RESTRICTION ENZYMES

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What are restriction enzymes?

- Restriction endonuclease is an enzyme that cuts double or single stranded DNA molecules at specific sites. (Molecular scissors)

- Found naturally in a wide variety of prokaryotes

- Over 10,000 bacteria species have been screened for restriction enzymes
- From them over 3000 restriction enzymes have been studied in detail
- More than 600 of them are available commercially and are routinely used for DNA modification & manipulation in laboratories

Discovery

- Arbor and Dussoix in 1962 discovered that certain bacteria contain Endonucleases which have the ability to cleave DNA.

- In 1970 Smith and colleagues purified and characterized the cleavage site of a Restriction Enzyme.

- Werner Arbor, Hamilton Smith and Daniel Nathans shared the 1978 Nobel prize for Medicine and Physiology for their discovery of Restriction Enzymes.

Biological Role

- Most bacteria use Restriction Enzymes as a defense against bacteriophages.

- Restriction enzymes prevent the replication of the phage by cleaving its DNA at specific sites.

- The host DNA is protected by Methylation which add methyl groups(CH_3) to adenine or cytosine bases within the recognition site thereby modifying the site and protecting the DNA.

Restriction sites

- Also called cleavage sites or restriction endonuclease site
- They are specific nucleotide site that are recognized by restriction enzymes
- They are generally palindromic sequences

Palindromic sequence

- DNA palindromes

➢ The mirror like palindrome: in which the same forward and backward are found on a single DNA strand as in GTAATG.

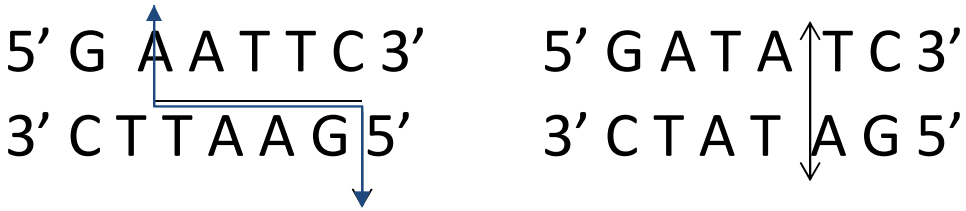
➢ Inverted repeats palindrome: is also a sequence that read the same forward and backwards but they found in complementary DNA strands

ex: GTATAC

CATATG

➢ Inverted repeats palindromes are more common and have greater biological importance than mirror like palindromes

Restriction fragments can be blunt ended or sticky ended



Sticky Ends Blunt Ends

Sticky ends or blunt ends can be used to join DNA fragments.

Sticky ends are more cohesive compared to blunt ends.

Isoschizomers and Neochizomers

- Restriction enzymes that have the same recognition sequence as well as the same cleavage site are Isoschizomers.

- 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

C|C C G G G
G G G C C|C
Xma I

C C C|G G G
G G G|C C C
Sma I

NOMENCLATURE

- ✓ first letter from genus
- ✓ Next two letters represents species
- ✓ Additional letters or numbers represents the strain or serotype

