GENETIC ENGINEERING

College of Science/ biology department
fourth class
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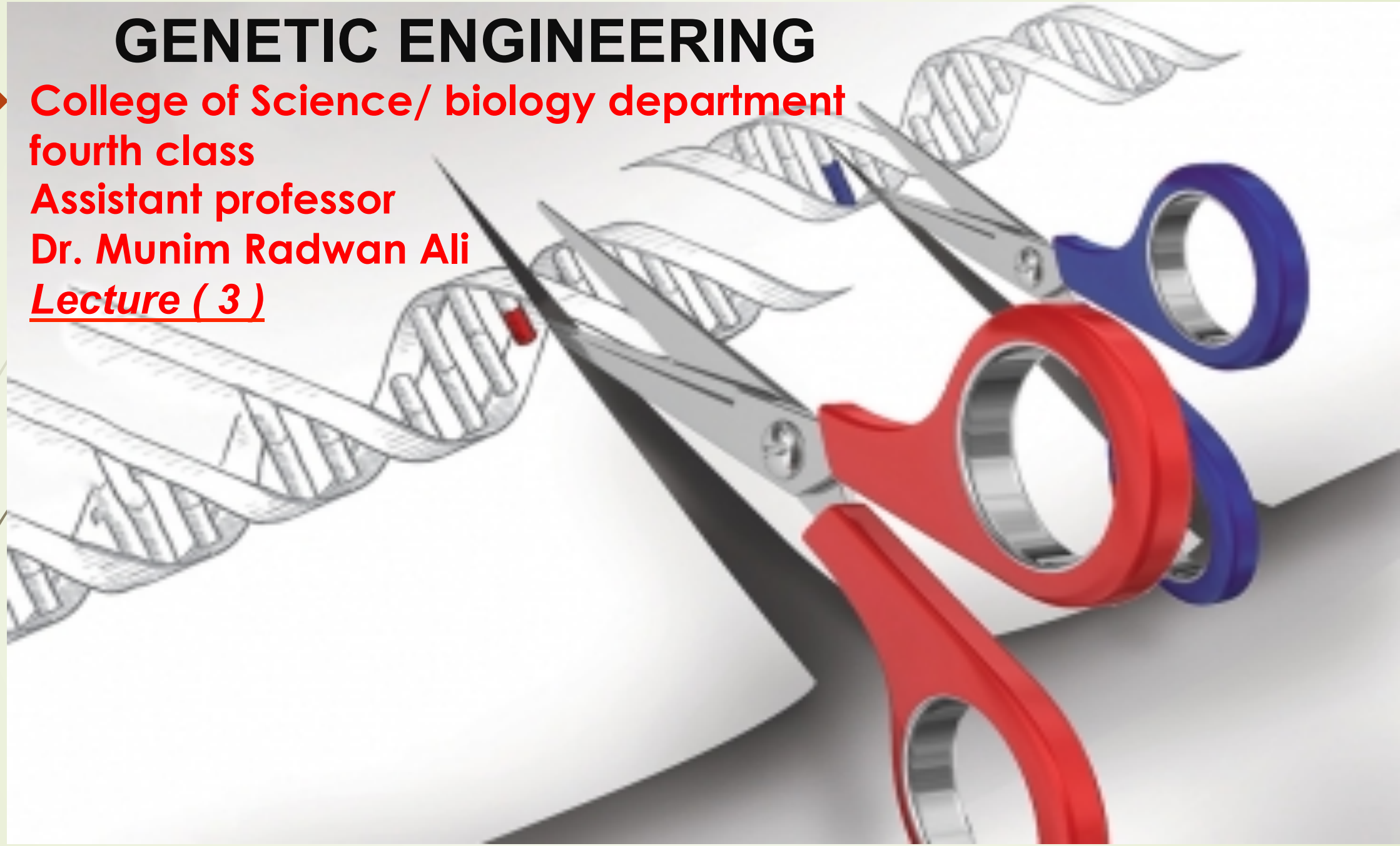
Lecture (3)



