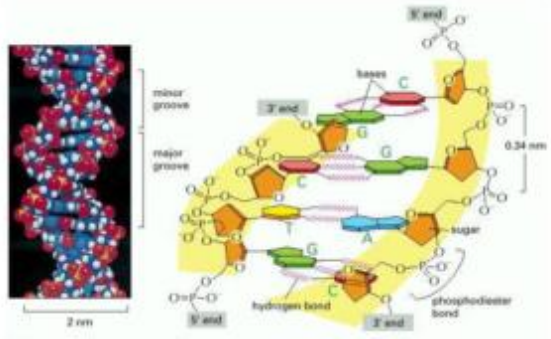
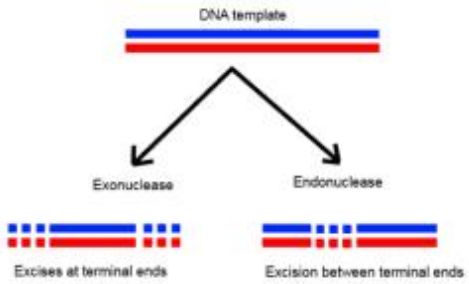
Genetic engineering

Restriction enzyme

What is endonuclease?

A restriction enzyme, restriction endonuclease, or restrictase is an enzyme that cleaves DNA into fragments at or near specific recognition sites within molecules known as **restriction sites. Endonucleases differ from Exonucleases**, which cleave the ends of recognition sequences instead of the middle (endo) portion. They found naturally in a wide variety of prokaryotes (Eubacteria and Archaea) and have an important tool for manipulating DNA.





Biological Roles:

1- Most bacteria use them as a defence against bacteriophages.
2- Restriction enzymes prevent the replication of the phage by cleaving its DNA at specific sites.
3- The host DNA is protected by Methylases which add methyl groups to adenine or cytosine bases within the recognition site thereby modifying the site and protecting the DNA.

Mechanism of Action:

These 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. The 3′OH and 5′ P are produced and Mg2+ is required as a cofactor for the catalytic activity of the enzyme.

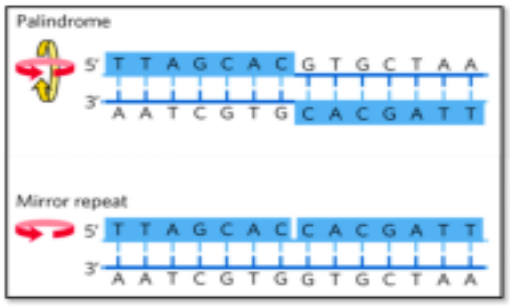
Recognition site

Restriction enzymes recognize a specific sequence of nucleotide and produce a double-stranded cut in the DNA. The recognition sequences can also be classified by the number of bases in its recognition site, usually between 4 and 8 bases. Many of them are palindromic, meaning the base sequence reads the same backwards and forwards. In theory, there are two

types of palindromic sequences that can be possible in DNA. **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. **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 doublestranded DNA), as in GTATAC (GTATAC being complementary toCATATG). Inverted repeat palindromes are more common and have greater biological importance than mirror-like palindromes.

Palindrome Sequences:

- **A palindrome** is a word, phrase, or sentence that is spelled identically read either forward or backward.
- This term is applied to regions of DNA with inverted repeats of base sequence having twofold symmetry over two strands of DNA.
- The mirror like palindrome in which the same forward and backwards are on a single strand of DNA strand, as in GTAATG.
- Inverted repeat palindromes are more common and have greater biological importance than mirror- like palindromes.



Ends of Restriction Fragments

Blunt ends Some restriction enzymes cut DNA at opposite base

- They leave blunt ended DNA fragments which can be joined to any other blunt ends' DNA fragment. These Enzymes are useful tools for certain types of DNA cloning experiments.