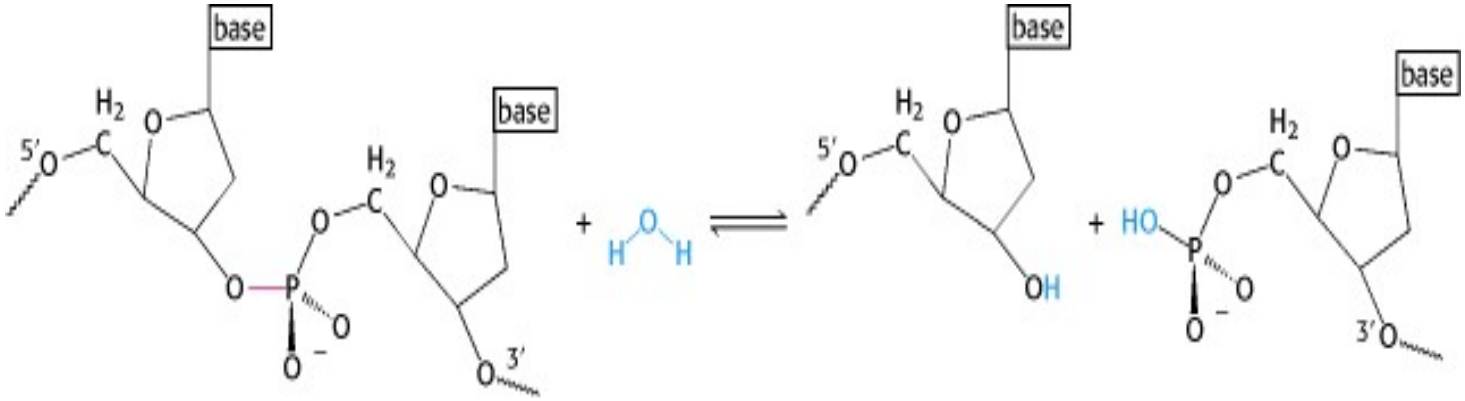
Ex: EcoRI isolated from Escherichia coli

Abbreviation	Meaning	Description
E	Escherichia	Genus
CO	Coli	Species
R	RY13	Strain
I	First identified	Order od identification in bacterium

Mechanism of Action

Restriction Endonuclease scan the length of the DNA , binds to the DNA molecule when it recognizes a specific sequence and makes one cut in each of the sugar phosphate backbones of the double helix – by hydrolyzing the phosphodiester bond. Specifically, the bond between the 3’ O atom and the P atom is broken.

Direct hydrolysis by nucleophilic attack at the phosphorous atom



3’OH and 5’ PO_4^{3-} is produced. Mg^{2+} is required for the catalytic activity of the enzyme. It holds the water molecule in a position where it can attack the phosphoryl group and also helps polarize the water molecule towards deprotonation .

Types of restriction enzymes

1. Type I: cleave at sites remote from recognition sites
2. Type II: cleave within or at short specific distances from recognition sites
3. Type III: cleave outside of their recognition sites
4. Type IV: target normal DNA

Type I :

- capable of both restriction & modification activities .
- The cofactor S-Adenosyl methionine (Adomet) , ATP & Mg+ are required for their activity

Contains :

- Two R (restriction) subunits
- Two M (methylation) subunits
- One S (specificity) subunit

Cleave DNA at random length from its recognition site

Type II:

- Mostly used for gene analysis & cloning
- More than 3500 Res
- Recognize 4-8 base sequence
- Need Mg2+ as a cofactor
- Cut the DNA at the recognition site
- Homodimer
- ATP hydrolysis is not required

EX: EcoRI , BamHI , Hind III

Type III:

- Large enzymes
- Combination of restriction & modification
- Cleave outside of their recognition sequences
- Required two recognition sequences in opposite orientation with the same DNA molecule
- No commercial use or availability

Type IV:

