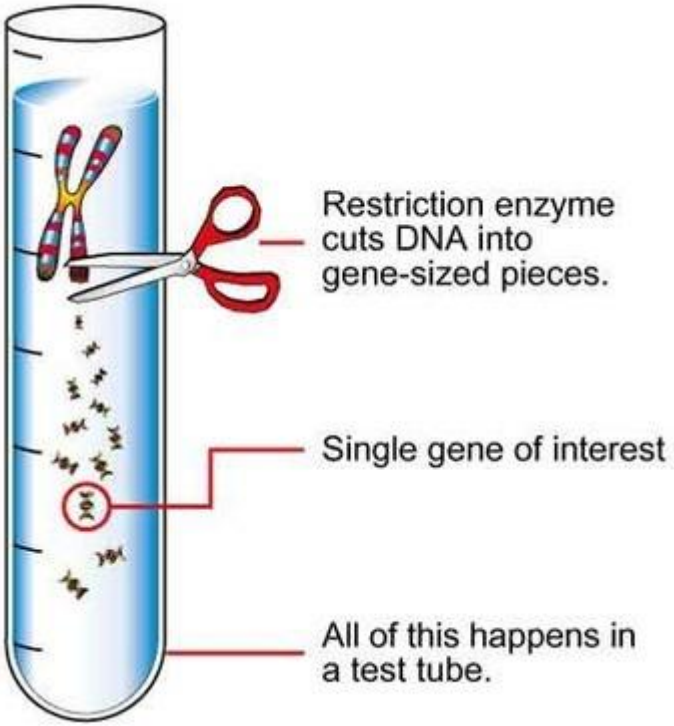
RESTRICTION ENZYMES



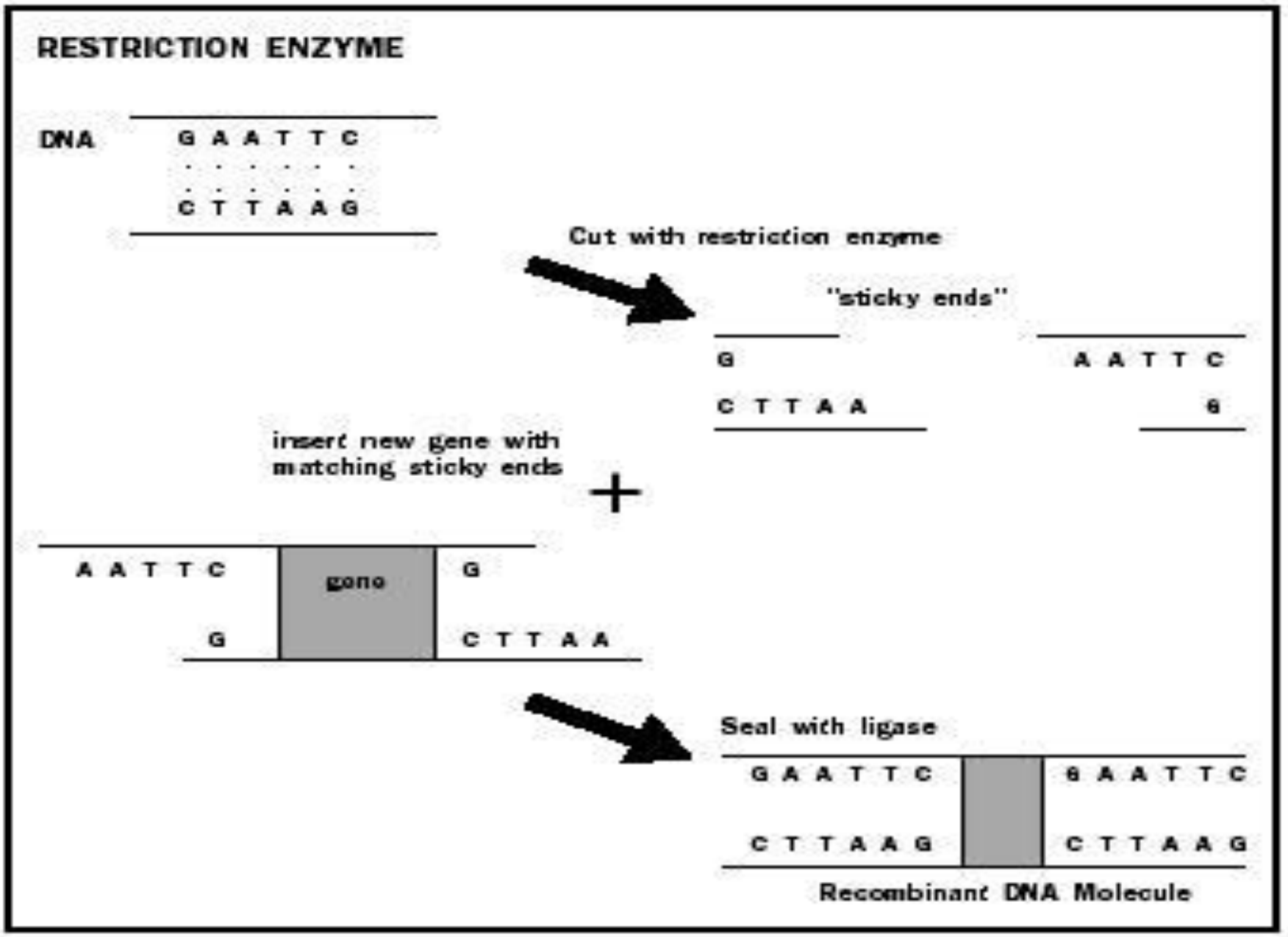
SAYANTI KAR

Why Restriction enzymes

Gene cloning requires that DNA molecules be cut in a very precise manner.

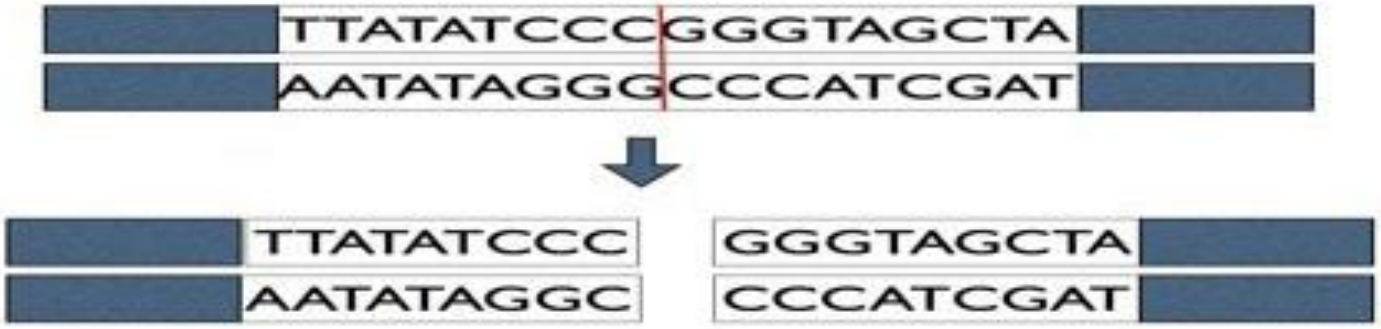
During construction of rDNA molecule, each vector must be cleaved in a single position.

Random cleavage is not satisfactory.


RESTRICTION ENZYME
DNA
G A A T T C
C T T A A G
Cut with restriction enzyme
"sticky ends"
G
A A T T C
C T T A A
G
insert new gene with matching sticky ends
+
A A T T C
G
gene
G
C T T A A
Seal with ligase
G A A T T C
C T T A A G
G A A T T C
C T T A A G
Recombinant DNA Molecule

