Restriction enzymes

- The restriction enzymes cut DNA at defined sites,
- represent one of the most important groups of enzymes for manipulation of DNA
- They work as a protective mechanism known as the restriction- modification system

Hydrolyse or breakdown any exogenous DNA enters the cells.

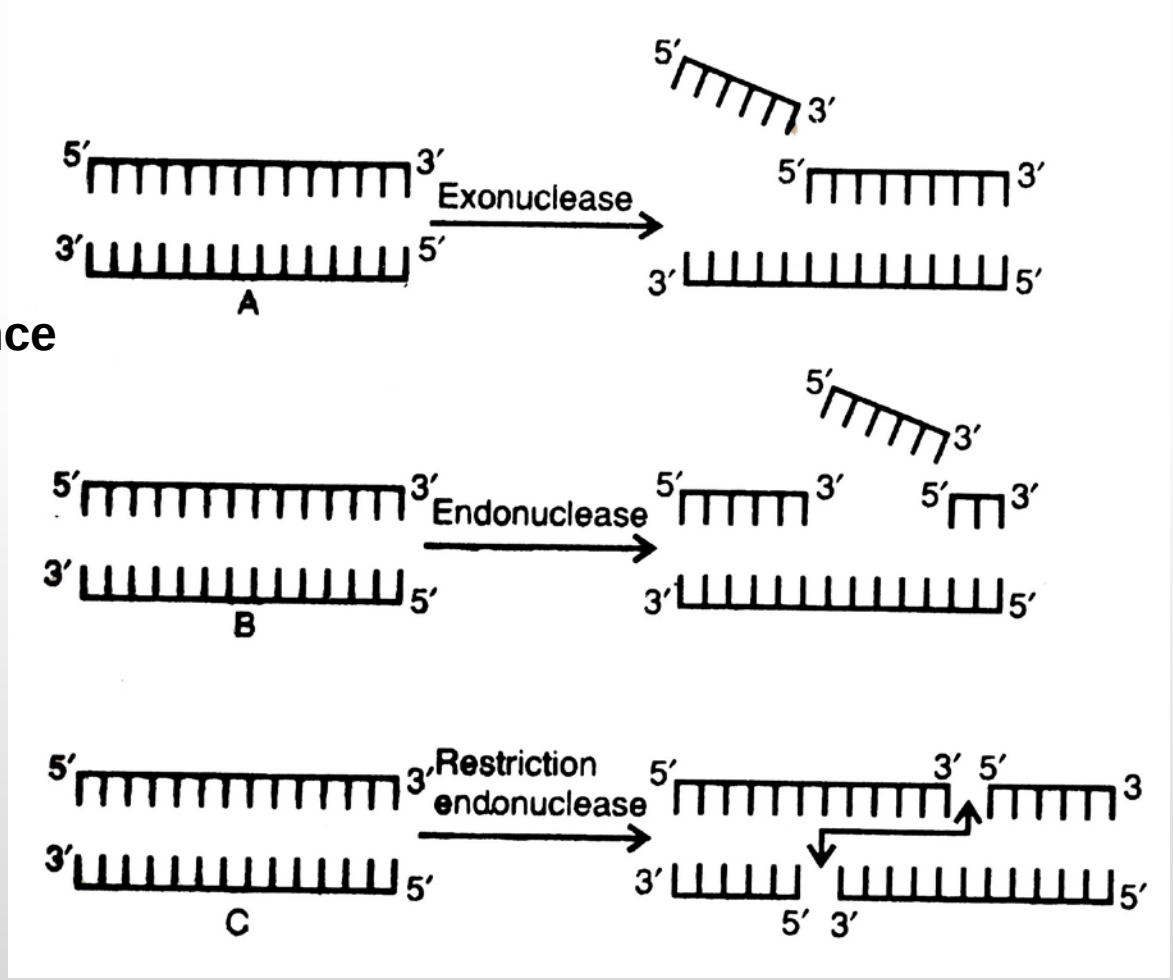
- The host DNA is protected from these enzymes by methylation of specific

bases in the restriction site. Therefore, These enzymes cut non-methylated DNA (exogenous)

