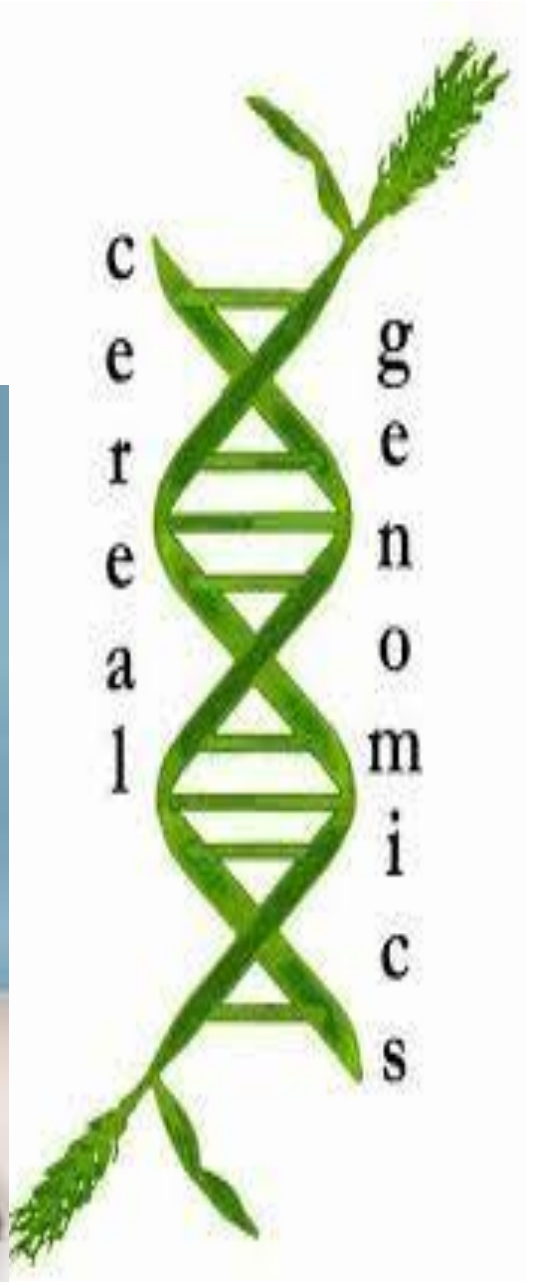
TOOLS USED IN GENETIC ENGINEERING



•GENETIC ENGINEERING : The simple addition, deletion, or manipulation of a single trait in an organism to create a desired change. Artificially copying a piece of DNA from one organism and joining this copy of DNA into the DNA of another organism.

•**Main Enzymes Involved in Genetic Engineering | Biotechnology.**

The following points highlight the five main enzymes involved in genetic engineering. The enzymes are: 1. Restriction Endonuclease 2. DNA Ligase 3. Alkaline Phosphatase 4. DNA Polymerase 5. Reverse Transcriptase.

Genetic engineering became possible with the discovery of mainly two types of enzymes: the cutting enzymes called restriction endonucleases and the joining enzymes called ligases.

Restriction endonucleases or restriction enzymes, as they are called popularly, recognize unique base sequence motifs in a DNA strand and cleave the backbone of the molecule at a place within or, at some distance from the recognition site. Whereas ligase is the enzyme that joins a 5′ end of a DNA with a 3′ end of the same or of another strand.

Restriction Enzymes : Enzymes that recognize a specific base sequence in DNA and cleave at that site ¬ Isolated from bacteria that leaves phosphate group on 5' end & OH group on the 3' end ¬ "Molecular scissors.

A large number of restriction enzymes have been identified and classified into three categories (type I, II, III) on the basis of their site of cleavage.

Restriction enzymes have three important features:

1. Restriction enzymes make breaks in palindromic sequences.
2. The breaks are usually not directly opposite to one another.
3. The enzymes generate DNA fragments with complementary ends.

