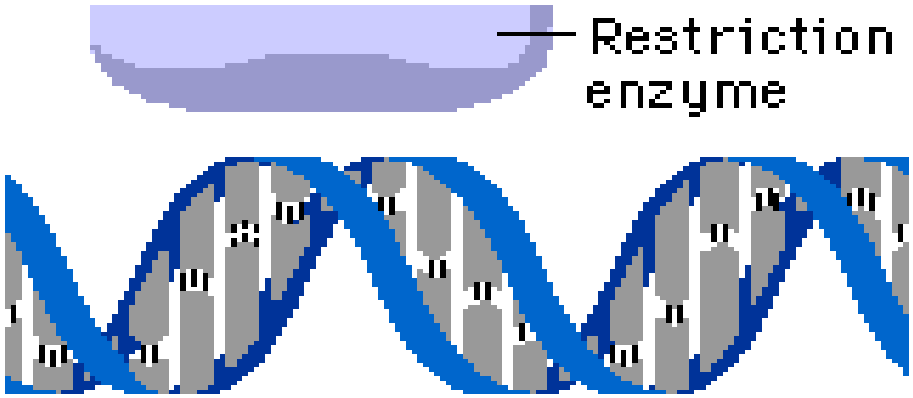
Restriction Endonuclease - DNA Cutting Enzyme



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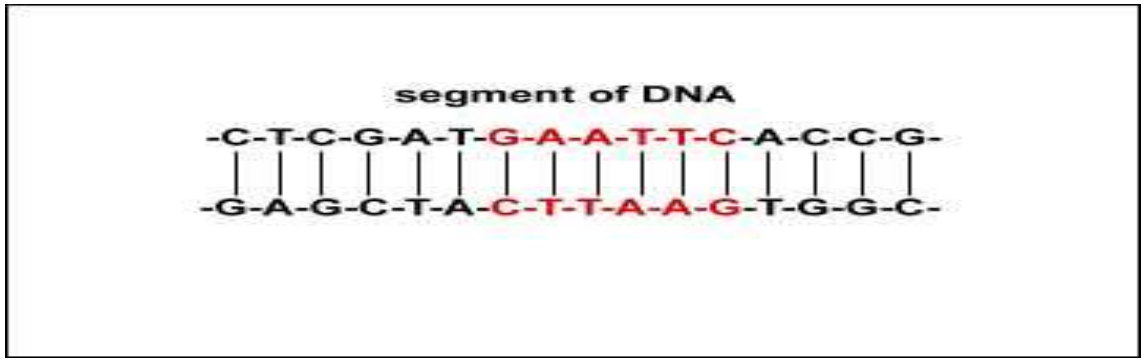
Introduction

- 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 evolved by bacteria to resist viral attack.
- Restriction Enzymes are also known as molecular scissor.



Historical Background

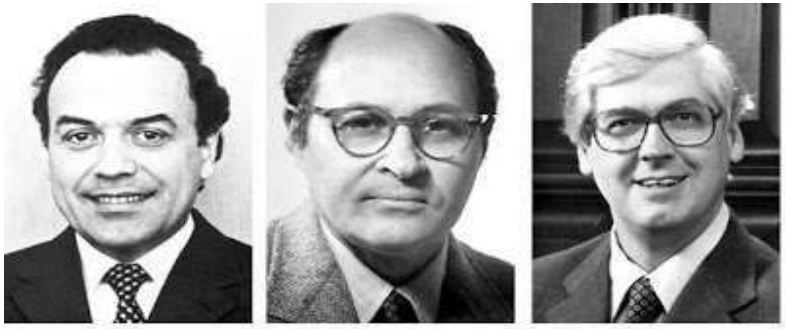
- The term restriction enzyme originated from the studies of phage λ and the phenomenon of host-controlled restriction and modification of a bacterial virus.

- The phenomenon was first identified in work done in the laboratories of Salvador Luria and Giuseppe Bertani in early 1950s.

- 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.

Historical Background

- In 1971, Daniel Nathans and Kathleen Danna showed that cleavage of simian virus 40 (SV40) DNA by restriction enzymes yields specific fragments that can be separated using polyacrylamide gel electrophoresis.

