

- These enzymes cut at specific positions closer to or within the restriction sites.
- Discrete restriction fragments and gel banding patterns are observed.
- They are exclusively used for DNA analysis and gene cloning in the laboratories.
- These are a family of unrelated proteins. They are named after the bacterial species from which they are isolated. For eg., EcoRI is isolated from bacterial species E.coli.
- The restriction enzymes generate two different types of cuts. Blunt ends are produced when they cut the DNA at the centre of the recognition sequence, and sticky ends produce an overhang.

Type III:

- These are multi-functional proteins with two subunits- Res and Mod.
- It is a modification methyltransferase.
- The DNA sequence specific for the system is recognized by the Mod subunit.


3'
5'
A A G C T T
T T C G A A
5'
3'
HindIII
3'
5'
A G C T T
A
T T C G A
5'
3'
5'
3'
5'
A G C T T
A
3'
5'
3'
A
T T C G A
5'


G A A T T C
5'
3'
3'
5'
C T T A A G
5'
3'
3'
5'
EcoRI, restriction endonuclease
G
5'
3'
A A T T C
5'
3'
3'
5'
3'
5'
C T T A A
G

Applications of Restriction Enzymes:

- They are used in RFLP techniques to cut the DNA into smaller fragments to study the fragment length differences among the individuals.

In Gene Cloning:

- During cloning, a gene is inserted into a plasmid. Restriction enzymes cut the plasmid producing single-stranded overhangs. The two DNA molecules are ligated with the help of DNA ligase to form a single DNA molecule.