Notes:-

PROCESSES OF RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology involves several steps in specific sequence such as isolation of DNA, fragmentation of DNA by restriction endonucleases, isolation of a desired DNA fragment, ligation of the DNA fragment into a vector, transferring the recombinant DNA into the host, culturing the host cells in a medium at large scale and extraction of the desired product.

Isolation of the Genetic Material (DNA)

In majority of organisms this is deoxyribonucleic acid or DNA. In order to cut the DNA with restriction enzymes, it needs to be in pure form, free from other macro-molecules. Since the DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids. This can be achieved by treating the bacterial cells/plant or animal tissue with enzymes such as lysozyme (bacteria), cellulase (plant cells), chitinase (fungus). You know that genes are located on long molecules of DNA interwined with proteins such as histones. The RNA can be removed by treatment with ribonuclease whereas proteins can be removed by treatment with protease. Other molecules can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol.

Cutting of DNA at Specific Locations :-

Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme, at the optimal conditions for that specific enzyme. Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion. The joining of DNA involves several processes. After having cut the source DNA as well as the vector DNA with a specific restriction enzyme, the cut out ‘gene of interest’ from the source DNA and the cut vector with space are mixed and ligase is added. This results in the preparation of recombinant DNA.

Amplification of Gene of Interest using PCR :

PCR stands for Polymerase Chain Reaction. In this reaction, multiple copies of the gene (or DNA) of interest is synthesised in vitro using two sets of primers (small chemically synthesised oligonucleotides that are complementary to the regions of DNA) and the enzyme DNA polymerase.

The enzyme extends the primers using the nucleotides provided in the reaction and the genomic DNA as template. If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made. Such repeated amplification is achieved by the use of a thermostable DNA polymerase (isolated from a bacterium, Thermus aquaticus), which remain active during the high temperature induced denaturation of double stranded DNA.