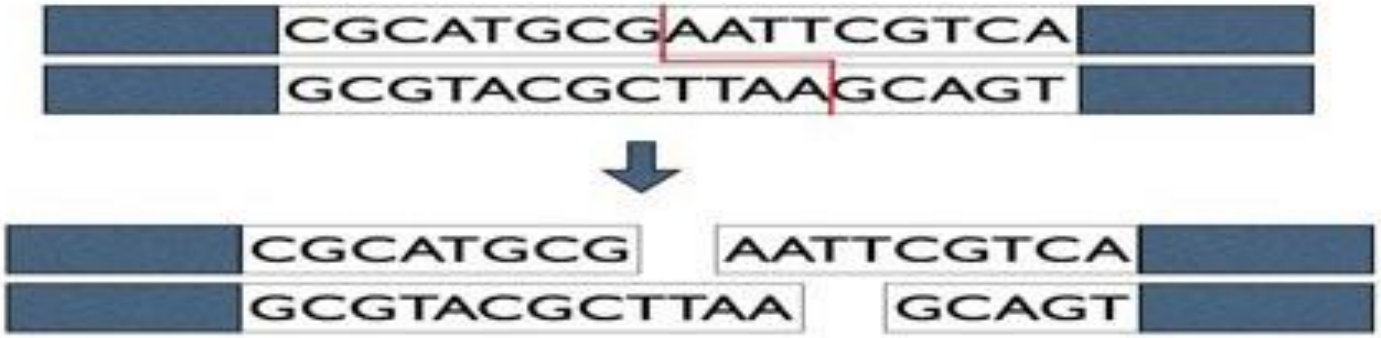
SmaI enzyme

Target Sequence: CCCGGG
End type: blunt

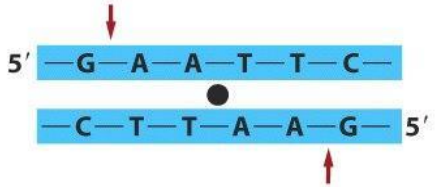
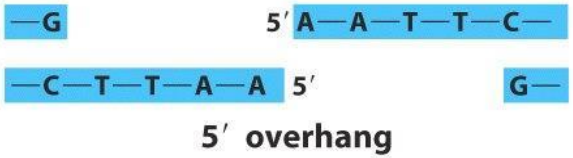
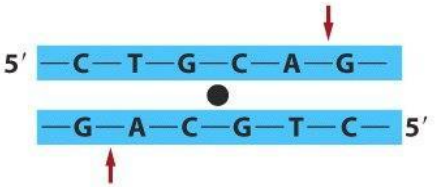
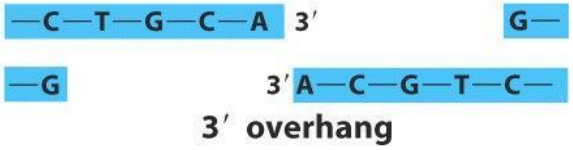
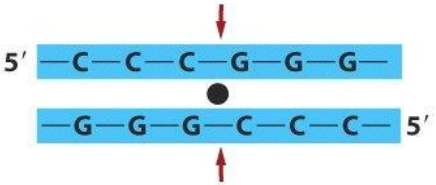
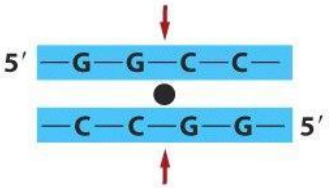
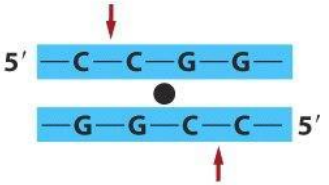


EcoRI enzyme

Target Sequence: GAATTC
End type: sticky



Some restriction enzymes

Enzyme	Source organism	Restriction recognition site in double-stranded DNA	Structure of the cleaved products
(a)			
*Eco*RI	*Escherichia coli*		
*Pst*I	*Providencia stuartii*		
*Sma*I	*Serratia marcescens*		
(b)			
*Hae*III	*Haemophilus aegyptius*		
*Hpa*II	*Haemophilus parainfluenzae*		

Restriction Enzymes

Restriction enzyme (or restriction endonuclease) is an enzyme that cuts DNA at specific recognition nucleotide sequences known as restriction sites.

Restriction sites, or restriction recognition sites, are locations on a DNA molecule containing specific (4-8 base pairs in length) sequences of nucleotides, which are recognized by restriction enzymes.

The discovery of RE

Host control restriction
(some strain of bacteria are immune to bacteriophage infection)

Restriction enzymes were discovered 40 years ago during investigations into the phenomenon of host-specific restriction and modification of bacterial viruses.