Restriction enzymes – cutting DNA

- The restriction enzymes, which cut DNA at defined sites.
- represent one of the most important groups of enzymes for the manipulation of DNA.
- These enzymes are found in bacterial cells, where they function as part of a protective mechanism called the restriction-modification system. In this system the restriction enzyme hydrolyses any exogenous DNA that appears in the cell.
- To prevent the enzyme acting on Restriction enzymes act as a ‘protection’ system for bacteria in that they hydrolyse exogenous DNA that is not methylated by the host modification enzyme. the host cell DNA, the modification enzyme of the system (a methylase) modifies the host DNA by methylation of particular bases in the restriction enzyme’s recognition sequence, which prevents the enzyme from cutting the DNA.

Restriction&Modification system

Immune system

- **Restrict**
- **Modifying**

Restriction-Modification (R-M) systems in *E. coli* evolved to protect bacteria from invading foreign DNA such as bacteriophage genomes. These R-M systems can be split into two broad classes.

1. *Those that protect bacterial DNA from restriction (degradation) by modification (methylation) of specific sequences that are recognized by the corresponding restriction enzymes of the R-M system.*
2. *Those that only cut DNA bearing foreign modifications. The host DNA is then protected from cleavage by the restriction enzymes of this system by virtue of it not being modified.*

3.1 Host-controlled restriction and modification

bacteria

Function of the restriction systems

- to monitor the origin of incoming DNA
- to destroy the foreigner, if it is recognized

Function of restriction endonucleases

- recognize specific sequences in the incoming DNA
- cleave the DNA into fragments, either at specific sites or more randomly.

3.1.1 Self-protection problem

The restrictive host must protect its own DNA from being broken down by the restriction endonuclease

How to?

its DNA be appropriately modified.