CCC|GGG
GGG|CCC

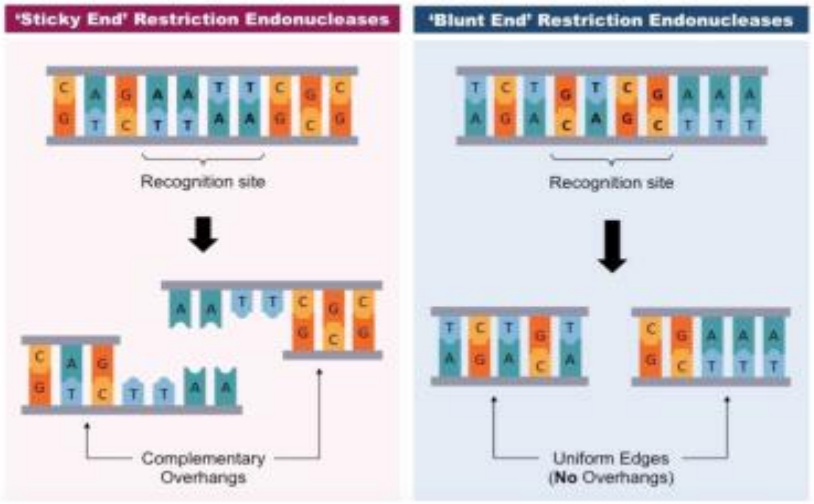
Sticky ends

-Most restriction enzymes make staggered cuts on the two DNA strands, leaving two to four nucleotides of one strand unpaired at each resulting end.

- Staggered cuts produce overhanging piece of single-stranded DNA, these products are called sticky ends or cohesive ends.

"**Sticky Ends"** Are Useful 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



Isoschizomers & Neoschizomers

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

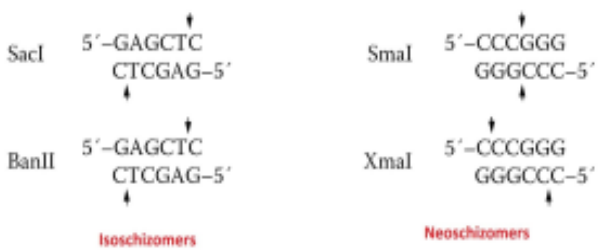
Neoschizomers are Restriction enzymes that have the same recognition sequence but cleave the DNA at a different site within that sequence.

Nomenclature of restriction enzyme

Each enzyme is named after the bacterium from which it was isolated using a naming system based on bacterial genus, species and strain.

For e.g. *Eco*RI

E	*Escherichia*	Genus
co	*coli*	Species
R	Ry 13	Strain
I	First identified	Order ID in bacterium



Examples:

***Haemophilus influenzae* D III = Hind III**

***Serratia marcescens* I= SmaI**

Types of restriction enzymes

There are four general groups of restriction endonucleases (I- IV), in which their classification according to:

1) Their composition.

2) Enzyme co-factor requirement.

3) the nature of their target sequence.

4) position of their DNA cleavage site relative to the target sequence.

Type I

• Capable of both restriction and modification activities

• The cofactors S-Adenosylmethionine (AdoMet), ATP, and Mg+ are required for their full activity

• Contain:

- two R (restriction) subunits
- two M (methylation) subunits
- one S (specificity) subunits

• Cleave DNA at random length from recognition sites

Type II

• These are the most commonly available and used restriction enzymes.

• They are usually composed of only one subunit.

• Their recognition sites are usually undivided and palindromic and 4-8 nucleotides in length.
• They generally recognize and cleave DNA at the same site.

• They do not use ATP for their activity.

• They usually require only Mg2+ as a cofactor.

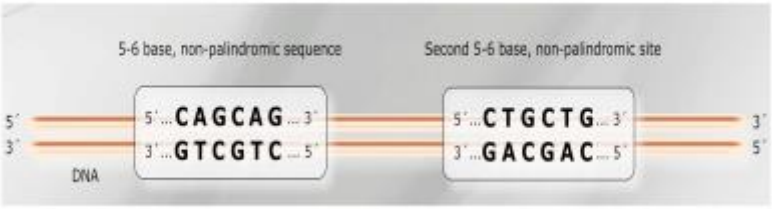
Type III

• recognize two separate non-palindromic sequences that are inversely oriented.

