**RECOMBINANT DNA**

**Dr. Praveenkumar N. Nasare,**

**Associate Professor, Department of Botany,**

**NilkanthraoShinde Science and Arts College, Bhadrawati, Dist. Chandrapur**

**Introduction**

Biotechnology is a branch of biology which deals with the techniques of using live organisms, enzymes or biological processes to produce products and provide services for human welfare. Making curds or bread which involves microorganisms can be considered as the oldest form of biotechnology. We also find application of fermentation in the production of wine and other alcoholic beverages. Most of the biotechnological processes gradually became more sophisticated. They are used to harvest various valuable materials like vitamins and antibiotics produced by microbes.

Today, biotechnology is undergoing change in the wide range of applications. Biotechnology involves DNA manipulations (recombinant DNA technology). Protoplast fusion, cell catalysis, tissue culture, immobilized enzymes, protein engineering etc.

DNA is a genetic material which contains all hereditary information needed to createan organism DNA actually does not make organisms, it only makes protein. DNA is transcribed into mRNA and mRNA is translated into protein and the protein then forms organism. The specific regions on the DNA molecule that direct the synthesis of proteins are called as genes by changing the nucleotide sequence in DNA, the protein formation is changed. This leads into either a different protein or inactive protein. Recombinant DNA is the general name for taking a piece of DNA and combining it with another strand of DNA. Therefore, recombinant DNA orrDNA. Recombinant DNA is sometimes also referred to as chimera DNA. By combining two or more strands of DNA from two different organisms, scientists are able to create a new strand of DNA.

Recombinant DNA Technology (rDNATech) or genetic engineering is concerned with the manipulation of genetic materials towards desired end in a directed way. It is also known as gene cloning. Genetic engineering aims at isolating DNA segments of one organisms of interest and recombining that with DNA of second unrelated organisms. In this process, the DNA molecules isolated and cut into pieces by one or more specialized enzymes and the fragments are joined together in a desired combination and resorted to a cell for replication and reproduction.

Recombinant DNA, thus is a composite DNA molecule that results from the physical combination of DNA segments derived from different sources. In other words, the Genetic engineering is biochemical manipulation of genes by which foreign genes or the genes of clones or organism are inserted into the DNA or unrelated recipient organisms through the plasmids of bacteria or through bacteriophages.

**Recombinant DNA Technology**

During sexual reproduction, variations are created. Some of the variations are useful. Such variations are not produced during asexual reproduction. Genetic engineering involves a manipulation of the genetic material towards a desired end and in a pre-determined way. To engineer means to design, construct and manipulate to a set plan. This technology bypasses the restriction in the gene transfer mechanisms between unrelated organisms. It differs from genetic recombination in that it does not occur through natural processes but is engineered.

The recombinant DNA technique was first prepared by Peter Lobhan, a graduate student, with A. Dale Kaiser at the Department of Biochemistry, Standford University. The present day, rDNA technology actually flourished after Stanley Cohen and Herbert Boyer (1972) could successfully link gene coding for antibiotic resistance with a native plasmid of *Salmonella typhimurium* with the vector plasmid and then cloning it in *Escherchia coli* (E.coli).

**Recombinant DNA Technology Procedure**

Recombinant DNA (rDNA) technology is the technique of manipulating the genome of a cell or organisms so as to change the phenotype desirably.

The following are the basic steps involved in the process

1. DNA fragments to be cloned or target gene sequence of desired type.
2. Restriction endonucleases for cutting DNA molecule into fragments.
3. Inserting the fragments with desired gene into a cloning vector (a plasmid cosmid, or phage DNA) so as to develop a recombinant DNA orChimeric DNA.
4. DNA ligase enzyme for splicing DNA segments.
5. Prokaryotic or eukaryotic cells to serve as host. Introducing the recombinant vector into a component host cell.
6. Culturing these cells to obtain multiple copies or clones of desired fragment of DNA.
7. Using these copies to transform suitable host cells so as to express the desired gene.