⇩

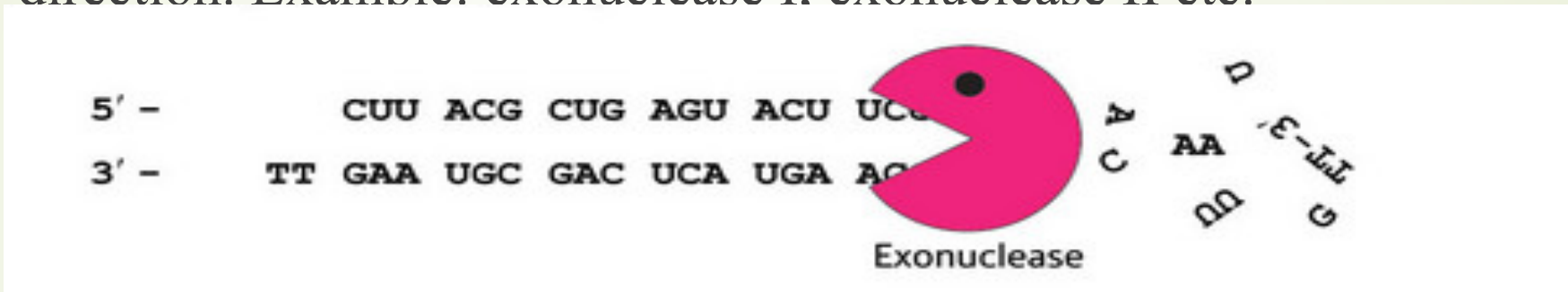
make methylation of certain bases

at a very limited number of sequences within DNA

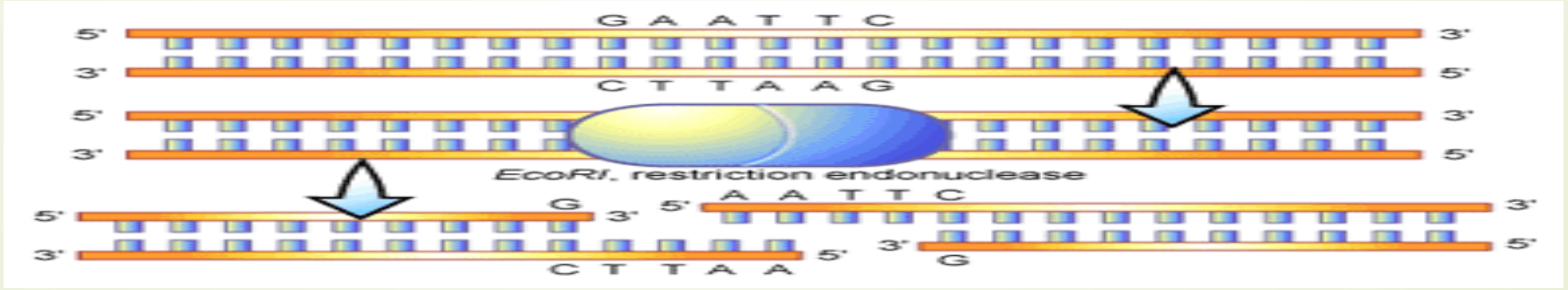
which constitute the recognition sequences of the restriction endonuclease.

Kinds of restriction enzymes

- (1) Exonucleases catalyses hydrolysis of terminal nucleotides from the end of DNA or RNA molecule either 5'to 3' direction or 3' to 5' direction. Example: exonuclease I, exonuclease II etc.



- (2) Endonucleases can recognize specific base sequence (restriction site) within DNA or RNA molecule and cleave internal phosphodiester bonds within a DNA molecule. Example: EcoRI, Hind III, BamHI etc.



Types of restriction endonucleases

- **Type I restriction endonucleases** are complex endonucleases, and have recognition sequences of about 15 bp; they cleave the DNA about 1000 bp away from the 5′-end of the sequence "TCA" located within the recognition site, e.g., Eco K, Eco B, etc.
- **Type II restriction endonucleases** are remarkably stable and induce cleavage either, in most cases, within or immediately outside their recognition sequences, which are symmetrical. More than 350 different type II endonucleases with over 100 different recognition sequences are known. They require Mg^{2+} ions for cleavage. The first type II enzyme to be isolated was HindII in 1970. Only type II restriction endonucleases are used for restriction mapping and gene cloning in view of their cleavage only at specific sites.
- **Type III restriction endonucleases** are intermediate between the type I and type II enzymes; they cleave DNA in the immediate vicinity of their recognition sites, e.g., EcoP1, EcoP15, HinfIII, etc. Type 1 and Type III restriction enzymes are not used in gene cloning. The Type III enzymes recognize asymmetric target sites, and cleave the DNA duplex on one side of the recognition sequence up to 20 bp away.

3.2.2 Three varieties of restriction endonucleases

❖ Type Ⅰ

- cleavage site is located more than 1,000 bp away from the recognition site
- cleavage does not occur at a specific sequence

❖ Type Ⅱ ✓

- the recognition sites are short (4-5 bp) and often palindromic.
- Require no ATP for restriction.

❖ TypeⅢ

- have 2 subunits, one for recognition and methylation and the other for restriction.
- Cleavage occurs 24-26 bp downstream from the recognition site.

Major classes of restriction endonucleases

There are three major classes of restriction endonucleases: Their grouping is based on:

1. the types of sequences recognized.
2. the nature of the cut made in the DNA.
3. the enzyme structure.

Type I and III restriction endonucleases are not useful for gene cloning because they cleave DNA at sites other than the recognition sites and thus cause random cleavage patterns.

In contrast, **type II endonucleases** are widely used for mapping and reconstructing DNA *in vitro* because they recognize specific sites and cleave just at these sites .In addition, the type II endonuclease and methylase activities are usually separate, single subunit enzymes. Although the two enzymes recognize the same target sequence, they can be purified separately from each other.

