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Tools of Recombinant DNA Technology includes

• Restriction Enzymes

• Polymerase enzymes

• Ligases

• Vectors

• Host organisms

Restriction Enzymes (Molecular Scissors):

Restriction enzymes belong to a larger class of enzymes called **Nucleases**. There are of two kinds; **Exonucleases and Endonucleases**. Exonucleases remove nucleotides from the ends of the DNA whereas, endonucleases make cuts at specific position within the DNA.

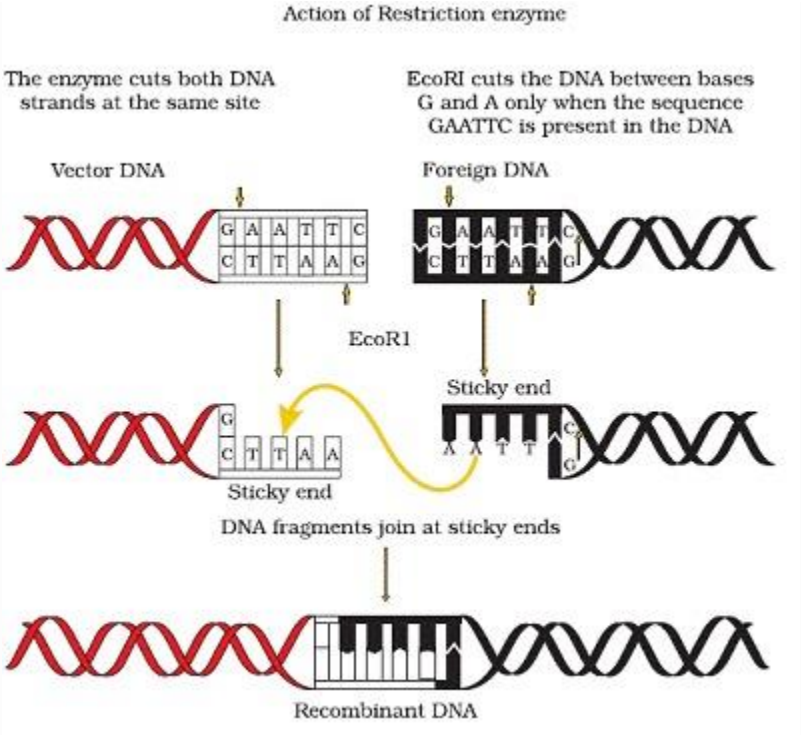
Example, the first restriction endonuclease – Hind II, always cut DNA molecules at a particular point by recognizing a specific sequence of six base pairs. This specific base sequence is known as the Recognition Sequence for Hind II.

• Each restriction endonuclease recognises a specific palindromic nucleotide sequence in the DNA. Palindromes are group of letters that form the same words when read both forward and backward for example "MALYALAM".

5′ —— GAATTC —— 3′

3′ —— CTTAAG —— 5′

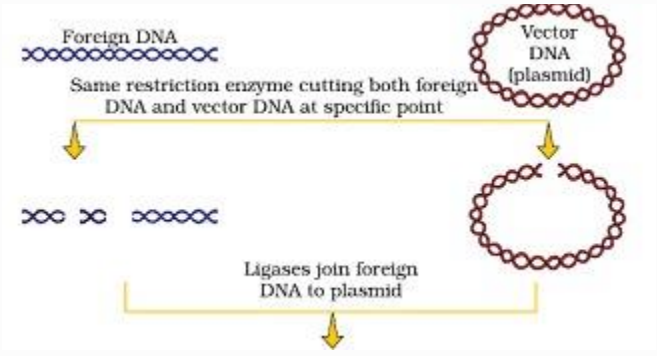
The palindrome in DNA is a sequence of base pairs that reads same on two stands when orientation of reading is kept the same.

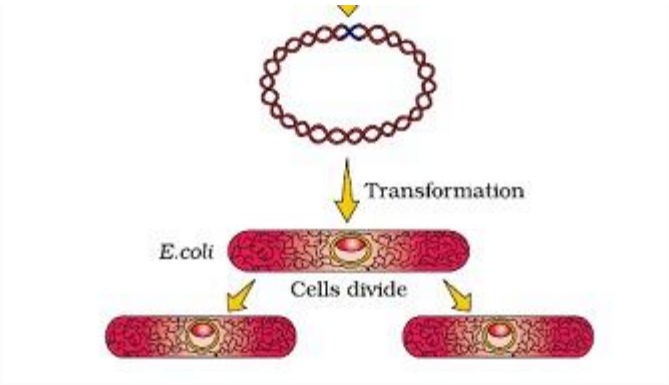


• Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome site between the same two bases on the opposite strands having sticky strand. The stickiness of the strands facilities the action of the enzyme DNA ligase.

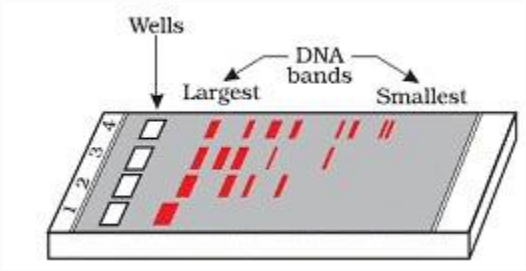
• Restriction endonucleases are used in genetic engineering to form recombinant molecules of DNA which are composed of DNA from different sources or genome.

• When cut the same restriction enzyme the resultant DNA fragments have the same kind of Sticky-ends and can be joined together using DNA ligases.





Diagrammatic representation of Recombinant DNA technology



Separation and isolation of DNA fragments

The fragment of DNA obtained by cutting DNA using restriction enzyme is separated by technique called **gel electrophoresis**. Negatively charged DNA fragments can be separated by forcing them to move towards the anode under an electric field through medium. DNA fragments separate according to their size through sieving effect provided by agarose gel.

• The separated DNA fragment can be visualized after staining the DNA with ethodium bromide followed by exposure to UV light. Separated bands of DNA are separated from agarose gel and extracted from gel, called **elution**. The DNA fragment purified this way is used for recombination.

Cloning Vector

Plasmids and Bacteriophages is commonly used vector for cloning. They have ability to replicate within bacterial cells independent of the control of chromosomal DNA. Bacteriophages because of their high number per cell, have very high copy numbers of their genome within the bacterial cells.