Gene Manipulation & Genetic Engineering

Genetic engineering is a broad term referring to manipulation of an organisms' nucleic acid. Organisms whose genes have been artificially altered for a desired affect is often called genetically modified organism (GMO). Plant genetic engineering is defined as the isolation, introduction and expression of foreign DNA in the plant. In other words, it refers to direct introduction or foreign gene (DNA) into a plant's system by micromanipulation at the cellular level.

Recombinant DNA technology (rDNA) is technology that is used to cut a known DNA sequence from one organism and introduce it into another organism thereby altering the genotype (gene manipulation) of the recipient.

Steps/Methods of rDNA Technology (Genetic Engineering)

1. Isolation of Genetic Material

- The first step in rDNA technology is to isolate the desired DNA in its pure form i.e., free from other macromolecules.
- Since DNA exists within the cell membrane along with other macromolecules such as RNA, polysaccharides, proteins, and lipids, it must be separated and purified which involves enzymes such as lysozymes, cellulase, chitinase, ribonuclease, proteases etc.

2. Restriction Enzyme Digestion

- Restriction enzymes act as molecular scissors that cut DNA at specific locations.
- They involve the incubation of the purified DNA with the selected restriction enzyme, at conditions optimal for that specific enzyme.
- The technique 'Agarose Gel Electrophoresis' reveals the progress of the restriction enzyme digestion.
- The vector DNA is also processed using the same procedure.

3. Amplification Using PCR

- Polymerase Chain Reaction or PCR is a method of making multiple copies of a DNA sequence using the enzyme – DNA polymerase in vitro.
- It helps to amplify a single copy or a few copies of DNA into thousands to millions of copies.