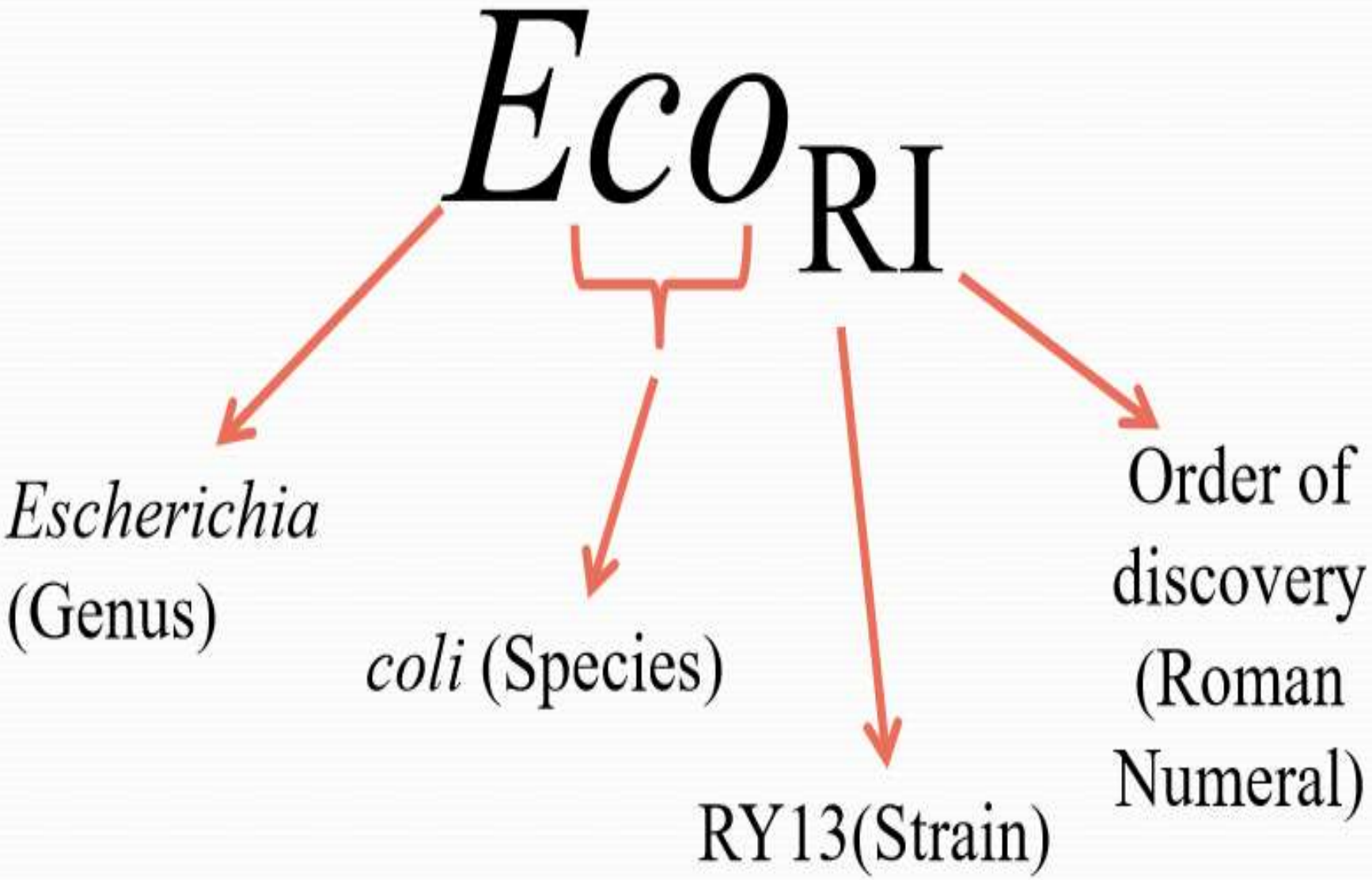


- For their work in the discovery and characterization of restriction enzymes, the 1978 Nobel Prize for Physiology or Medicine was awarded to Werner Arber, Daniel Nathans and Hamilton O. Smith.

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


EcoRI
Escherichia (Genus)
coli (Species)
RY13(Strain)
Order of discovery (Roman Numeral)

RESTRICTION SITES

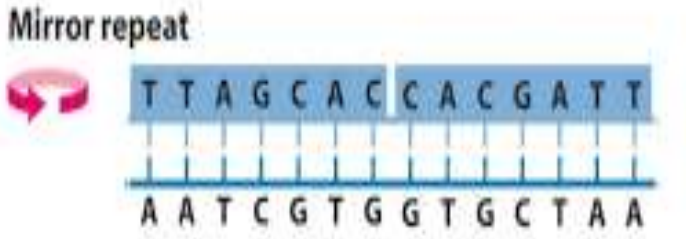
- Also called restriction recognition sites.
- These are locations on DNA molecule which contains specific nucleotide sequences, and are recognized by restriction enzymes.
- A particular restriction enzyme cuts the sequence between two nucleotide within or near its recognition site.
- Restriction enzymes recognize a specific sequence of nucleotides and produce a double-stranded cut in the DNA.
- These are generally palindromic sequences.

Palindromes

- It is a sequence made up of nucleic acids within double helix of DNA or RNA.
- Two types of palindromic sequences that can be possible in DNA:

1. The mirror-like palindrome is similar to those found in ordinary text, in which a sequence reads the same forward and backward on a single strand of DNA, as in GTAATG.

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



Recognition sequence of R.E.