Restriction enzymes protect bacteria from infections by viruses, and it is generally accepted that this is their role in nature. They function as microbial immune systems.

Bacterial host defence

In addition, adenosine or cytosine methylation is part of the restriction modification system of many bacteria. Bacterial DNAs are methylated periodically throughout the genome.

A methylase is the enzyme that recognizes a specific sequence and methylates one of the bases in or near that sequence.

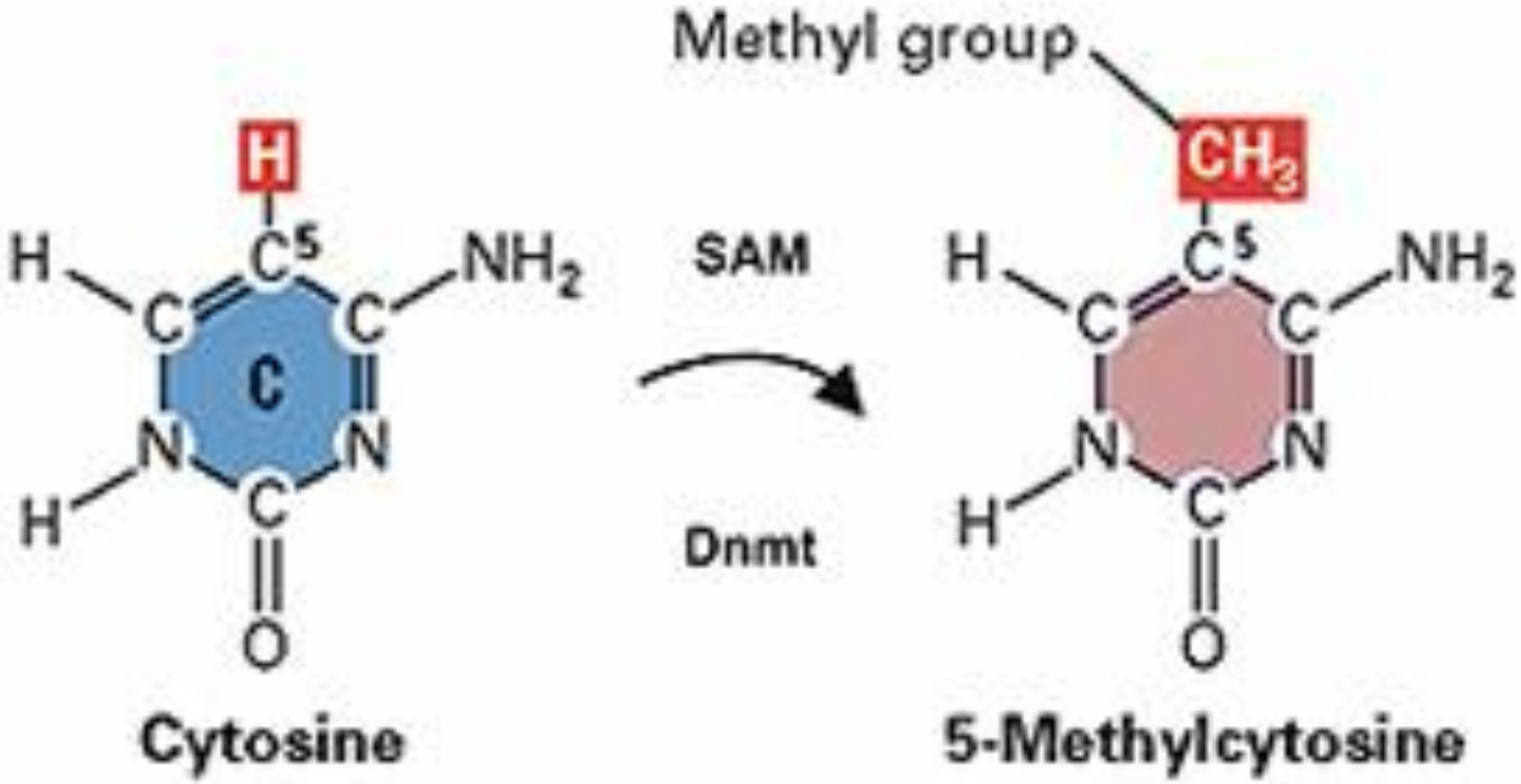
Foreign DNAs (which are not methylated in this manner) that are introduced into the cell are degraded by sequence-specific restriction enzymes. Bacterial genomic DNA is not recognized by these restriction enzymes. The methylation of native DNA acts as a sort of primitive immune system, allowing the bacteria to protect themselves from infection by bacteriophage.