• They cut DNA about 20-30 base pairs after the recognition site.

• These enzymes contain more than one subunit.

• And require AdoMet and ATP cofactors for their roles in DNA methylation and restriction.



Type IV

• Cleave modified DNA (methylated, hydroxymethylated and glucosylhydroxymethylated bases).

• Recognition sequences have not been well defined.

• Cleavage takes place ~30 bp away from one of the sites.

Application of restriction enzymes

• They are used in gene cloning and protein expression experiments.

• 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.

What is RFLP?

Restriction fragment length polymorphism (RFLP) is a technique that exploits variations in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples.

This method involves the following steps:

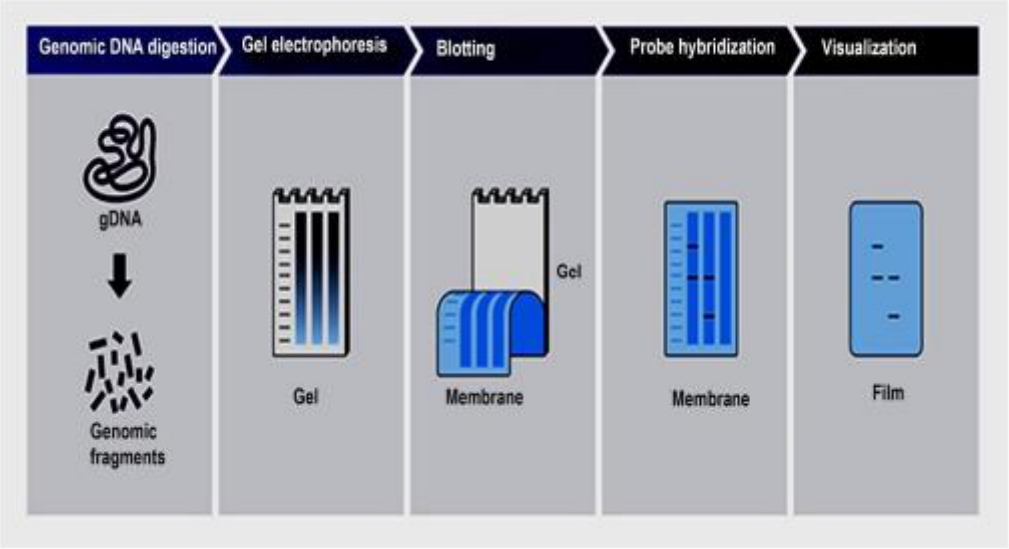
1. In the first step fragmentation of a sample of DNA is done by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction digest

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 labelled 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

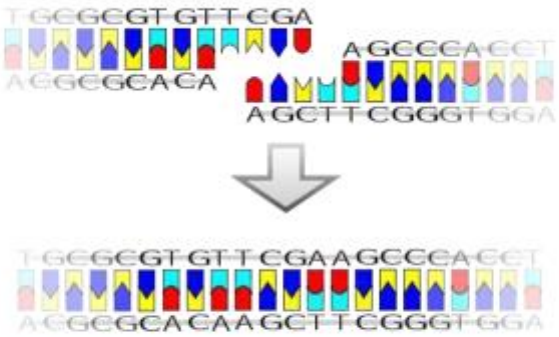


DNA ligase

DNA ligase is a specific type of enzyme that facilitates the joining of DNA strands together by catalysing the formation of a phosphodiester bond. It plays a role in repairing single-strand breaks in duplex DNA in living organisms. Single-strand breaks are repaired by DNA ligase using the complementary strand of the double helix as a template, with DNA ligase creating the final phosphodiester bond to fully repair the DNA.

DNA ligase is used in both (DNA repair and DNA replication). In addition, DNA ligase has extensive use in molecular biology laboratories for recombinant DNA experiments.

Purified DNA ligase is used in gene cloning to join DNA molecules together to form recombinant DNA.



A pictorial example of how a ligase works (with sticky ends)