

③ Synthesizing enzymes. Synthesizing enzymes are used to synthesize new strands of DNA. These are of two types.

i) Reverse transcriptase. These helps to synthesize complementary DNA strands on RNA template.

ii) DNA polymerase. These helps to synthesize complementary DNA strand on DNA template.

④ Joining enzymes. They help in joining the DNA fragments by forming phosphodiester bonds, so called as molecular glues, e.g. DNA ligase.

⑤ Alkaline Phosphatase. These are needed to cut off phosphate group from 5' end of circular DNA molecule, and prevents recircularisation of vector.

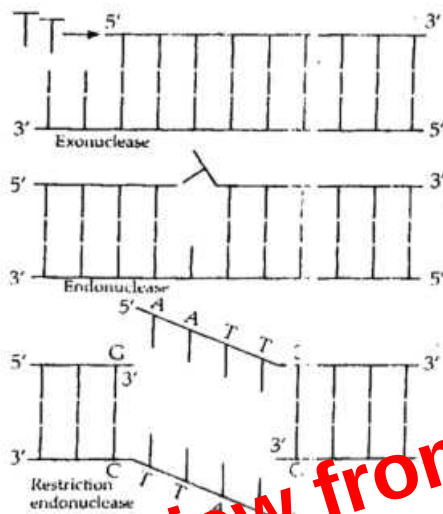


Fig. 1.1. DNA cleaving enzymes.

2. Vector DNA or Vehicle DNA:

The DNA, which is used as carrier to transfer a fragment of foreign DNA into a suitable host, is called vector or vehicle DNA. (cloning vector or gene carrier).

Vectors used in genetic engineering are plasmids (DNA), bacteriophages (DNA), DNA of plant and animal viruses, Transposons or jumping genes, Artificial DNA of bacteria, yeast, mammals etc.

Plasmids and bacteriophages are common vectors.

Plasmids can take up genes of one bacterium and transfer it to another and retains the same property in another organism also. e.g. T1 plasmid.

3. Passenger DNA or Donor DNA:

The DNA, which is combined with vector DNA and transferred from one organism into another, is called passenger DNA or Donor DNA.

They may be,

i) Complementary DNA (cDNA). It is synthesized with the help of necessary nucleotides and reverse transcriptase on mRNA. It is shorter than the actual gene because of the absence of non-coding regions.

ii) Synthetic DNA (sDNA). It is synthesized on DNA template or from free nucleotides without a template with the help of DNA polymerase.

iii) Random DNA. These are the small fragments formed by breaking a chromosome with the help of restriction endonuclease.

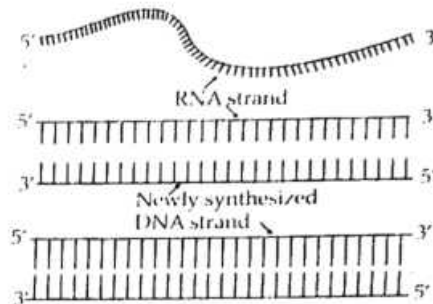


Fig. Synthesis of complementary DNA (c-DNA) from RNA

Methodology (Technique) of Genetic engineering

Genetic engineering is the construction of recombinant DNA molecule which contains genes from different and unrelated organisms.

Main steps of genetic engineering are,

a) Isolation of desired gene-

Specific DNA fragments or desired genes are identified. Then, they are isolated and purified for transfer into proper host. Desired isolated DNA is also called as Donor DNA, Passenger DNA or Target DNA.

b) Selection and isolation of vector-

The desired gene introduced into a carrier molecule, which transport it into host cell. Carrier molecule is called vector, cloning vehicle, which is self-replicating molecule of DNA to which donor DNA links. Commonly used vectors are plasmids and DNA of viruses.

c) Construction of recombinant vectors-

Donor DNA inserted into vector to form donor vector hybrid DNA molecule, known as recombinant DNA or chimeric DNA or recombinant vector.

d) Introduction of Recombinant vector into host cell-

Recombinant vector is introduced into suitable host cell. Host cells or target