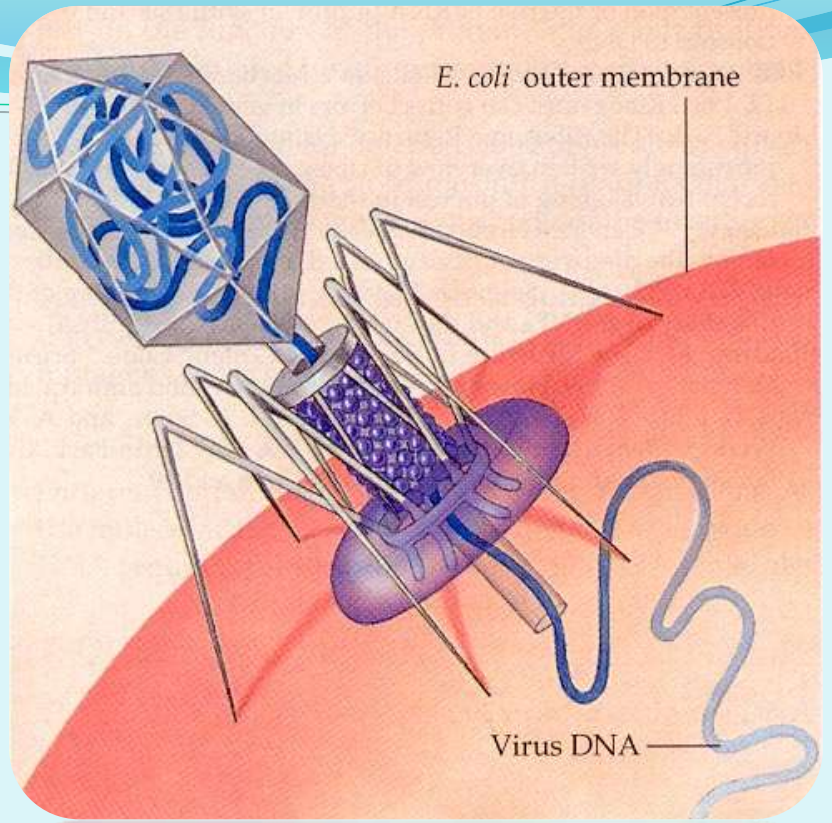
PRESENTATION ON RESTRICTION ENZYME



Presented by:-

MOHD ANAS

DEPT.-BIOSCIENCE

SHRI RAM COLLEGE,MUZAFFARNAGAR

Contents :-

Introduction :-

- ***Restriction Endonucleases*** **are enzymes that produce internal cuts, called *cleavage,* In the DNA molecule.**
- ***Restriction Endonuclease*** **(Restriction Enzyme) is a bacterial enzyme that cuts *dsDNA* into fragments after recognizing specific nucleotide sequence known as *recognition or restriction site*.**
- ***Restriction Enzymes*** **are beleved to be evolved by bacteria to resist *viral attack*.**
- ***Restriction Enzymes*** **are also known as molecular scessor.**

Discovery Of Restriction Enzyme :-

The term restriction enzyme originated from the studies of ***phage λ.***

In 1960 it was shown by **Werner Arber** and **Matthew Meselson** but they studied only about type I restriction enzyme.

In 1970, ***Hamilton O. Smith, Thomas Kelly*** and Kent Wilcox isolated and characterized the first type II restriction enzyme, ***HindII,*** from the bacterium ***Haemophilus influenzae***

For their work in the discovery and characterization of restriction enzymes, the 1978 ***Nobel Prize for Physiology or Medicine*** was awarded to ***Werner Arber, Daniel Nathans***, and ***Hamilton O. Smith***.

Types of Restriction Enzymes :-

- **Restriction enzymes are categorized into three general groups.**

Type I Restriction Endonucleases

Type II Restriction Endonucleases

Type III Restriction Endonucleases

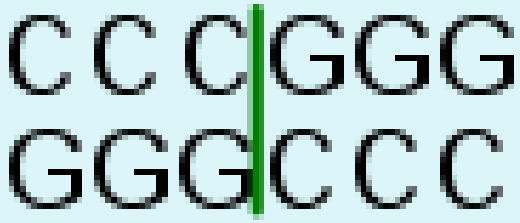
Categorization of Restriction Enzymes on the bases of :-

