RAMA UNIVERSITY

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FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

RESTRICTION ENZYMES

➢Restriction endonucleases (REs) are bacterial enzymes that cleave double-stranded DNA.

➢In 1968 **Stuart Linn** and **Werner Arber** showed in vitro restriction of fd phage DNA by an *E. coli* cell extract.

➢in the year 1970 **Hamilton Smith** and **Kent Wilcox** isolated the first restriction enzyme from *Hemophilus influenzae*, endonuclease R (later renamed as HindII),.

➢**Kathleen Dana** and **Daniel Nathans** pioneered the applications of restriction enzymes by showing specific cleavage of SV40 DNA by HindII.

➢**Werner Arber**, **Hamilton Smith** and **Daniel Nathans** were awarded Nobel Prize in Physiology or Medicine in 1978 for the discovery

➢ Information about restriction enzymes is available in the database known as **REBASE** given by R. J. Roberts in 2007.

➢According to the database, 3805 restriction enzymes have been biochemically or genetically characterized.

➢Restriction enzymes are classified in four major groups known as Type I, Type II, Type III and Type IV, on the bases of their composition, cofactor requirement, target sequence, position of cleavage site, etc.

➢**Type I REs** are important in bacterial function but do not cleave DNA at specific sequences.

➢**Type II REs**, require highly specific sites for DNA cleavage and are thus extremely useful tools in molecular biology.

➢These enzymes allow the cloning and purification of defined DNA fragments. The 500 or so known REs are typically isolated from a variety of bacterial strains.

TYPES OF RESTRICTION ENDONUCLEASE

Sr. No.	Type	Enzyme Name
1	Type I	EcoK , EcoB
2	Type II	EcoRI , EcoRV
3	Type III	EcoPI , EcoP15
4	Type IV	McrBC, Mrr

PALINDROMIC RECOGNITION SEQUENCES

Sequences having two-fold axis of dyad symmetry.
i.e. PHRASES THAT READ THE SAME BACKWARDS AS FORWARD ,

Continuous : two half-sites of recognition sequence are adjacent.
For Example: 5' G AATTC 3'

Discontinuous : two half-sites are separated or interrupted.
For Example: 5' GCCNNNN NGGC 3'

Frequency of occurrence of recognition sequence in the random sequence of DNA
– 1/4n
n= length (in bp) of recognition sequence.
For Example a 4 base cutter that recognizes a tetra nucleotide recognition sequence, would cleave DNA every
4^4=256bp