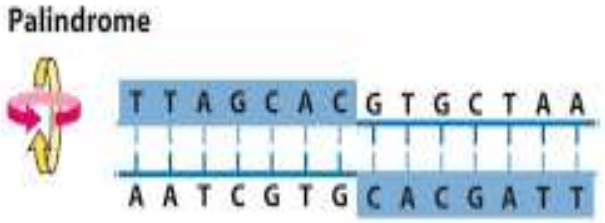
Palindromes

2.The inverted repeat palindrome is also a sequence that reads the same forward and backward, but the forward and backward sequences are found in complementary DNA strands (i.e., of double-stranded DNA), as in GTATAC (GTATAC being complementary to CATATG). Inverted repeat palindromes are more common and have greater biological importance than mirror-like palindromes.

- An example –

5` GAATTC 3`

3` CTTAAG 5`



CLEAVAGE PATTERNS

- Most Type II REases cleave the DNA molecules and produce two type of cuts.

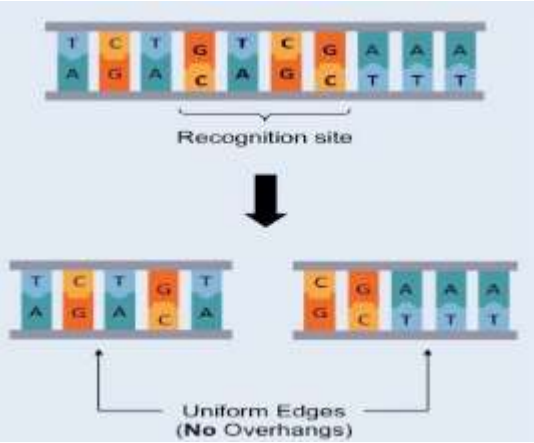
1. Staggered Cuts
2. Blunt or Even Cuts

- **Staggered Cuts-** Two DNA strands are cleaved at different locations. This generates 3` or 5` protruding ends which readily pair with each other under annealing conditions. These ends are called **cohesive or sticky ends.**

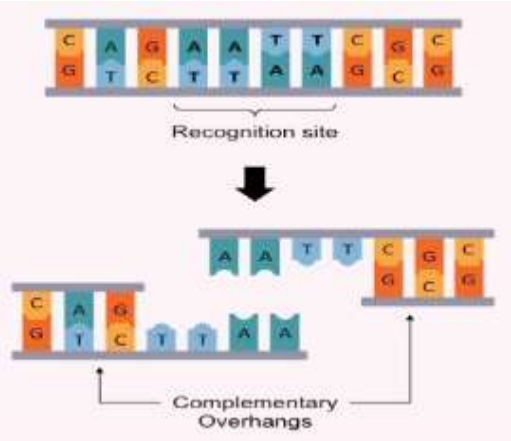
How Restriction Endonucleases work

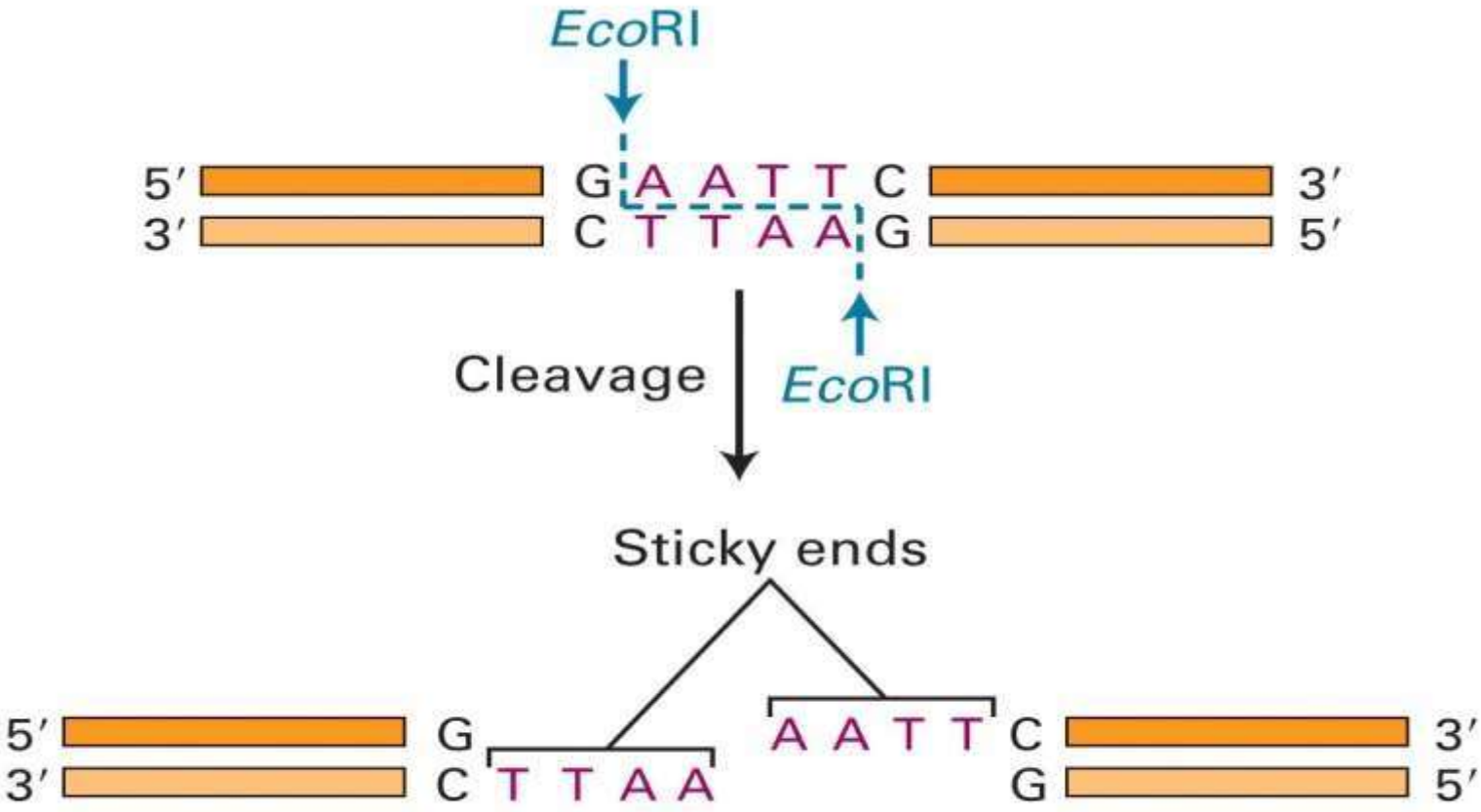
- Restriction enzymes recognize a specific sequence of nucleotides, and produce a double-stranded cut in the DNA. these cuts are of two types:

1. BLUNT END

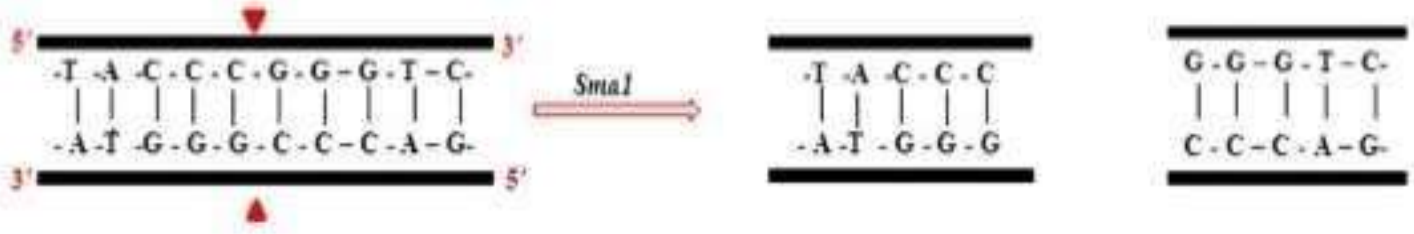


2. STICKY END/ STAGGERED



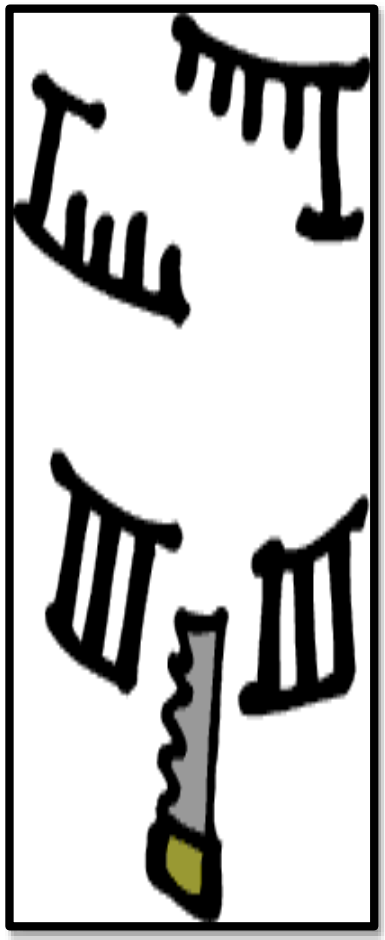

EcoRI
5′
3′
G A A T T C
C T T A A G
3′
5′
Cleavage
EcoRI
Sticky ends
5′
3′
G
C T T A A
A A T T C
G
3′
5′

Blunt Cuts- Some restriction endonucleases cut both strands the strands of DNA molecule at the same site producing blunt, even or flush ends.