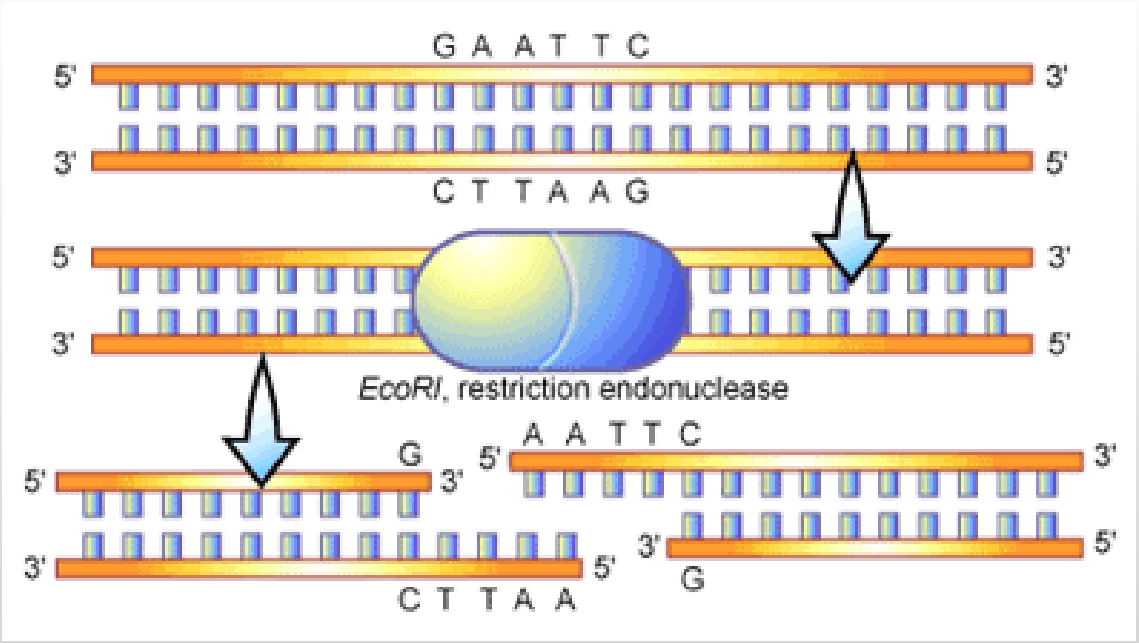
Bacteria found to be resistant to some bacteriophage (bacterial virus). Restriction enzymes would cut viral DNA, not its own genome.

Types of restriction enzymes

1. **Exonucleases :catalyses hydrolysis of terminal nucleotides from the end of DNA or RNA**



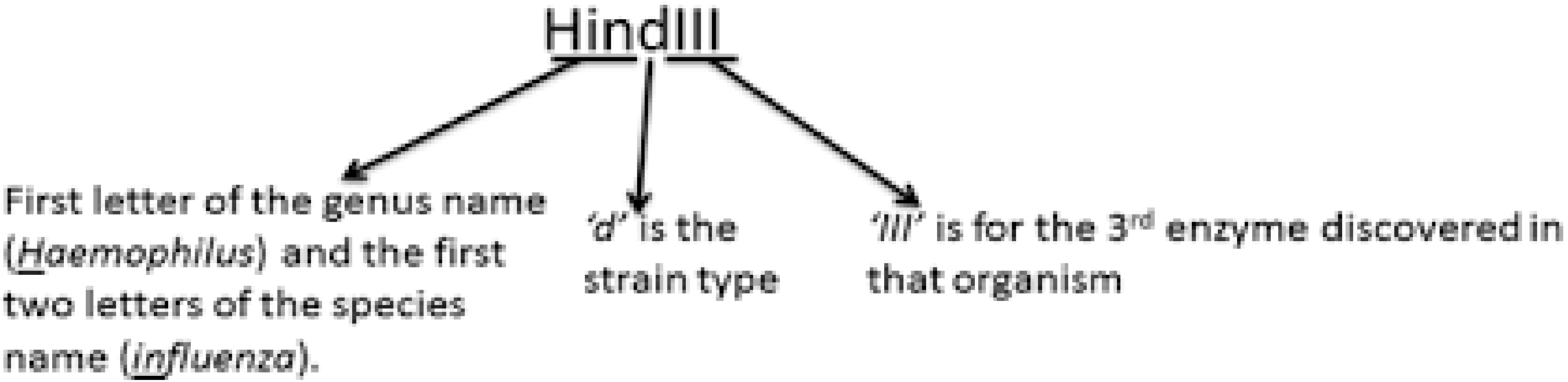
2. **Endonucleases : Recognise specific base sequence**



Types of restriction endonucleases

	Cleavage site	Location of methylase	Examples
Type I	Random, Recognition site is of 15bp in length Methylate A* in rec site Cleavage site is around 1000bp away from recognition site	Endonuclease and methylase located on a single multifunctional protein molecule Require Mg++, ATP and S-adenocyle methionine as cofactor	*EcoK I* *EcoA I* *CfrA I*
Type II	Specific palindromic sequences Within the recognition site	Simple enzymes of single polypeptide, Endonuclease and methylase are separate entities Very stable and require only Mg++ as cofactor	*EcoR I* *BamH I* *Hind III*
Type III	Random, non-palindromic sequences 24-26 bp downstream of the recognition site	Endonuclease and methylase located on a single protein molecule Require Mg++ & ATP as cofactor	*EcoP I* *Hinf III* *EcoP15 I*