DNA methylation is a biochemical process that is important for normal development in living organisms. It involves the addition of a methyl group to the 5 positionof the cytosine pyrimidine ring or the number 6 nitrogen of the adenine purine ring

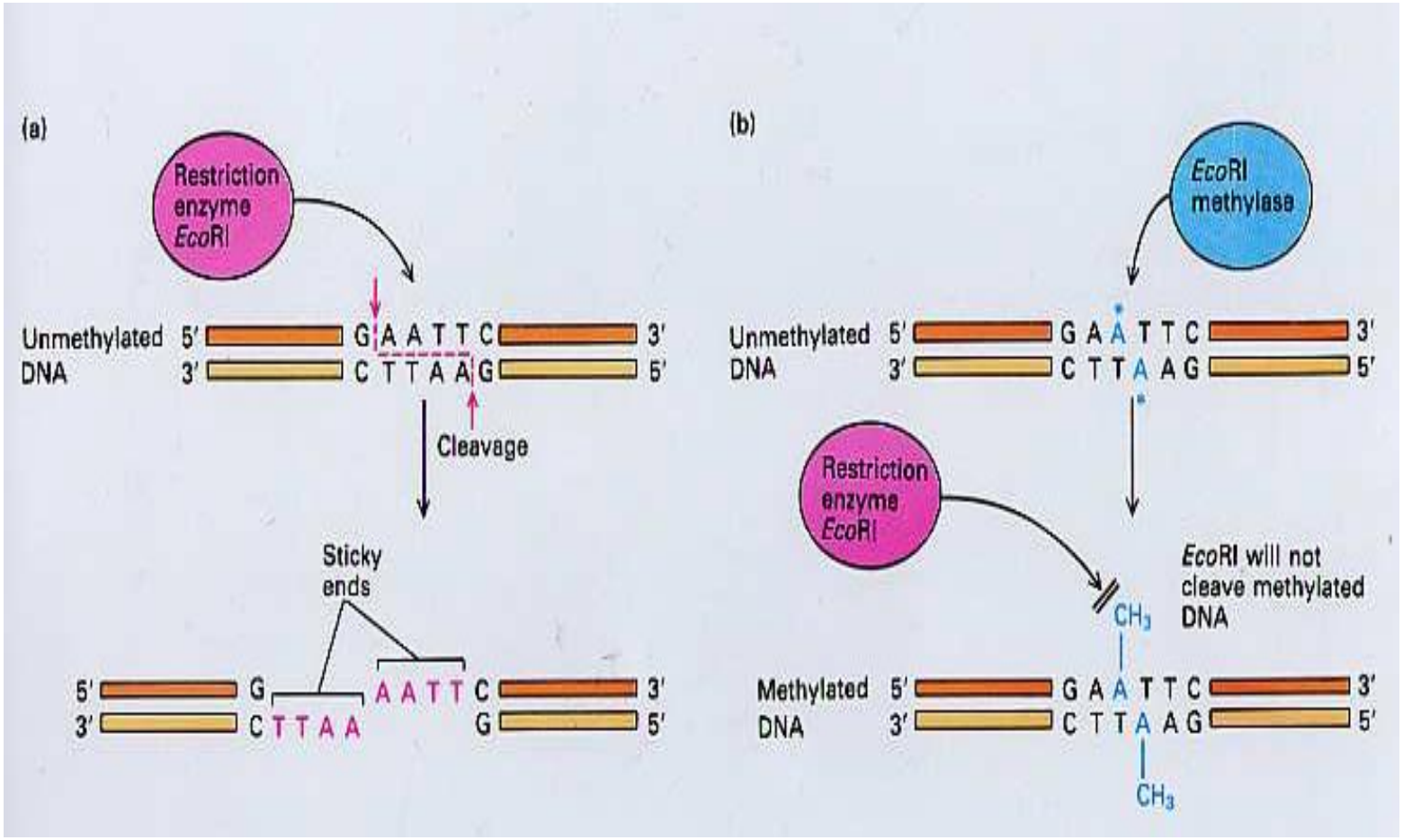
In bacteria

Adenine or cytosine methylation is part of the restriction modification system of many bacteria, in which specific DNA sequences are methylated periodically throughout the genome.