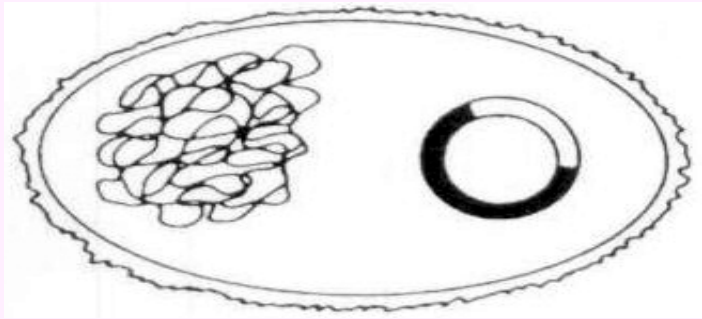
3.2.3 Nomenclature of Restriction endonucleases

The enzyme are named by the binomial nomenclature (genus and species).

- **First letter (uppercase) represented the genus ,**
- **Second and third letters (lowercase) are the first two letters of the species**
- **Roman numerals represent different R-M systems,**

Ex: *HpaI* and *HpaII* represent the first and second type II restriction enzymes isolated from *Haemophilus parainfluenzae*.

Nomenclature



*Eco*RI

Eschreichia *coli* *strain* *Extra-chromosomal element*

- Another example:

  *Bam*HI from *Bacillus amyloliquefaciens.*

Enzyme Activity

Scanning

Recognition Sequence

Cleavage

GGACGCTAGCTGATG
CCTGCGATCGACTACTTAA

AATTCGCATCGGATCCGAATCCGCTCTTTCAA
GCGTAGCCTAGGCTTAGGCGAGAAAGTT

3.2.1 Restriction enzymes ′s properties (2)

The enzymes act like scissors, cut DNA at specific sites (recognition sites)

1. cut DNA into fragments of a size suitable for cloning.

2. many restriction enzymes make staggered cuts

- The nucleotide sequence at these sites is palindromic
- consists of 4, 5, 6, or 8 nucleotide pairs.
- Blunt or sticky ends.

- The sticky ends be re-annealed through complementary binding with the T4 DNA ligase

Pay attention (3)

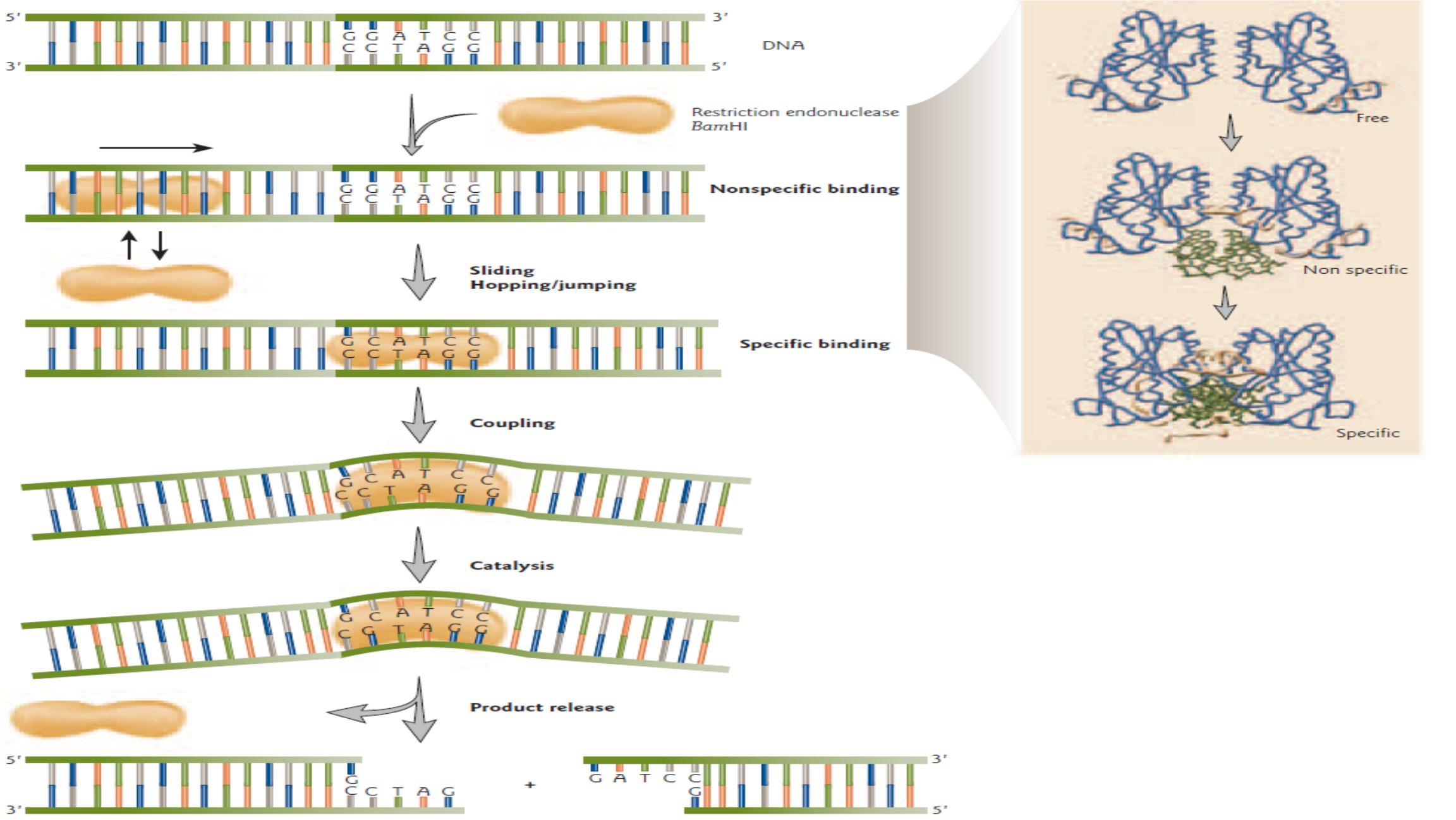
most organisms have not restriction enzymes.

