Course- M.Sc. Botany Part -II Paper- XVI

Topic- Restriction Enzymes (RE)

(Biotechnology & Bioinformatics)

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Restriction Enzymes or Restriction Endonuclease:

Restriction enzyme, also called restriction endonuclease, is a protein produced by bacteria that cleaves DNA at specific sites along the molecule.

Restriction endonucleases cut the DNA double helix in very precise ways. It cleaves DNA into fragments at or near specific recognition sites within the molecule known as restriction sitesor the target sequence.

They have the capacity to recognize specific base sequences on DNA and then to cut each strand at a given place. Hence, they are also called as **'molecular scissors'**.

In the bacterial cell, restriction enzymes cleave foreign DNA, thus eliminating infecting organisms. Restriction enzymes can be isolated from bacterial cells and used in the laboratory to manipulate fragments of DNA, such as those that contain genes; for this reason they are indispensible tools of recombinant DNA technology (genetic engineering).

What is a Restriction Enzyme?

A restriction enzyme is a protein that recognizes a specific, short nucleotide sequence and cuts the DNA only at that specific site, which is known as **restriction site or target sequence.**

More than 400 restriction enzymes have been isolated from the bacteria that manufacture them. In live bacteria, restriction enzymes function to defend the cell against invading viral bacteriophages. Restrictions sites in the viral genome (a "happy accident" of nature, as far as the bacteria are concerned, since they don't appear to have any specific function in the virus) are cleaved by the bacterium's restriction enzymes, fragmenting and destroying the DNA of invading bacteriophages before it can incorporate into the host's genome and take over the cell.

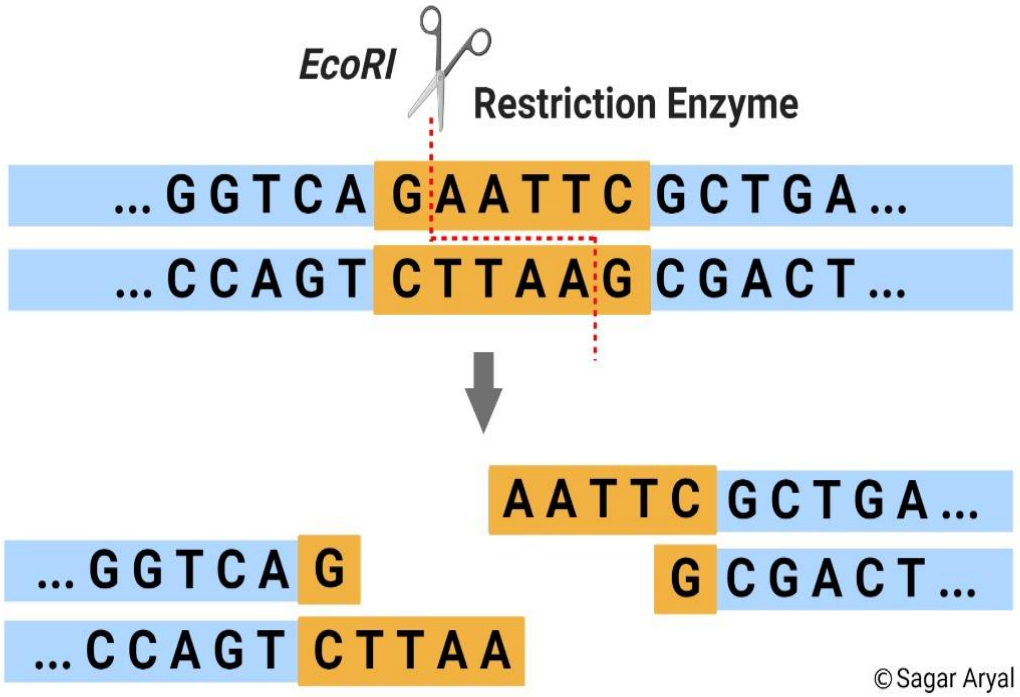
A bacterium is immune to its own restriction enzymes, even if it has the target sequences ordinarily targeted by them. This is because the bacterial restriction sites are highly methylated, making them unrecognizable to the restriction enzyme.

Isn't evolution fantastic?