A methylase is the enzyme that recognizes a specific sequence and methylates one of the bases in or near that sequence. Foreign DNAs (which are not methylated in this manner) that are introduced into the cell are degraded by sequence-specific restriction enzymes and cleaved. Bacterial genomic DNA is not recognized by these restriction enzymes.


Methyl group
H
CH3
H
C5
NH2
C
C
C
N
N
H
C
O
SAM
Dnmt
Cytosine
5-Methylcytosine

EcoRI restriction endonuclease-methylase system (type II)



	Restriction Enzyme	Organism	Recognition sequence	Cut Site	Blunt or Sticky end
1	*Eco* RI	*Escherichia coli*	5'GAATTC 3'CTTAAG	5'---G▼AATTC---3' 3'---CTTAA▲G---5'	Sticky
2	*Bam*HI	*Bacillus amyloliquefaciens*	5'GGATCC 3'CCTAGG	5'---G▼GATCC---3' 3'---CCTAG▲G---5'	Sticky
3	*Bgl*II	*Bacillus globigii*	5'AGATCT 3'TCTAGA	5'---A▼GATCT---3' 3'---TCTAG▲A---5'	Sticky
4	*Pvu*II	*Proteus vulgaris*	5' CAGCTG 3' GTCGAC	5'---CAG▼CTG---3' 3'---GTC▲GAC---5'	Blunt
5	*Hind*III	*Haemophilus influenzae Rd*	5'AAGCTT 3'TTCGAA	5'---A▼AGCTT---3' 3'---TTCGA▲A---5'	Sticky
6	*Sau*3A	*Staphylococcus aureus*	5'GATC 3'CTAG	5'---▼GATC---3' 3'---CTAG▲---5'	Sticky
7	*Alu*I	*Arthrobacter luteus*	5'AGCT 3'TCGA	5'---AG▼CT---3' 3'---TC▲GA---5'	Blunt
8	*Taq*I	*Thermus aquaticus*	5'TCGA 3'AGCT	5'---T▼CGA---3' 3'---AGC▲T---5'	Sticky
9	*Hae*III	*Haemophilus aegyptius*	5'GGCC 3'CCGG	5'---GG▼CC---3' 3'---CC▲GG---5'	Blunt
10	*Not*I	*Nocardia otitidis-caviarum*	5'GCGGCCGC 3'CGCCGGCG	5'---GC▼GGCCGC---3' 3'---CGCCGG▲CG---5'	Sticky

Type I enzymes are complex, multisubunit, combination restriction-and-modification enzymes that cut DNA at random far from their recognition sequences. ATP, SAM (S-adenosylmethione) and Mg2+ required.

Type II enzymes cut DNA at defined positions close to or within their recognition sequences. Type II enzymes and their corresponding modification methyltransferases act as separate proteins.

Type III enzymes :- Like Class I enzymes, Type III enzymes possess both restriction and modification activities.They recognize specific sequences and cleave 25 - 27 base pairs outside of the recognition sequence, in a 3´ direction. They require Mg2+ ions for their activity.

	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

Isoschizomers are pairs of restriction enzymes specific to the same recognition sequence. For example, Sph I (CGTAC/G) and Bbu I (CGTAC/G) are isoschizomers of each other.

Isoschizomers are isolated from different strains of bacteria and therefore may require different reaction conditions.

Neoschizomer

An enzyme that recognizes the same sequence but cuts it differently is a neoschizomer. Neoschizomers are a specific type (subset) of Isoschizomers. For example, Sma I (CCC/GGG) and Xma I (C/CCGGG) are neoschizomers of each other.