**Major steps involved in the genetic engineering**

The following are the major steps involved in the genetic engineering

1. To break open the living cells

Several methods are available to break open the living cells. One of the popular methods involves mechanical shearing, the cell in a blender and then treating them with detergents.

2. Isolation and identification of desired genes or DNA sequence.

The genetic information is stored in DNA, since the DNA molecule are much longer than all other molecules found in the cells, it has become possible to develop techniques of purifying DNA. DNA molecules are spooled on a glass rod. The glass rod bearing the DNA molecules is then lifted out from the mixture of broken cells.

3. Cutting of DNA molecules into segments containing specific genes from the rest of DNA.

DNA is cut into gene size segments with the help of molecules scissors called as restriction endonucleases. The restriction endonucleases recognized specific base sequences in DNA molecules and make two cuts, one in each strand. Such an action results in generating 3’ hydroxyl and 5’ phosphate terminals.

4. Insertion of a foreign DNA fragment into a vector

The specific section of DNA generated by the action of restriction enzyme is spliced incorporated into an agent called as cloning vehicle or vectors are short DNA molecules that can penetrate the wall of living host cells and can multiply inside the cells. Currently,small DNA molecules of bacterial plasmids, Lambda and M13 bactriophages of *E.coli* and several animal viruses are used as cloning vectors to transfer the DNA fragment from test tube into the living host cell. To be useful in the cloning process a vector or cloning vehicle must have the following characteristics.

1. It must be small and well characterized molecule having means of introducing DNA into a cell.
2. It must have a replication origin enabling self-replication as well as the replication of foreign DNA segment.
3. It should be capable of selection of hybrid molecules by a straight forward assay preferably by the growth of a host cell on a solid culture medium.

**Insertion of Vector**

Insertion of veoctor into the target cell is usually called transformation.For bacterial cells, transfection for eukaryotic cells, although insertion of a viral vector is often called transduction.Some of the common vectors are bacteria are plasmids, cosmids, lambda phage, *Baculovirus* is useful in insect.Ti-plasmid is for plants and YAC (Yeast Artificial Chromosome) is for yeast cells.

**Process**

DNA taken from both the sources is fragmented by restriction endonuclease. The restriction enzyme cuts both molecules at the specific site. The ends of the cut have an overhanging piece of single stranded DNA called sticky ends. These sticky ends are able to base pair with any DNA fragment that contains the complimentary sticky ends. Enzyme DNA ligase is used to covalently link the two strand into a molecule of recombinant DNA. This recombinant DNA needs to be replicated many times. Cloning can be done in vitro, via polymerase chain Reaction (PCR), or in vivo using unicellular prokaryotes (e.g. *E.coli*) unicellular eukaryotes, (e.g Yeast), or Mammalian tissue culture cells.

**Some Examples**: some examples of the therapeutic products made by recombinant DNA techniques include-

1. Blood Proteins: Erythoprotien, Factors, VII, VIII IX, Tissue plasminogen activator,Urokinase.
2. Human Hormones: Epidermal growth factor, Follicle stimulating hormone, Insulin, Nerve growth factor, Relaxin, Somatotropin.
3. Human Modulators, α-Interferon; β-Interferons Colony stimulating factor, lysozyme, Tumor necrosis factor.
4. Vaccines: Cytomegalovirus,Hepatitis B, Measles Rabies.

**References**

Arora, M.P. (2013): Biotechnology, Sixth Reprint 2013, Himalaya Publishing House Pvt. Ltd. Mumbai.

Dubey, R.C. (2014): Advanced Biotechnology, First Edition 2014. S. Chand & Company Pvt. Ltd. New Delhi.

Jogdand, S.N. (2005): Advances in Biotechnology, Fifth Revised edition 2005, Himalaya Publishing House, Mumbai.

Kumar, H.D.(2003): Modern concepts of Biotechnology, Third Reprint 2003, Vikas Publishing House Pvt. Ltd. New Delhi.