When a restriction enzyme cleaves a restriction site, the reaction creates highly reactive **"sticky ends"** on the broken DNA. This is useful to the biotechnologist!

By cutting open vector DNA with the same with restriction enzymes used to cleave the target DNA, complementary "sticky ends" are created. This fosters the insertion of the target DNA into the vector:

The fragment is "glued in" with DNA ligase, which creates the phosphodiester bonds necessary to complete the sugar-phosphate backbone of the newly transgenic DNA.



- Some restriction enzymes also cut DNA to form **"blunt" ends**" (without single-stranded tails), which also can be inserted into target DNA via the action of DNA ligase.
- DNA ligase isn't picky: it can't tell the difference between foreign and host DNA (who'd figure it would ever have to?), and this enables the creation of chimeric DNA--DNA from two separate sources.
- Each enzyme recognizes and cuts specific DNA sequences. For example, BamHI recognizes the double stranded sequence:

  5'--GGATCC--3'
  3'--CCTAGG--5'

Here's another artist's conception of how this works. (Notice the "sticky ends.")

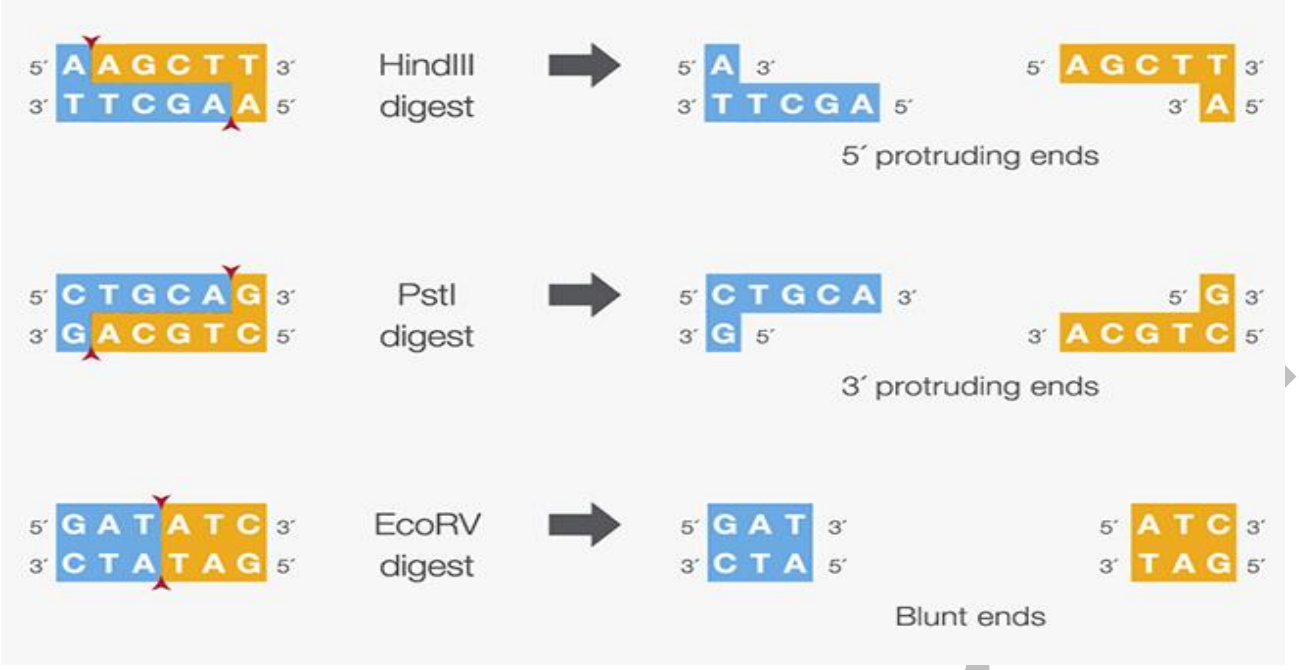


Figure: Sticky or protruding ends (5′ or 3′) or blunt ends produced by Specific Restriction Enzyme

Source of Restriction Enzymes:

The natural source of restriction endonucleases are bacterial cells.

