

## Steps in Gene cloning

The entire procedure of gene cloning or recombinant DNA technology may be classified into the following steps for the convenience of in description and on the basis of the chief activity performed.

1. Production and isolation of the DNA fragments to be cloned.
2. Insertion of the isolated gene in a suitable vector to obtain recombinant DNA.
3. Introduction of recombinant DNA into a suitable organism/cell called host (transformation)
4. Selection of the transformed host cells, and identification of the clone containing the desired gene.
5. Multiplication of the introduced gene in the host.

## Tools used for gene cloning

The following four types of enzymes are used.

- 1) Nucleases
- 2) Ligases
- 3) Polymerases
- 4) DNA modifying enzymes

The tools basic to almost all gene cloning experiments are listed below -

- a) Restriction endonucleases to cut the DNA at specific sites, These are often called molecular scissors.
- b) A suitable DNA molecule capable of autonomous-replication in the selected host cell.
- c) DNA ligase to seal the nicks that remain in the recombinant DNA molecule. (Molecular glue)
- d) A suitable organisms that serves as a host for propagation of recombinant DNA.
- e) Reverse transcriptase is used to produce cDNA (Complementary DNA) copies of mRNA that are used for creation of cDNA libraries.
- f) Alkaline phosphatase for removing 5'-phosphate from DNA ends.
- g) T4 polynucleotide kinase for addition of phosphate group to an end having a free 5'-OH.



## Coenzyme :-

Coenzymes are part of the active site of the enzyme, they supply reactive groups that are not available on the side chains of the amino acids.

Coenzymes that can be synthesized from common metabolites are ~~ref~~ referred to as metabolite co-enzymes. Those that cannot be synthesized and are derived from vitamins are known as Vitamin derived co-enzymes.

Coenzymes that remain bound, either covalently or non covalently to an enzyme are called ~~p~~ prosthetic groups.

We will discuss in details about  $\text{NAD}^+$ ,  $\text{NADP}^+$ , Coenzyme A, Biotin.