- restriction enzymes cut at specific DNA target sequences, not randomly;

is one of the key features that make them suitable for DNA manipulation.

- Any DNA molecule (from viral to human) , contains very few restriction-enzyme target sites

may be cut into defined fragments of a size suitable for cloning


5′
3′
3′
5′
DNA
Restriction endonuclease
BamHI
Nonspecific binding
Sliding
Hopping/jumping
Specific binding
Coupling
Catalysis
Product release
5′
3′
+
3′
5′
Free
Non specific
Specific

Type Ⅱ /three different ends

5 ' overhang sticky end

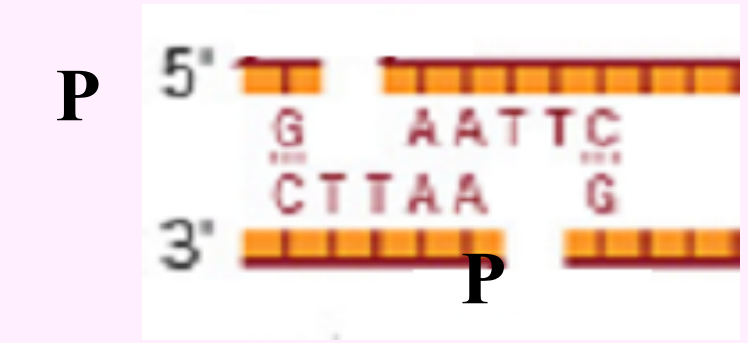
***Eco*R I**

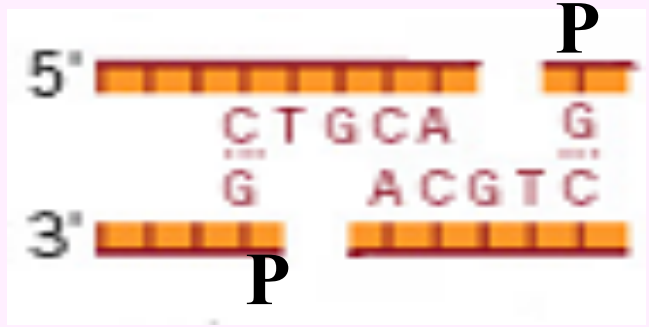
3' overhang sticky end

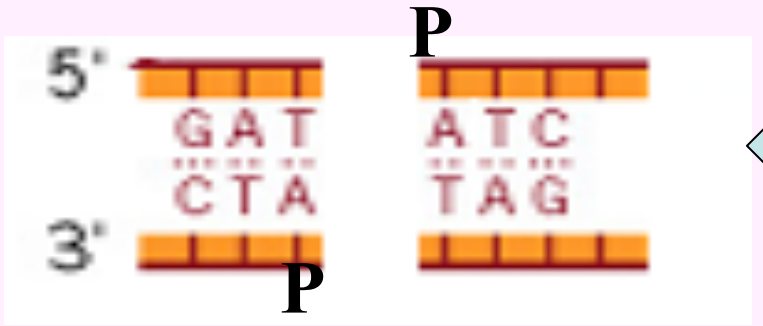
***Pst* I**

Blunt end

***Eco*R V**







imPerfect Isoschizomers

Perfect Isoschizomers

an example:

the restriction enzyme *Eco*RI (from *E. coli*) recognizes six-nucleotide-pair sequence "GAATTC" in the DNA

"sticky ends"

(stick)

they can <u>hydrogen bond</u> to a <u>complementary sequence</u>.

- The enzyme *Eco*RI cuts within this sequence
- result in a pair of staggered cuts between the G and the A nucleotides.

In DNA technology

two different DNA molecules are cut with the same restriction enzyme,

both produce fragments with the same complementary sticky ends

form DNA chimeras[kaimiere]

There are cases where two different restriction enzymes recognize the same target sequence, but one of them is able to recognize both methylated as well non-methylated target sequences, while the other enzyme can recognize only the non-methylated target sequence; such enzymes are known as isoschizomers.

For example, restriction enzymes HpaII and MspI

5'CCGG3'

methylated

HpaII can no longer recognize it, while MspI recognizes it just as well as it does the unmethylated sequence

Frequency of cutting of recognition enzymes

Sau 3A (GATC) cuts (¼)(¼)(¼)(¼) = once every 256 base pairs
(assuming G/C = A/T, which is often does not)

