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التقانة الحيوية
Biotechnology

انزيمات التقييد
Restriction Endonucleases

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Restriction Endonucleases

What are restriction enzymes?

- Molecular scissors that cut double stranded DNA molecules at specific points
- Found naturally in a wide variety of prokaryotes
- An important tool for manipulating DNA.

Biological Role

- Most bacteria use Restriction Enzymes as a defence against bacteriophages.
- Restriction enzymes prevent the replication of the phage by cleaving its DNA at specific sites.
- 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.

History of Restriction Enzyme

- First restriction enzyme isolated was *Hin*dll in 1970 by Hamilton Smith.
- He also done the subsequent discovery and characterization of numerous restriction endonucleases.
- From then more than 3000 restriction enzymes have been studied in detail, and more than 600 of these are available commercially and are routinely used for DNA modification and manipulation in laboratories.

Mechanism of Action

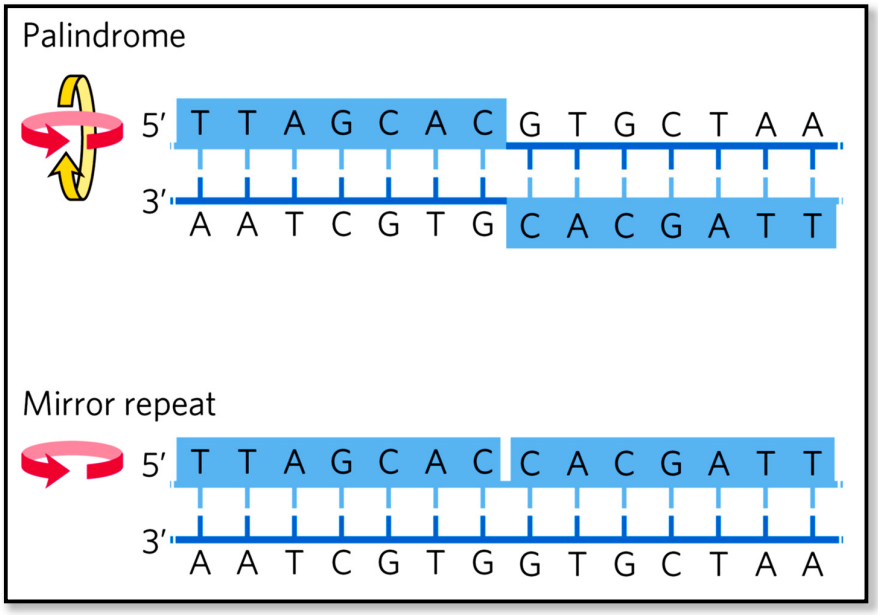
- Restriction Endonuclease scan the length of the DNA, binds to the DNA molecule when it recognizes a specific sequence and makes one cut in each of the sugar phosphate backbones of the double helix – by

hydrolyzing the phosphodiester bond. Specifically, the bond between the 3' O atom and the P atom is broken.

- 3'OH and 5' P is produced. Mg^{2+} is required as a cofactor for the catalytic activity of the enzyme.

Palindrome Sequences

- A **palindrome** is a word, phrase, or sentence that is spelled identically read either forward or backward.
- The 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.

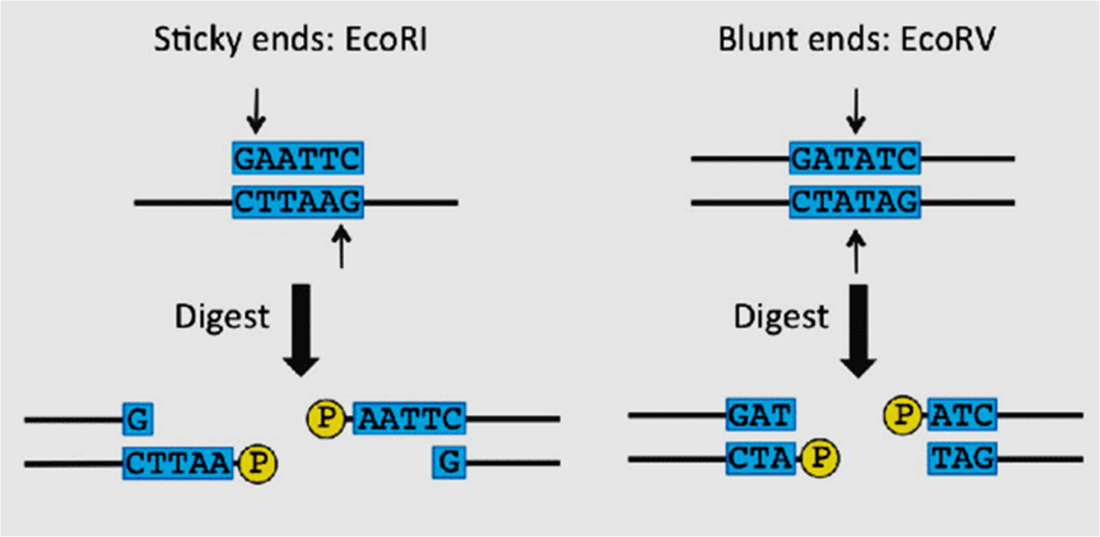


Ends of Restriction Fragments

Blunt ends

- Some restriction enzymes cut DNA at opposite base

- They leave blunt ended DNA fragments
- 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 ends