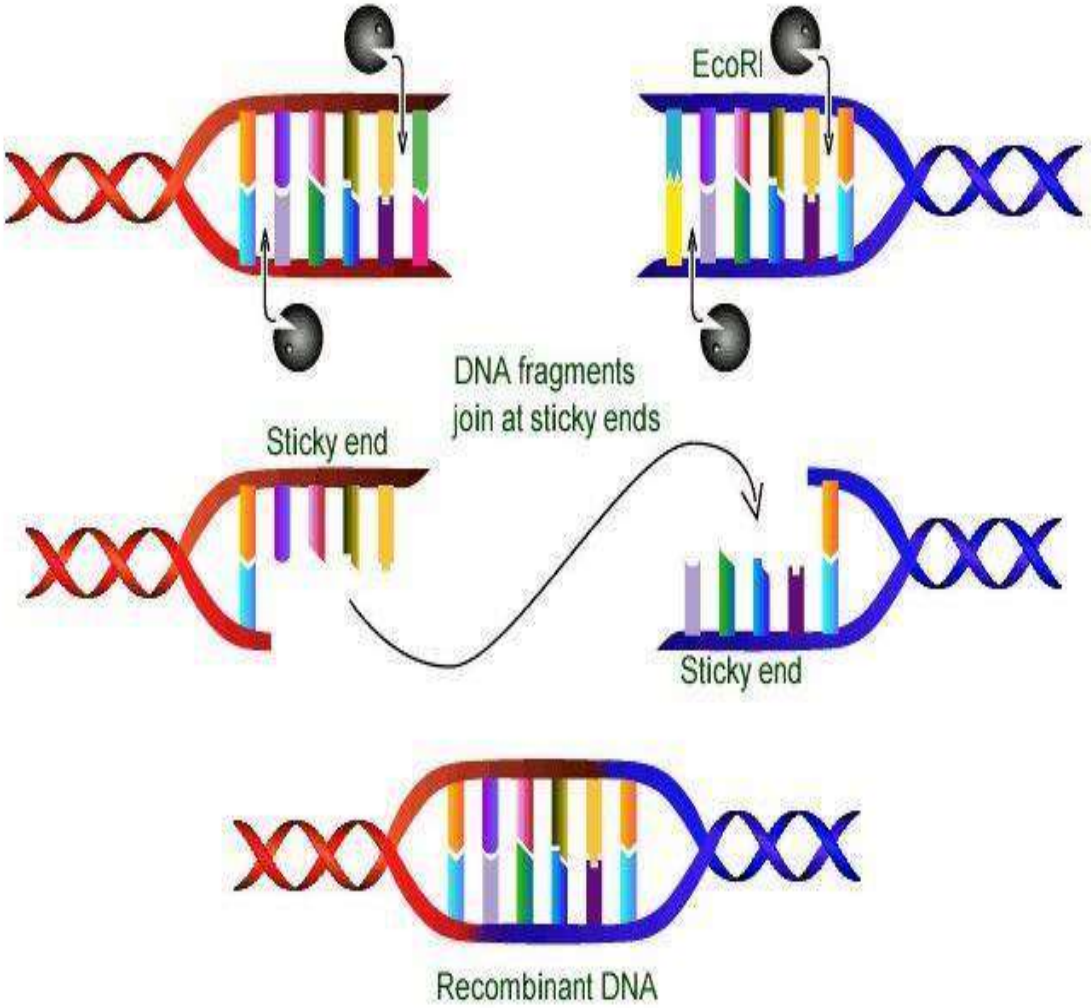
BLUNT END

- 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.