These enzymes are called restriction enzymes because they restrict infection of bacteria by certain viruses (i.e., bacteriophages), by degrading the viral DNA without affecting the bacterial DNA. Thus, their function in the bacterial cell is to destroy foreign DNA that might enter the cell.

The restriction enzyme recognizes the foreign DNA and cuts it at several sites along the molecule.

Each bacterium has its own unique restriction enzymes and each enzyme recognizes only one type of sequence.

Recognition Sites

The DNA sequences recognized by restriction enzymes are called palindromes. **Palindromes** are the base sequences that read the same on the two strands but in opposite directions.

For example, if the sequence on one strand is GAATTC read in 5'→3′ direction, the sequence on the opposite strand is CTTAAG read in the 3'→5′ direction, but when both

strands are read in the 5'→ 3′ direction the sequence is the same. The palindrome appears accordingly -

5′ GAATTC 3′

3′ CTTAAG 5′

In addition, there is a point of symmetry within the palindrome. In the example, this point is in the center between the AT/AT.

The value of restriction enzymes is that they make cuts in the DNA molecule around this point of symmetry. Some enzymes cut straight across the molecule at the symmetrical axis producing **blunt ends.**

Of more value, however, are the restriction enzymes that cut between the same two bases away from the point of symmetry on two strands, thus, producing a **staggering break.**

Nomenclature of Restriction Enzymes:

Since their discovery in the 1970s, many restriction enzymes have been identified while Type II restriction enzymes have been characterized.

Each enzyme is named after the bacterium from which it was isolated, using a naming system based on bacterial genus, species and strain. For example, the name of the EcoRI restriction enzyme was derived as:

E – Escherichia: Genus

co- coli: specific species

R- RY13: strain

I- First identified: order of identification in the bacterium

One of the most popular restriction enzymes is EcoRI from E. coli (bacterium).

BamHI is isolated from Bacillus amyloliquefaciens strain H

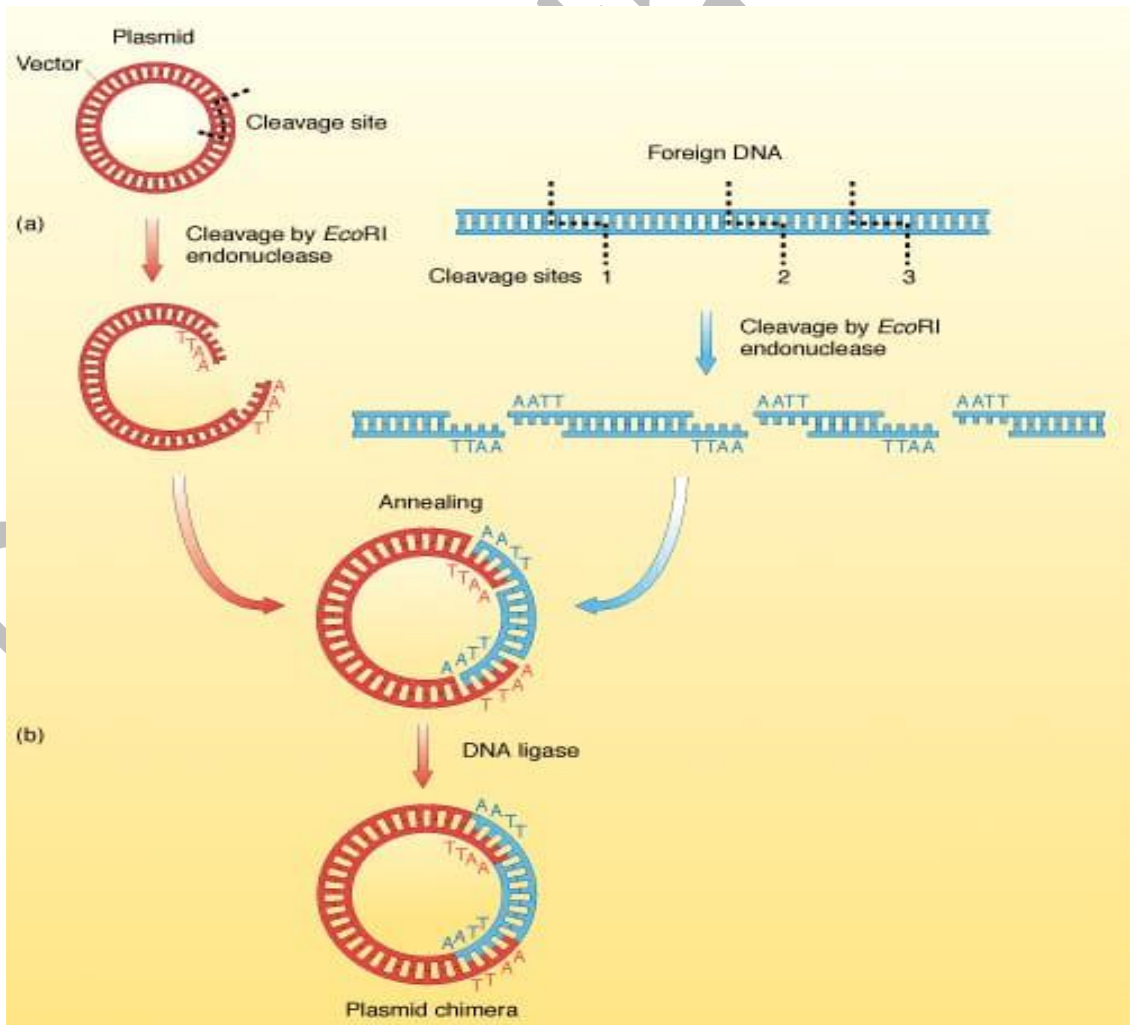
Sau3A is isolated from Staphylococcus aureas strain 3A. And so on.