4. Ligation of DNA Molecules

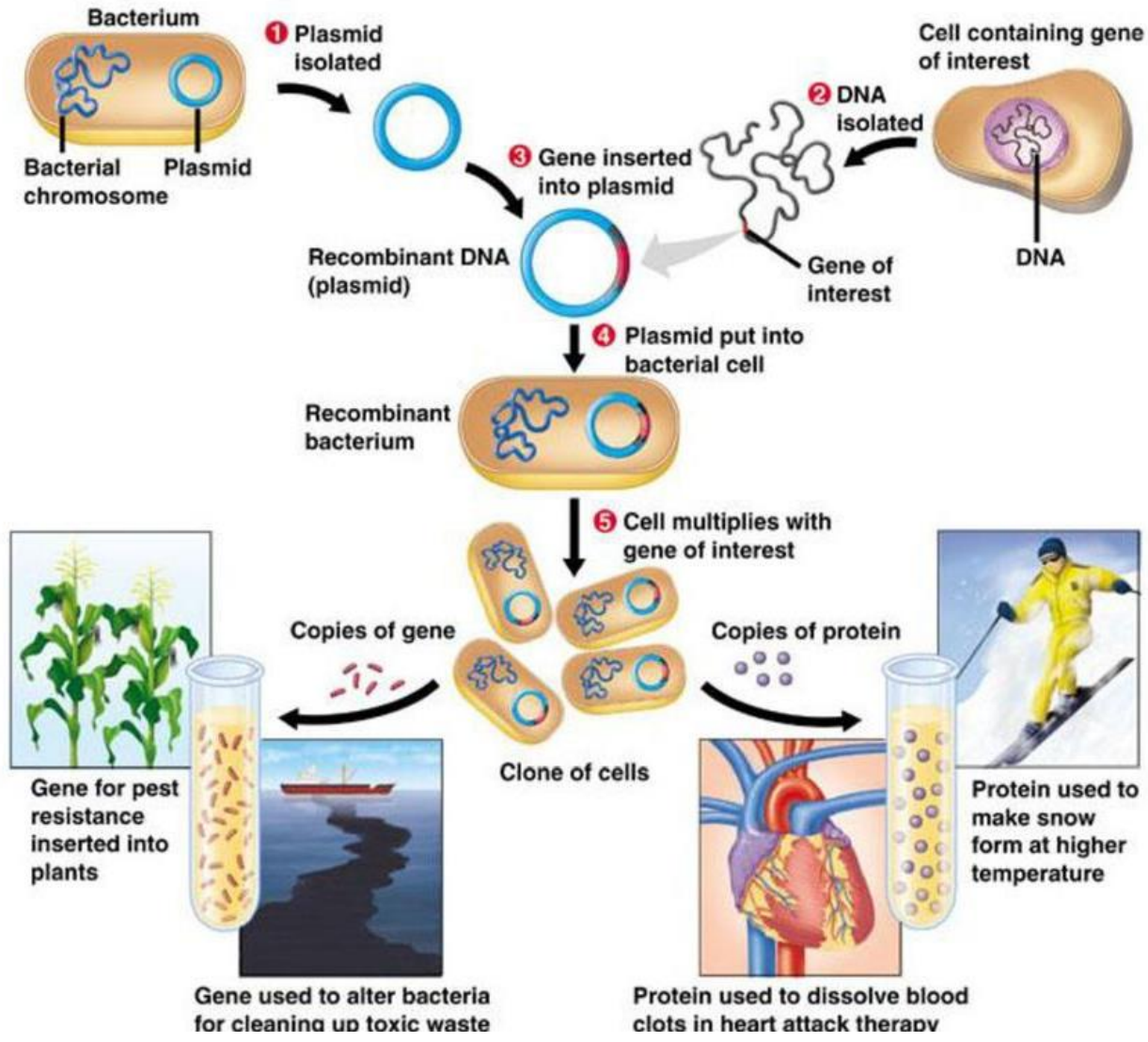
- The purified DNA and the vector of interest are cut with the same restriction enzyme.
- This gives us the cut fragment of DNA and the cut vector, that is now open.
- The process of joining these two pieces together using the enzyme 'DNA ligase' is ligation.
- The resulting DNA molecule is a hybrid of two DNA molecules – the interest molecule and the vector. In the terminology of genetics this intermixing of different DNA strands is called recombination.
- Hence, this new hybrid DNA molecule is also called a recombinant DNA molecule and the technology is referred to as the **recombinant DNA technology**.

5. Insertion of Recombinant DNA Into Host

- In this step, the recombinant DNA is introduced into a recipient host cell mostly, a bacterial cell. This process is 'Transformation'.
- Bacterial cells do not accept foreign DNA easily. Therefore, they are treated to make them 'competent' to accept new DNA. The processes used may be thermal shock, Ca^{++} ion treatment, electroporation etc.

6. Isolation of Recombinant Cells

- The transformation process generates a mixed population of transformed and non-trans- formed host cells.
- The selection process involves filtering the transformed host cells only.
- For isolation of recombinant cell from non-recombinant cell, marker gene of plasmid vector is employed.
- For examples, PBR322 plasmid vector contains different marker gene (Ampicillin resistant gene and Tetracycline resistant gene. When pst1 RE is used it knock out Ampicillin resistant gene from the plasmid, so that the recombinant cell become sensitive to Ampicillin.



Applications of Genetic Engineering:

- Genetic engineering has applications in medicine, research, industry and agriculture and can be used on a wide range of plants, animals and microorganisms.
- In medicine, genetic engineering has been used to mass-produce insulin, human growth hormones, human albumin, monoclonal antibodies, antihemophilic factors, vaccines, and many other drugs.
- In research, organisms are genetically engineered to discover the functions of certain genes.
- Industrial applications include transforming microorganisms such as bacteria or yeast, or insect mammalian cells with a gene coding for a useful protein. Mass quantities of the protein can be produced by growing the transformed organism in bioreactors using fermentation, then purifying the protein.
- Genetic engineering is also used in agriculture to create genetically-modified crops or genetically-modified organisms.