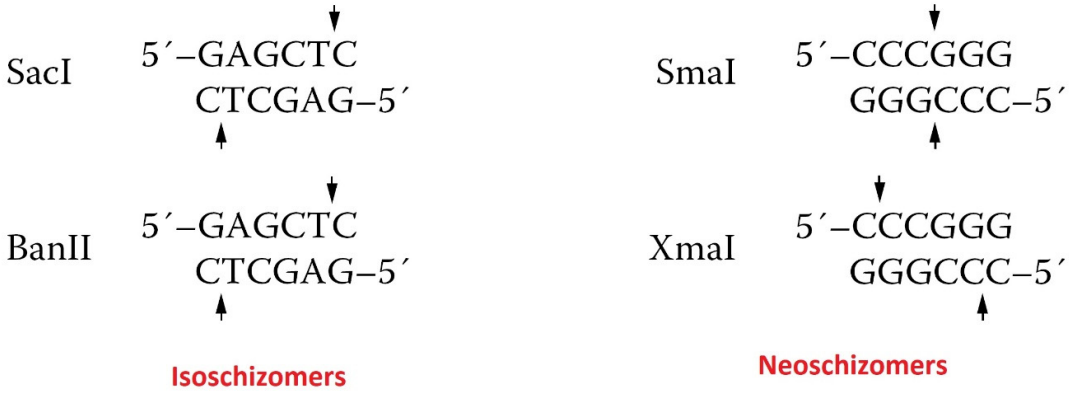
- ✓ Most restriction enzymes make staggered cuts on the two DNA strands, leaving two to four nucleotides of one strand unpaired at each resulting end.
- ✓ Staggered cuts produce overhanging piece of single-stranded DNA, these products are called sticky ends or cohesive ends.

“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.

ISOSCHIZOMERS & NEOSCHIZOMERS

- 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 Neoschizomers



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**

TYPES OF RESTRICTION ENZYMES

Restriction endonucleases are categorized into four general groups.

- Type I
- Type II
- Type III

These types are categorization based on:

- Their composition.
- Enzyme co-factor requirement.
- the nature of their target sequence.
- 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

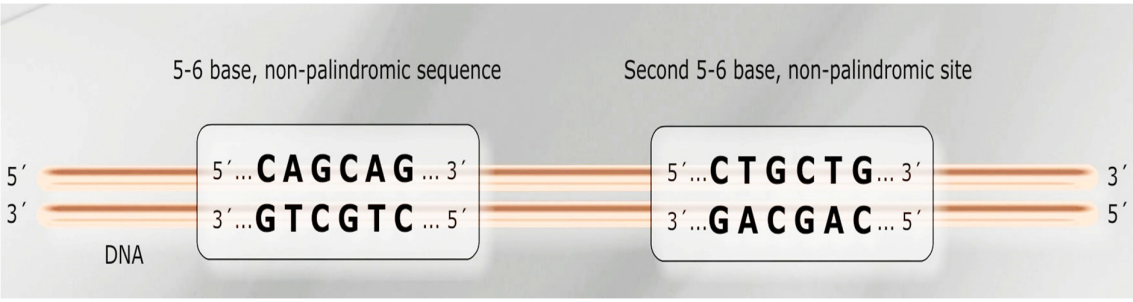
Type II

- 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 Mg^{2+} as a cofactor.

Type III

- Type III restriction enzymes, 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.
- ✓ 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.

What is RFLP

RFLP is a difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples.

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

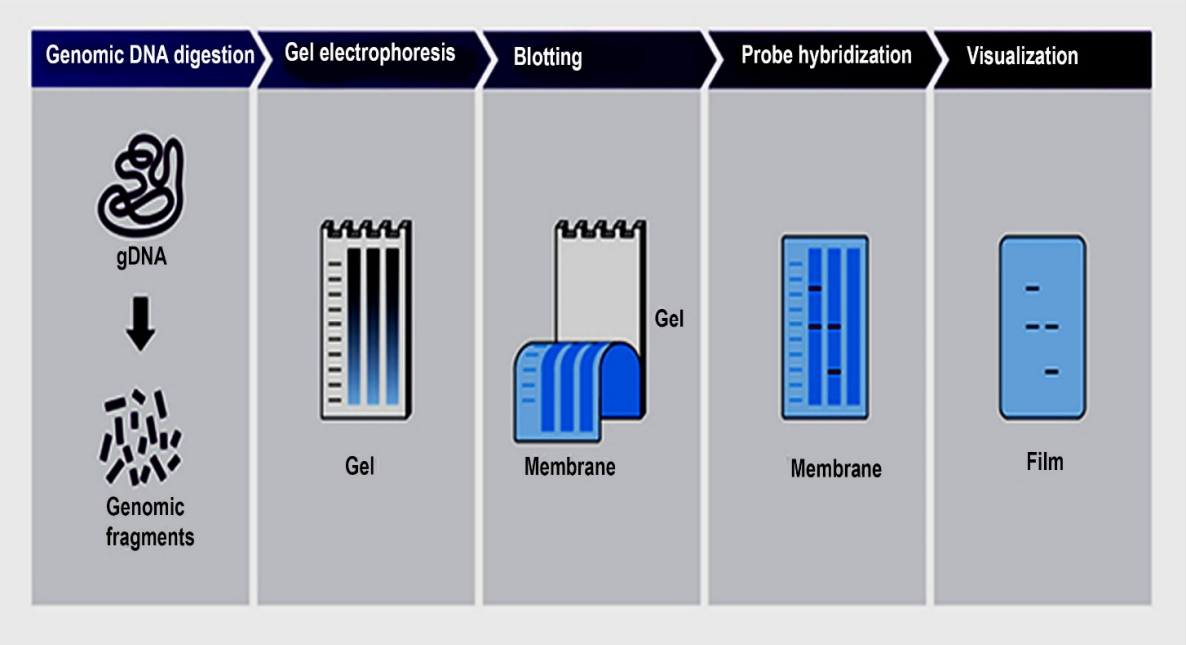
2- The resulting DNA fragments are then separated by length through a process known as agarose gel electrophoresis.

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

4- Hybridization of the membrane to a 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



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