*Bam*H1 (GGATCC) cuts (¼)(¼)(¼)(¼)(¼)(¼) = once every ~4Kb

$$P=(1/4)^{n}$$

P:Cutting Probability
4:represent the essential fore bases
N : number of base paring belong to recognition sequence

Restriction mapping:

Most pieces of DNA will have recognition sites for various restriction enzymes, and it is often beneficial to know the relative locations of some of these sites. The technique used to obtain this information is known as restriction mapping. This involves cutting a DNA fragment with a selection of restriction enzymes, singly and in various combinations. The fragments produced are run on an agarose gel and their sizes determined. From the data obtained, the relative locations of the cutting sites can be worked out. A fairly simple example can be used to illustrate the technique, as outlined in the following.

Table 4.2. Digestion of a 15 kbp DNA fragment with three restriction enzymes

*Bam*HI	*Eco*RI	*Pst*I	*Bam*HI + *Eco*RI	*Bam*HI + *Pst*I	*Eco*RI + *Pst*I	*Bam*HI + *Eco*RI + *Pst*I
14	12	8	11	8	7	6
1	3	7	3	6	5	5
			1	1	3	3
						1

Note: Data shown are lengths (in kbp) of fragments that are produced on digestion of a 15 kbp DNA fragment with the enzymes *Bam*HI, *Eco*RI, and *Pst*I. Single, double, and triple digests were carried out as indicated. Fragments produced by each digest are listed in order of length.

Applications

In various applications related to genetic engineering DNA is cleaved by using these restriction enzymes.

- They are used in the process of insertion of genes into plasmid vectors during gene cloning and protein expression experiments.
- Restriction enzymes can also be used to distinguish gene alleles by specifically recognizing single base changes in DNA known as single nucleotide polymorphisms (SNPs). This is only possible if a mutation alters the restriction site present in the allele.
- Restriction enzymes are used for Restriction Fragment Length Polymorphism (RFLP) analysis for identifying individuals or strains of a particular species.