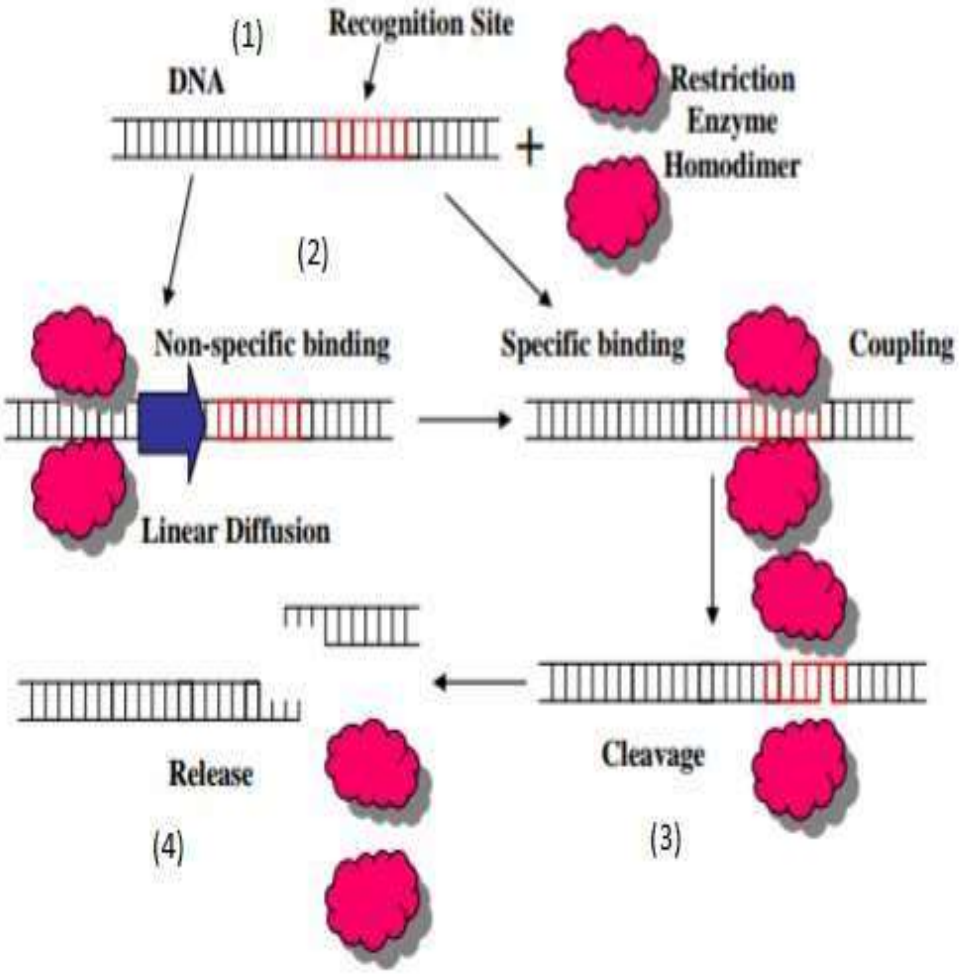


CCC|GGG
GGG|CCC

G|AATTC
CTTAA|G

MODE OF ACTION

- Generally the process consists of the following steps :
  1. Recognition of the binding sites
  2. Binding of the enzyme dimer to the DNA
  3. Cleavage of the DNA
  4. Enzyme release.



Categorization of Restriction Enzymes on the basis of

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

Type 1 : Enzymes recognize DNA sequences but cut the DNA in random sites that can be as far as 1,000 base pairs away from the recognized site.

Type 2 : Enzymes recognize and cut within the recognized site.

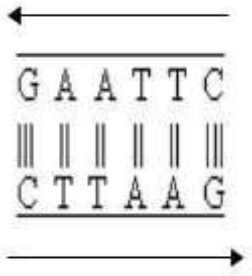
Type 3 : Enzymes recognize sequences but cut at a different location within 25 base pairs of the recognized site.

Type 4 : Recognition sequences have not been well defined.

Types of Restriction Enzymes

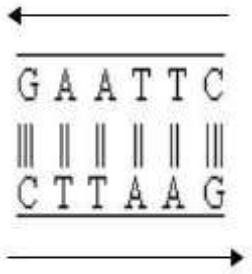
- **Restriction enzymes are categorized into three general groups.**
  - Type I Restriction Endonucleases
  - Type II Restriction Endonucleases
  - Type III Restriction Endonucleases
  - Type IV Restriction Endonucleases
  - Type V Restriction Endonucleases

Type I Restriction Endonucleases



- These have recognition sequence of about 15 bp.
- They cleave the DNA about 1000 bp away from 5'- end of the sequence.
- The sequence of cutting is nonspecific.
- It requires S- Adenosylmethionine, ATP, Mg2+ for its optimal activity.
- Type I restriction enzymes have a complex structure with three different subunits (endonuclease, methyl transferase and recognition).

Type I Restriction Endonucleases

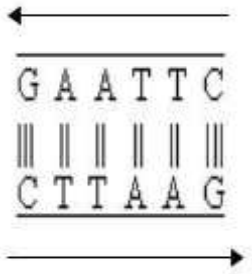


- Consists of 3 subunits –
  1. HsdR (required for restriction)
  2. HsdM (required for methylation of host DNA) and
  3. HsdS is important for specificity of recognition (DNA binding) site.

- Examples – EcoK, EcoB etc.

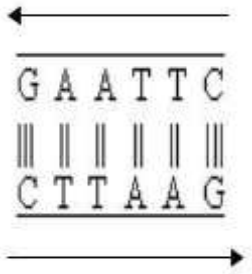
- They may have biological significance but are of very little practical value since the site where the DNA is going to be digested cannot be predicted.

Type II Restriction Endonucleases



- These are the simplest and commonly available and used restriction endonucleases.
- These are made up of two separated identical subunits having endonuclease and methylase activity.
- They cut DNA at defined positions close to within their recognition sequences.
- They donot use ATP for their activity but usually require only Mg2+ as cofactor for their activity.
- Their sequence of cutting is specific.
- They produce discrete (specific) recognition fragments.
- The recognition site is mostly symmetric, showing two fold symmetry.

Type II Restriction Endonucleases



- Type II restriction endonucleases cleave DNA at very precise and defined positions close to or within the recognition sequence.
- They are smaller in size, in comparison to type I and type III enzymes and mostly bind to DNA as homodimers.
- Most of the type II enzymes cleave within the symmetric recognition sequence, e.g. HhaI, HindIII and NotI, but there are other classes also that cleave outside their recognition sequence.
- A few other type II restriction enzymes cleave on both sides of the recognition sequence releasing a small fragment.