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

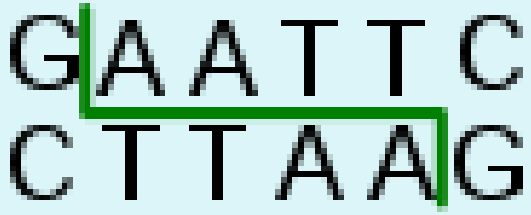
How Restriction Endonucleases work

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

1. BLUNT END

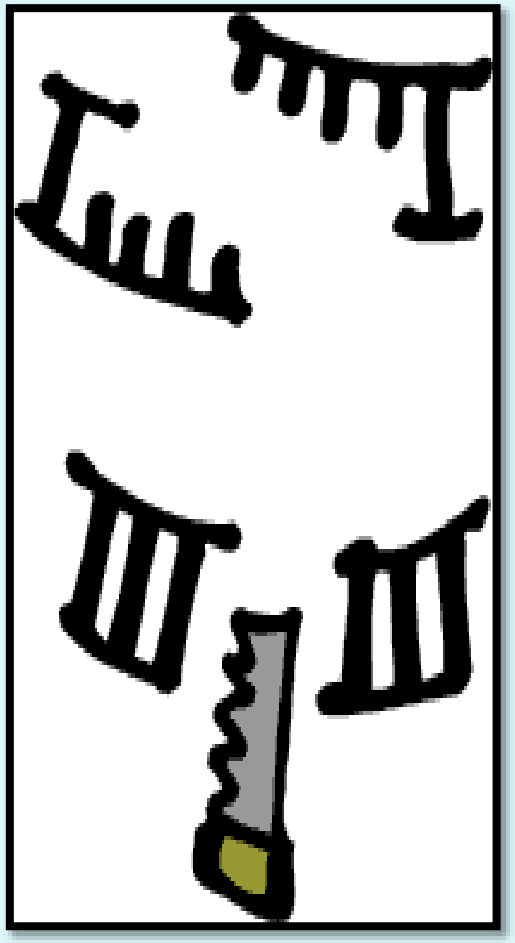


2. STICKY END



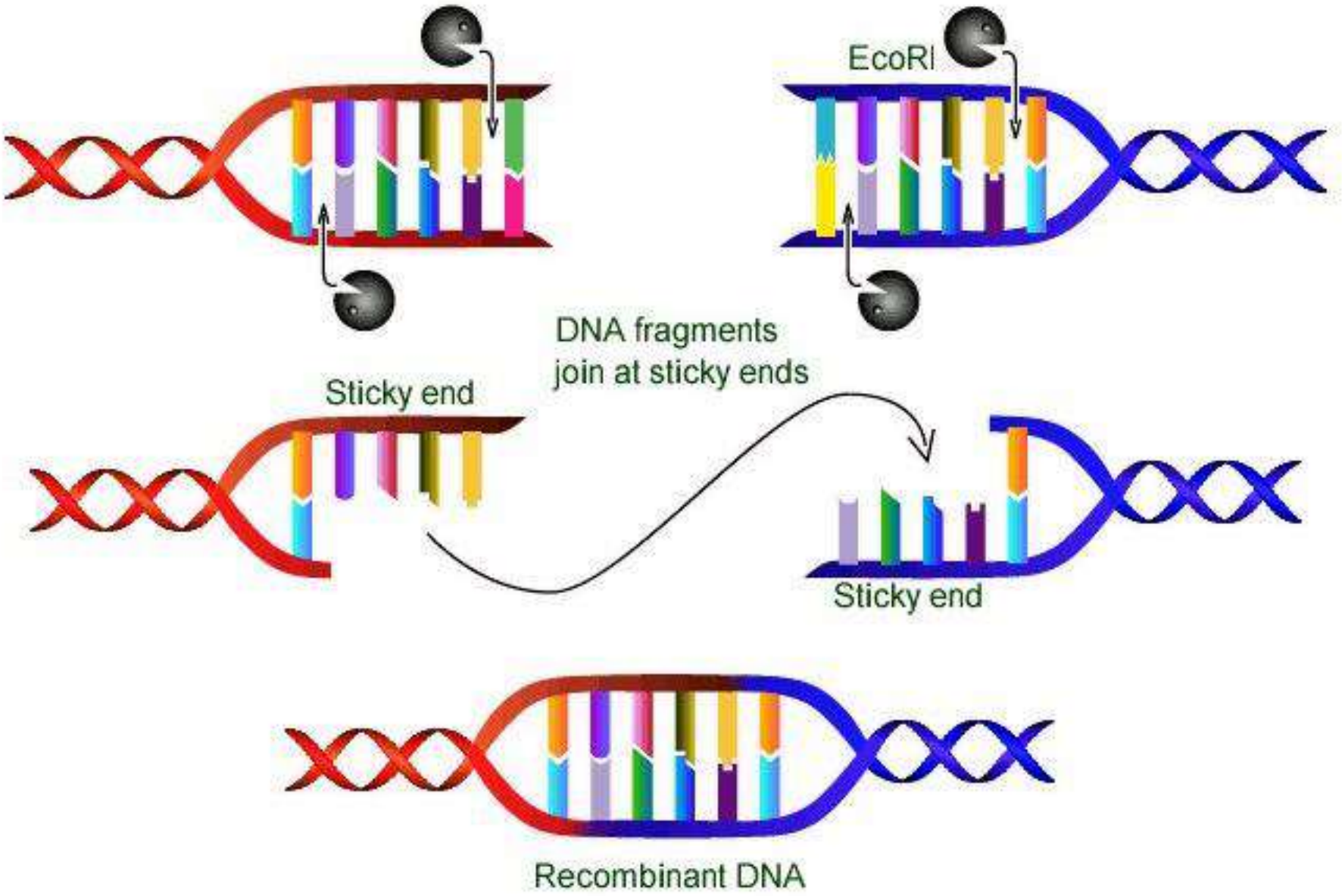
BLUNT END

- 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 END

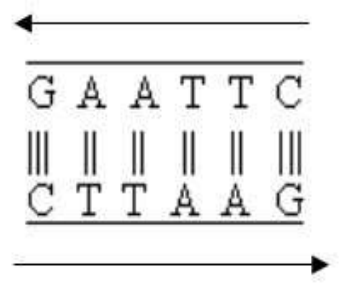
DNA fragments with complimentary sticky ends can be combined to create new molecules which allows the creation and manipulation of DNA sequences from different sources.



G A A T T C
C T T A A G

C C C G G G
G G G C C C

Type I Restriction Endonucleases



- Type I restriction enzymes are complex endonucleases, and have recognition sequence of 15 bp; they cleave the DNA about 1000 bp away from the 5` end of the sequence “TCA” located within the recognition site. Eg:- EcoK-12,EcoB,etc.
- The type I Restriction Enzyme work with the help of many co factor like as S-Adenosyl Methionine, Hydrolysed Adenosine Triphosphate (ATP) ad Magnesium (Mg2+).
- The type I Restriction Enzyme methylates the DNA sequence at the site of reognition.
- The type I Restriction Enzyme translocate the DNA sequence.

Subunits of Type I Restriction Endonucleases

- **Type I restriction enzymes possess three subunits:**

- **HsdR**: is required for restriction.

- **HsdM**: necessary for adding methyl groups to host DNA (methyltransferase activity).

- **HsdS**: important for specificity of cut site recognition in addition to its methyltransferase activity.

Type II Restriction Endonucleases

