ENZYMES INVOLVED IN GENETIC ENGINEERING

The technique of genetic engineering needs several enzymes. The technique is basically possible due to the discovery of two main enzymes: the cutting enzymes called restriction endonucleases and the joining enzymes called ligases. Along with these two, some other important enzymes required for the process are-

1. Restriction Endonuclease

These are the enzymes that recognise specific nucleotide sequences in DNA and cleave the DNA double helix at or near the specific restriction sites, called the target sites. These were first reported by W. Arber (1962), who noticed that when DNA of a bacteriophage entered a host bacterium, it was cut into smaller pieces. However, first restriction enzyme was isolated by Meselson and Yuan from *E. coli*. Real breakthrough came when restriction enzyme ***Hind II*** was isolated from bacterium, Haemophilus influenza Rd. By Kelly and Smith (1970) and Smith. This enzyme is basically of three types-

i) Type I –restriction endonucleases- These interact with an unmodified reconation sequence in double stranded DNA. These cleave only one strand of DNA and at an apparently random site. These enzymes create a gap of about 75 nucleotides in length by releasing acid soluble oligonucleotides. Enzyme action required Mg^{2+} ions, ATP and s-adenosyl methionine cofactors.

ii) Type-II- restriction endonucleases- These enzyme cleave both polynucleotide chains within or near to the palindromic sequences. Paliendromes are base pair sequences that read the same forward and backward or in 5′- 3′ direction in both strands of DNA. For eg-

A) 5′ .....NNGAATTCNN..... 3′
3′ .....NNCTTAAGNN .....5′

B) 5′ .....NNAAGCTTNN..... 3′
3′ .....NNTTCGAANN .....5′

These enzymes are mostly use in gene manipulation.

iii) Type- III - restriction endonucleases- These enzymes cleave double stranded DNA at well defined sites and require Mg^{2+} ions , ATP and partially s-adenosyl methionine cofactors.

Following are few examples of restriction enzyme, their source and target site-

Enzyme name	Source organism	Target DNA sequence
Eco RI	*Escherichia coli* Ry13	5′.....G↓ AATTC.....3′ 3′.....CTTAA↑ G..... 5′

Eco RII	*Escherichia coli* R245	5′.....↓ CCAGG.....3′ (T) (A) 3′..... GGTCC ↑..... 5′
Hind III	*Haemophilus influenza(Rd)*	5′.....A↓AGCTT.....3′ 3′.....TTCGA↑ A..... 5′
BgI	*Bacillus globigii*	5′.....A↓GATCT.....3′ 3′.....TCTAG↑ A..... 5′

2. DNA Ligase:

Mutz and **Davis (1972)** demonstrated that cohesive terminus of the cleaved DNA molecule could be covalently tied with *E.coli* DNA ligase and were able to produce recombinant DNA molecule.

An extensively ligase is T4 DNA ligase., which is used for covalently joining restriction fragments, extracted from *E. coli* that is encoded by T4 phage. Depending on the source this DNA ligase is known as T4 DNA ligase.

The ends of DNA strands may be joined by the enzyme polynucleotide ligase, called 'glue' of the recombinant DNA molecule. The enzyme catalyses the formation 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 and that encoded by T4 phage. As the main source of DNA ligase is T4 phage, hence, the enzyme is known as T4 DNA ligase.

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, the kinetics of joining depend on the number of ends (concentration) available for joining.

3. Alkaline Phosphatase:

This enzyme help to join the passenger DNA to vehicle DNA. The broken fragments of plasmids, instead of joining with foreign DNA, join the cohesive end of the same DNA molecules. The treatment with alkaline phosphatase prevents re-circularisation of plasmid vector and increases the frequency of production of recombinant DNA molecule.

4. DNA Polymerase and the Klenow Fragment:

The DNA polymerase that is generally utilized is either the DNA Pol I *from E. coli* or the T4 DNA polymerase encoded by the phage gene. The E. coli enzyme is basically a proof-reading and repairing enzyme. It is composed of 3 subunits each with a specific activity. They are: 5′-3′ polymerase, 3′-5′ exonuclease and 5′-3′ exonuclease.

The enzyme is useful for synthesizing short length of a DNA strand, especially by the nick translation method. The 5-3′ exonuclease activity may be deleted, this edited enzyme is referred to as the klenow fragment. The T4 DNA Pol possesses, like the klenow fragment, only the polymerase and proofreading (3′-5′ exonuclease) functions.

5. 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 complementary long chain of DNA.

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

These enzymes along with other components like plasmid, bactriophage, required DNA for cloning etc. perform the process of artificial manipulation of gene or the technique genetic engineering.