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 nucleotide overhangs.

- 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.
- They cut DNA upto 20-30 base pairs away from the recognition site.
- These enzymes contain more than one subunit.
- Consists of 2 subunits-

  1. Mod subunit recognizes the DNA sequence and is a modification methyl-transferase.
  2. Res subunit is required for restriction.

- They require AdoMet and ATP cofactors (Mg^{2+})for their role. Examples- EcoP1, EcoP15
- Type I and Type III endonucleases are not of any experimental value in genetic engineering, but have biological significance.

Type IV Restriction Endonucleases

- Type IV enzymes recognize modified, typically methylated DNA and are exemplified by the McrBC and Mrr systems of E. coli.(methylated, hydroxymethylated and glucosyl- hydroxymethylated bases).

- Recognition sequences have not been well defined
- Cleavage takes place ~30 bp away from one of the sites

- (McrBC is an endonuclease which cleaves DNA containing methylcytosine* on one or both strands. Sites on the DNA recognized by McrBC consist of two half-sites of the form.
- The Mrr protein of Escherichia coli is a laterally acquired Type IV restriction endonuclease with specificity for methylated DNA)

Type V Restriction Endonucleases

- These enzymes utilize guide RNAs to target specific non- palindromic sequences found on invading organisms.
- They can cut DNA of variable length, provided that a suitable guide RNA is provided.
- Example - the cas9-gRNA complex from CRISPRs.

TYPE II RESTRICTION ENDONUCLEASES ARE WIDELY USED IN RECOMBINANT TECHNOLOGY

This is due to their ability to catalyse phosphodiester bond cleavage with very large rate enhancements while also maintaining exquisite sequence selectivities.

Characteristic features of different classes of restriction enzymes

Type of enzyme	DNA recognition sequence	Cleavage site	Example	Co-factor required	Experimental value
I	Asymmetric and complex; composed of two parts separated by a spacer	Non-specific; >1000 base pairs from recognition site	*Eco*KI	Mg^{2+}, S-adenosyl methionine and ATP	Only biological significance
II	Two-fold rotational symmetry (Palindrome)	Specific; at the recognition site	*Eco*RI, *Hind*III	Mg^{2+}	In DNA analysis and molecular cloning
III	Short and asymmetric	Non-specific; 25-28 base pairs 3' to the recognition site	*Eco*PI	S-adenosyl methionine and ATP	Only biological significance