- The type II restriction enzymes are remarcably stable and induce cleavage either, in most cases, within or outside their recognition sequences,which are symmetrical.

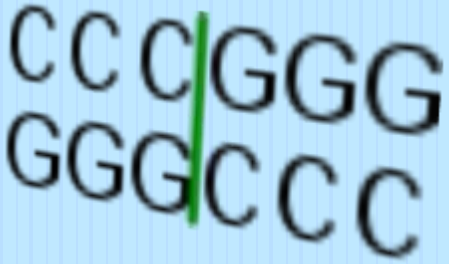
- The type I Restriction Enzyme work with the help of only single co factor like as Magnesium (Mg2+).

Type II Restriction Endonucleases

- The Ist type II Enzyme to be isolated was Hind II in 1970.
- Only type II restriction endonucleases are used for restriction mapping and gene cloning in view of their cleavage only at specific site .

Cut of Type II Restriction Endonucleases

- **Type II restriction enzymes can generate two different types of cuts depending on whether they cut both strands at the centre of the recognition sequence:**
- **The former cut will generate “blunt ends” with no nucleotideoverhangs.**
- **The latter, generates “sticky” or “cohesive” ends**



Subunits of Type II Restriction Endonucleases

- These subgroups are defined using a letter suffix.
- Type II B restriction enzymes.
- Type II E restriction endonucleases.
- Type II M restriction endonucleases.
- Type II T restriction enzymes

Type III Restriction Endonucleases

- Type III restriction enzymes are intermediate between type I and type II endonucleases, they cleave DNA in the immediate vacinity of their recognition sites, Eg;- EcoP1, EcoP15, Hind III etc.
- They cut DNA upto 20-30 base pairs away from the recognition site.
- These enzymes contain more than one subunit.(ATP & Mg^{2+})
- And require AdoMet and ATP cofactors for their roles in DNA methylation and restriction.

