Restriction Enzymes

The two enzymes responsible for restricting the growth of bacteriophage in Escherichia coli were isolated in the year 1963. One was the enzyme which added methyl groups to DNA, while the other cut DNA. The later was called restriction endonuclease. A **restriction** enzyme or **restriction endonuclease** is an enzyme that cleaves DNA into fragments at or near specific recognition sites within the molecule known as **restriction** sites. Based on their mode of action restriction enzymes are classified into Exonucleases and Endonucleases.

a. Exonucleases are enzymes which remove nucleotides one at a time from the end of a DNA molecule. e.g. Bal 31, Exonuclease III.

b. Endonucleases are enzymes which break the internal phosphodiester bonds within a DNA molecule. e.g. Hind II, EcoRI, Pvul, BamHI, TaqI.

Restriction endonuclease: Molecular scissors

The restriction enzymes are called as molecular scissors. These act as foundation of recombinant DNA technology. These enzymes exist in many bacteria where they function as a part of their defence mechanism called restriction-modification system.

There are three main classes of restriction endonuclease : Type I, Type II and Type III, which differ slightly by their mode of action.

Only type II enzyme is preferred for use in recombinant DNA technology as they recognise and cut DNA within a specific sequence typically consisting of 4-8 bp. Examples of certain enzymes are given in table below.

Restriction enzyme	Microbial source	Recognition sequence	Fragments
Alu I	Arthrobacter luteus	5'AG/CT3' 3'TC/GA5'	A-G C-T T-C G-A Blunt ends
BamHI	Bacillus amyloliquefaciens	5'G/GATCC3' 3'CCTAG/G5'	G G-A-T-C-C C-C-T-A-G G Sticky ends
EcoRI	Escherichia coli	5'G/AATTC3' 3'CCTAG/G5'	G A-A-T-T-C C-T-T-A-A G Sticky ends
HaeIII	Haemophilus aegyptus	5'GG/CC3' 3'CC/GG5'	G-G C-C C-C G-G Blunt ends
HindIII	Haemophilus influenza	5'A/AGCTT3' 3'TTCGA/A5'	A A-G-C-T-T T-T-C-G-A A Sticky ends