Hundreds of other restriction enzymes with different sequence specificities have been isolated from several bacteria and are commercially available.

Some examples of Restriction Enzymes are:

Enzyme	Obtained from	Recognition Sequence
EcoRI	Escherichia coli	5'GAATTC 3'CTTAAG
EcoRII	Escherichia coli	5'CCWGG 3'GGWCC
BamHI	Bacillus amyloliquefaciens	5'GGATCC 3'CCTAGG
HindIII	Haemophilus influenza	5'AAGCTT 3'TTCGAA

Mechanism of Cleavage of Restriction Enzymes



When a restriction endonuclease recognizes a particular sequence, it snips through the DNA molecule by catalyzing the hydrolysis (splitting of a chemical bond by addition of a water molecule) of the bond between adjacent nucleotides. To cut DNA, all restriction enzymes make two incisions, once through each sugar-phosphate backbone (i.e. each strand) of the DNA double helix.

Types of Restriction Enzymes:

Traditionally, four types of restriction enzymes are recognized, designated I, II, III, and IV, which differ primarily in structure, cleavage site, specificity, and cofactors.

Type I enzymes cleave at sites remote from a recognition site; require both ATP and S-adenosyl-L-methionine to function; multifunctional protein with both restriction and methylase activities.

Type II enzymes cleave within or at short specific distances from a recognition site; most require magnesium; single function (restriction) enzymes independent of methylase.

Type III enzymes cleave at sites a short distance from a recognition site; require ATP (but do not hydrolyze it); S-adenosyl-L-methionine stimulates the reaction but is not required; it exists as part of a complex with a modification methylase.

Type IV enzymes target modified DNA, e.g. methylated, hydroxymethylated and glucosyl-hydroxymethylated DNA.

	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

Applications of Restriction Enzymes:

Restriction enzymes can be isolated from bacterial cells and used in the laboratory to manipulate fragments of DNA, such as those that contain genes; for this reason, they are indispensable tools of recombinant DNA technology (genetic engineering).

The most useful aspect of restriction enzymes is that each enzyme recognizes the same unique base sequence regardless of the source of the DNA. It means that these enzymes establish fixed landmarks along an otherwise very regular DNA molecule. This allows dividing a long DNA molecule into fragments that can be separated from each other by size with the technique of gel electrophoresis.

Each fragment, thus generated, are also available for further analysis, including the sequencing.

One value of cutting DNA molecule up into discrete fragments is being able to locate a particular gene on the fragment where it resides which is done by the general technique of Southern blotting.