Restriction endonucleases nomenclature



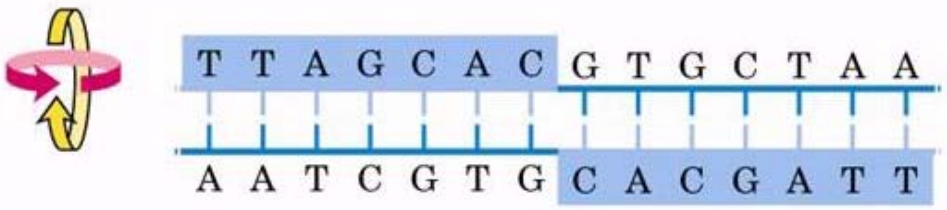
Restriction enzyme nomenclature

Why the funny names?

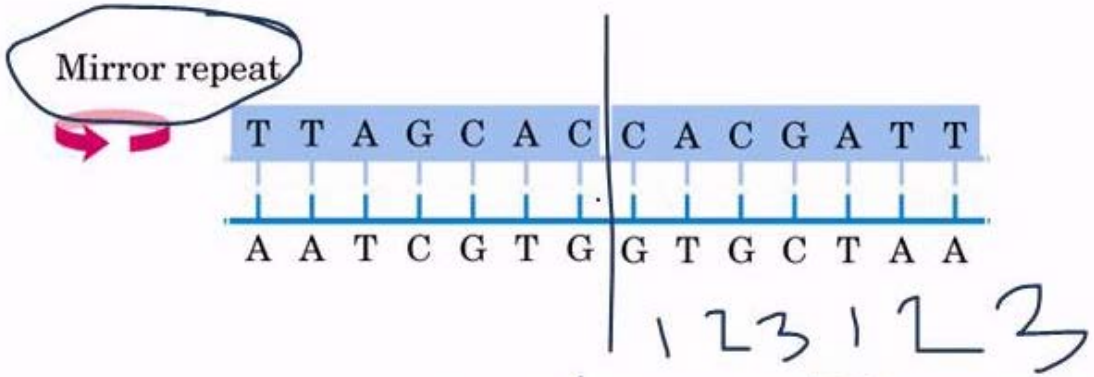
- *Eco*RI – *Escherichia coli* strain R, 1st enzyme
- *Bam*HI – *Bacillus amyloliquefaciens* strain H, 1st enzyme
- *Dpn*I – *Diplococcus pneumoniae*, 1st enzyme
- *Hin*dIII – *Haemophilus influenzae*, strain D, 3rd enzyme
- *Bgl*II – *Bacillus globigii*, 2nd enzyme
- *Pst*I – *Providencia stuartii* 164, 1st enzyme
- *Sau*3AI – *Staphylococcus aureus* strain 3A, 1st enzyme
- *Kpn*I – *Klebsiella pneumoniae*, 1st enzyme