Nomenclature of Restriction Enzymes :-

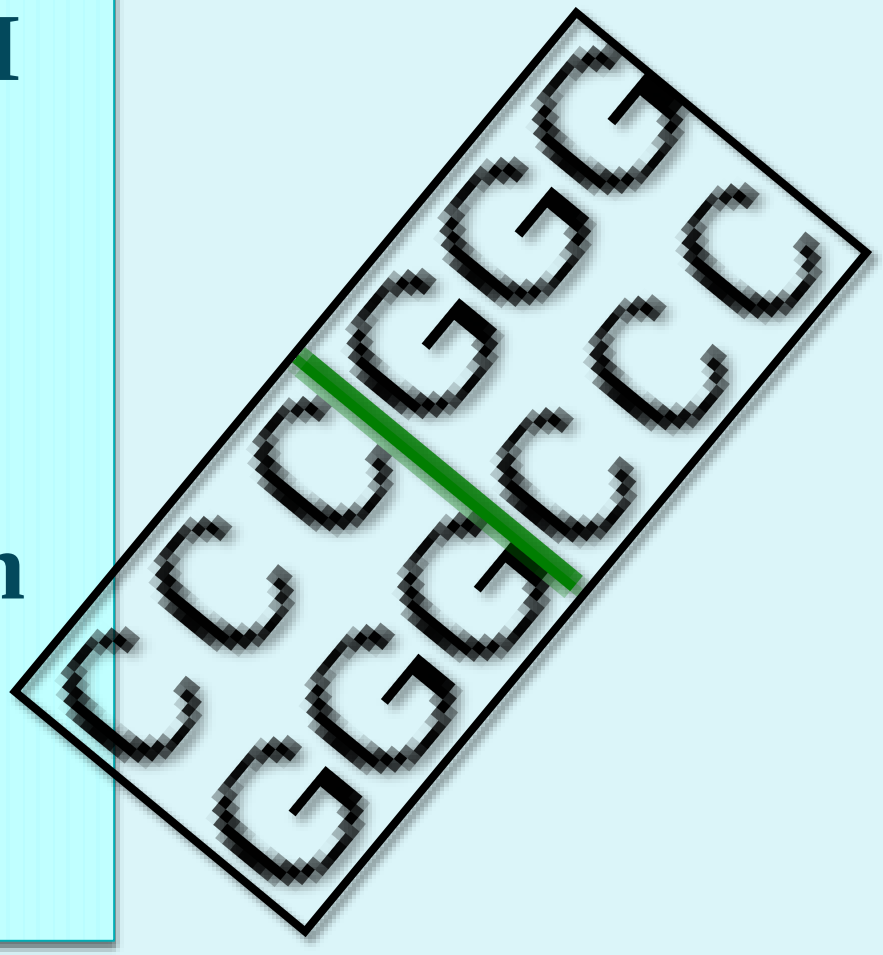
- After bacteria which produces them.

	EcoRI	HindIII	BamHI
Genus	Escherichia	Haemophilus	Bacillus
Species	coli	influenzae	amylo.
Strain	R	d	H
Order Isolated	I	III	I
Recognition Site	G^AATTC	A^AGCTT	G^GATGC

Recognition sequence of Res. Enz. :-

- **The recognition sequence of Type II endonucleases form palindrome with rotational symmetry.**

- **Mostly Type II endonucleases have recognition sites of 4,5 or 6 bp. Which are predominantly GC rich.**



Properties of Restriction Enzymes :-

PROPERTIES	TYPE I Re Enz	TYPE II Re Enz	TYPE III Re Enz
➢ Nature of enzyme	▪ It show endonuclease & methylase activity.	▪ separate endonuclease & methylase activity.	▪ It show endonuclease & methylase activity.
➢ Protein structure	▪ 3 different subunits	▪ 2 Identical subunits	▪ 2 different subunits
➢ Restriction requirement	▪ ATP ,Mg2+ S Adenosyle methionine	▪ Mg2+	▪ ATP ,Mg2+
➢ Cleavage Site	▪ Random,upto 1000 bp away from restriction site .	▪ AT or near restriction site .	▪ 24 – 26 bp 3`to restriction site .
➢ Example	▪ Eco B	▪ EcoR I	▪ Eco PI

Applications of Restriction Enzymes :-

- 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).
- Each of these methods depends on the use of agarose gel electrophoresis for separation of the DNA fragments.

Applications of Restriction Enzymes :-

- Provides different ways of manipulating DNA such as the creation of recombinant DNA, which has endless applications
- Allows for the large scale production human insulin for diabetics using E. coli, as well as for the Hepatitis B and HPV vaccines
- Cloning DNA Molecules
- Studying nucleotide sequence

THANK YOU