Nomenclature EcoRI • E = Escherichia genus name • co = coli species name • R = strain RY12 strain or serotype • I = Roman numeral one = first enzyme

HinDIII • Haemophilus influenza serotype d 3rd enzyme

Table 22.1: Source of restriction enzymes, cleavage sites and productions of cleavage

Microorganisms	*Restriction enzymes*	*Cleavage sites*	*Cleavage products*	
Bacillus amyloliquefaciens H	Bam HI	5-G↓GATCC-3 3-CCTAGG↑-5	5-G 3-CCTAG	GATCC-3 G-5
B. globigii	Bgl II	5-A↓GATCT-3 3-TCTAGA↑-5	5-A 3-TCTAG	GATCT-3 A-5
Escherichia coli RY13	Eco RI	5-G↓AATTC-3 3-CTTAAG↑-5	5-G 3-CTTAA	AATTC-3 G-5
Haemophilus influenzae Rd	Hin dIII	5-A↓AGCTT-3 3-TTCGAA↑-5	5-A 3-TTCGA	AGCTT-3 A-5
H. parainfluenzae	Hpa I	5-GTTAAC-3 3-CAATTG-5	5-GTT 3-CAA	AAC-3 TTG-5
Klebsiella pneumoniae OK 8	Kpn I	5-GGTACC↓-3 3-C↑CATGG-5	5-GGTAC 3-C	C-3 CATGG-5
Streptomyces albus G	Sal I	5-G↓TCGAC-3 3-CAGCTG↑-5	5-G 3-CAGCT	TCGAC-3 G-5
Serratia marcescens	Sma I	5-CCC↓GGG-3 3-GGG↑CCC-5	5-CCC 3-GGG	GGG-3 CCC-5

DNA Ligase:

Ends of DNA strands may be joined by the enzyme polynucleotide ligase, called ‘glue’ of the recombinant DNA molecule. The enzyme catalyses the forma-tion of a phosphodiester bond between the 3’OH and 5’P terminals of two nucleotides. The enzyme is thus able to join unrelated DNA, repair nicks in single strand of DNA and join the sugar phosphate backbones of the newly repaired and resident region of a DNA strand.

The enzyme which is extensively used for covalently joining restriction fragments is the ligase from E. coli

The ligation reaction is controlled by several factors, such as pH, temperature, concentration and kinds of sticky ends, etc. As ligase uses the ends of DNA molecules as substrates rather than the entire DNA,

ALKALINE PHOSPHATASE • Alkaline phosphatase is an enzyme involved in the removal phosphate groups • This enzyme is useful to prevent unwanted ligation of DNA molecules which is a frequent problem encountered by cloning experiments

The treatment with alkaline phosphatase prevents re-circularisation of plasmid vector and increases the frequency of production of recombinant DNA molecule

•POLYMERASES

• The group of enzymes that catalyse the synthesis of nucleic acid molecules are collectively referred to as polymerases • DNA dependent DNA polymerase that copies of DNA from RNA • RNA dependent DNA polymerase (reverse transcriptase) that synthesis DNA from RNA • DNA dependent RNA polymerase that produces RNA from DNA

Reverse Transcriptase:

Retroviruses (possessing RNA) contain RNA dependent DNA polymerase which is called reverse transcriptase. This produces single stranded DNA, which in turn functions as template for complemen-tary long chain of DNA.

This enzyme is used to synthesize the copy DNA or complemen-tary DNA (cDNA) by using mRNA as a template. The enzyme is very useful for the syn-thesis of cDNA and construction of cDNA clone bank and to make short labelled probes.

• Plasmids are circular DNA molecules present in the cytoplasm of the bacteria Capable of autonomous replication ♣Can transfer genes from one cell to other ♣ Act as vectors in genetic engineering. ♣ Can also present in Yeasts

Plasmid vectors are double-stranded, circular, self-replicating, extra-chromosomal DNA molecules. • Advantages: – Small, easy to handle –Useful for cloning small DNA fragments (< 10kbp) • Disadvantages: – Less useful for cloning large DNA fragments (> 10kbp)