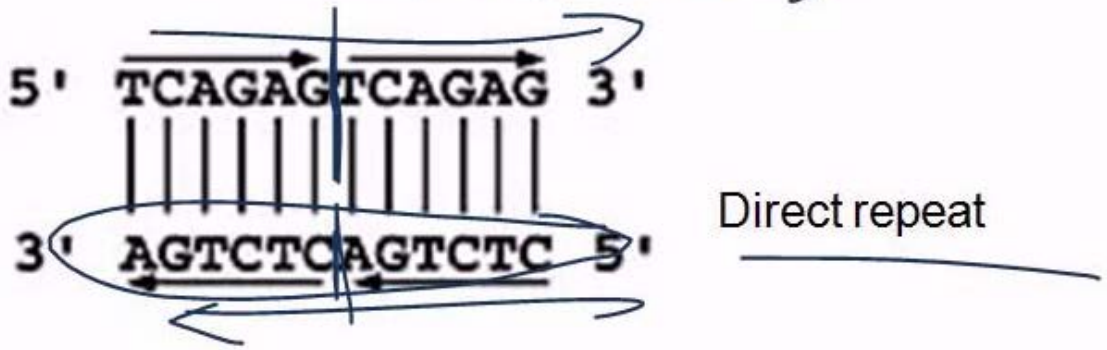
Recognition sequence

Palindrome

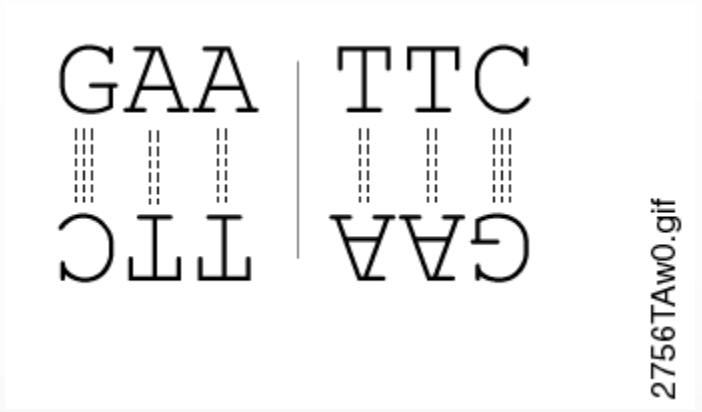


Mirror repeat

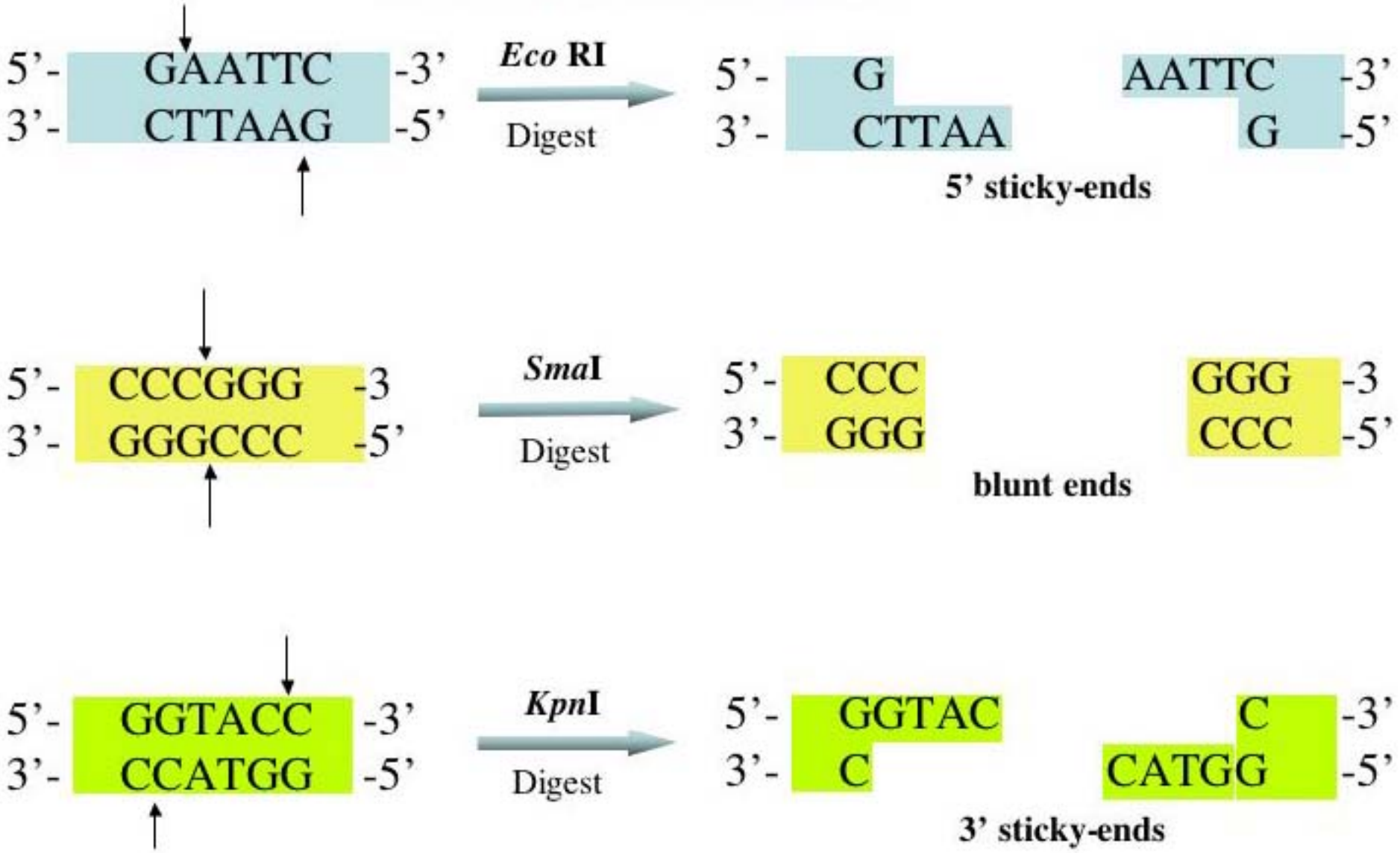




Direct repeat



Restriction Endonucleases



HpaII and Msp1 isoschizomers

MspI	Identify 5-mC; used with HpaII	5′... C▼CGG ...3′ 3′... GGC▲C...5′
HpaII	Identify 5-mC; used with MspI	5′... C▼CGG ...3′ 3′... GGC▲C...5′

Restriction mapping