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

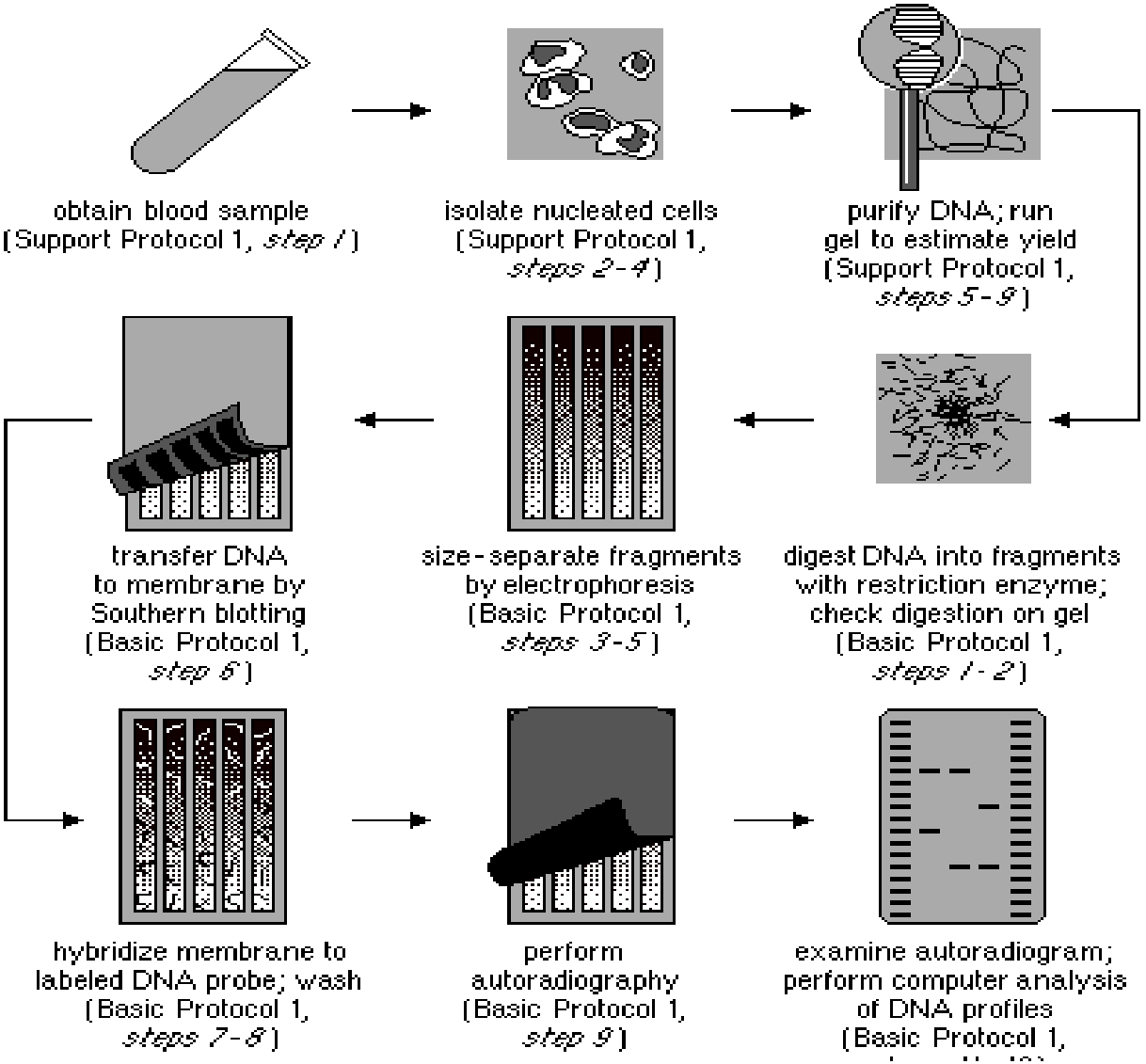
- Cloning DNA Molecules

- Studying nucleotide sequence

What is RFLP

RFLP (Restriction fragment length polymorphism)is an enzymatic procedure for separation and identification of desired fragments of DNA. Using restriction endonuclease enzymes fragments of DNA is obtained and the desired fragment is detected by using restriction probes. Southern hybridization using restriction endonuclease enzymes for isolation of desired length of DNA fragments is an example of RFLP.

Restriction fragment length polymorphism



Step I: Restriction digest

- Extraction of desired fragments of DNA using restriction endonuclease (RE).

- The enzyme RE has specific restriction site on the DNA, so it cut DNA into fragments.

- Different size of fragments are generated along with the specific desired fragments.

Step II: Gel electrophoresis

- The digested fragment are run in polyacrylamide gel electrophoresis or Agarose gel electrophoresis to separate the fragments on the basis of length or size or molecular weight.

- Different size of fragments form different bands.

Step III: Denaturation

•The gel is placed in sodium hydroxide (NaOH) solution for denaturation so that single stranded DNA are formed.

Step IV: Blotting

•The single stranded DNA obtained are transferred into charge membrane ie. Nitrocellulose paper by the process called capillary blotting or electro-blotting.

Step V: Baking and blocking

- The nitrocellulose paper transferred with DNA is fixed by autoclaving.
- Then the membrane is blocked by using bovine serum albumin or casein to prevent binding of labelled probe nonspecifically to the charged membrane.

Step VI: Hybridization and visualization

- The labelled RFLP probe is hybridized with DNA on the nitrocellulose paper.
- The RFLP probes are complimentary as well as labelled with radioactive isotopes so they form color band under visualization by autoradiography.

Application of RFLP test:

- **Genome mapping:** helps in analysis of unique pattern in genome for organism identification and differentiation. It also helps in determining recombination rate in the loci between restriction sites.

- **Genetic disease analysis:** After identification of gene for particular genetic or hereditary disease, that gene can be analyzed among other family members.

- To detect mutated gene.

- **DNA finger printing (forensic test):** It is the basis of DNA finger printing for paternity test, criminal identification etc.

Conclusion

- ✓ Restriction endonucleases are very essential enzymes which are able to cut the host DNA and create a favorable site for the insertion of a DNA segment of interest.
- ✓ It produces sticky or blunt ends as per their recognition sequence, and thus it is a valuable tool for modern biotechnological studies.
- ✓ Among of all its classes, Type II is more favorable for restriction mapping because of its ability to produce discrete fragments.
- ✓ Restriction endonucleases are indispensable for DNA cloning and sequencing.
- ✓ They serve as the tools for cutting DNA molecules at predetermined sites, which is the basic requirement for gene cloning or recombinant DNA technology

Thank You

Method of DNA analysis by RFLP

The method of analysis of DNA by RFLP involves the following steps:

1- In the first step fragmentation of a sample of DNA is done by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction digest.

2 The resulting DNA fragments are then separated by length through a process known as agarose gel electrophoresis.

3 Then transferred to a membrane via the Southern blot procedure.

4. Hybridization of the membrane to a labeled DNA probe will done and then determines the length of the fragments which are complementary to the probe.

5.Then we will observe the fragments of different length.

An RFLP occurs when the length of a detected fragment varies between individuals. Each fragment length is considered an allele, and can be used in genetic analysis.