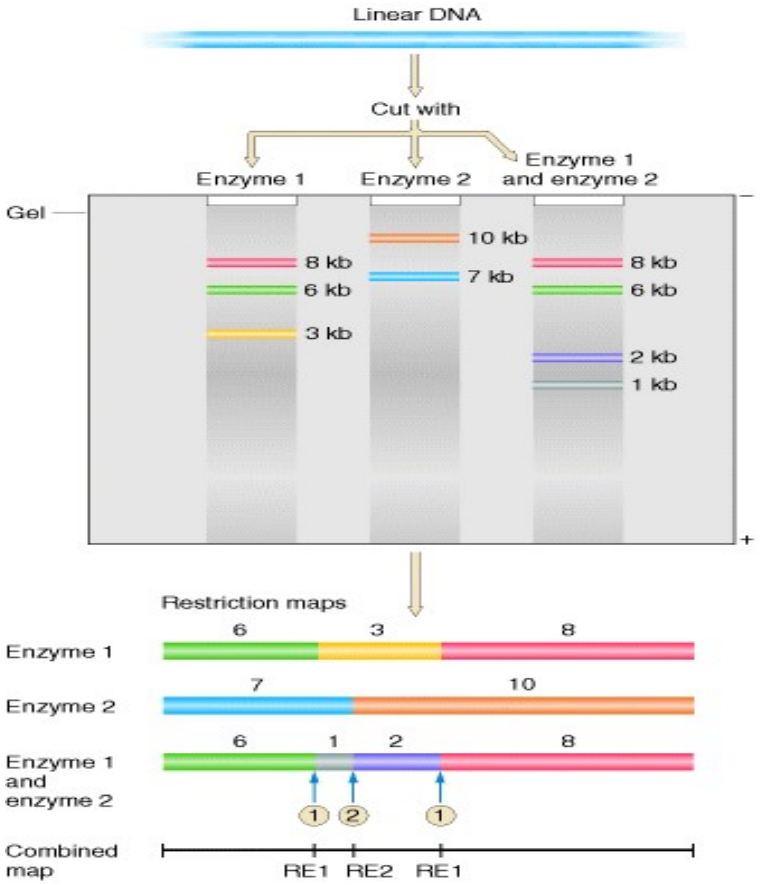
- Cleave only normal or modified DNA (methylated , hydroxymethylated & glucosyl hydroxymethylated)
- Recognition sequence have not been well defined
- Cleavage takes place about 30 bp away from one of the sites

APPLICATION 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.

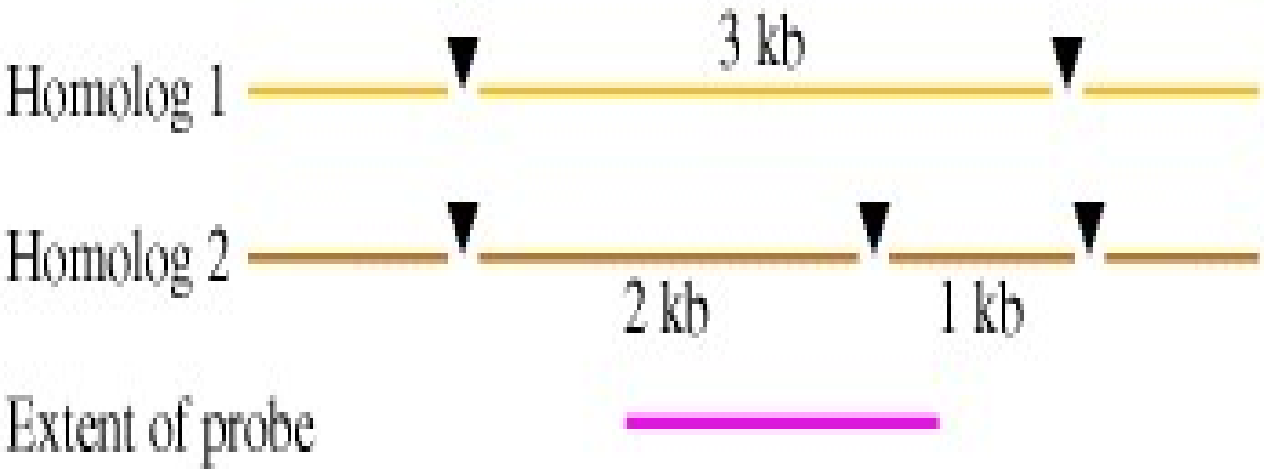
Uses of Restriction Enzymes

Restriction Enzymes can be used to generate a restriction map. This can provide useful information in characterizing a DNA molecule.



Uses….

Restriction Fragment Length Polymorphism is a tool to study variations among individuals & among species



Uses….

Restriction enzymes are most widely used in recombinant DNA technology.