Molecular Biology

2nd stage

LEC 6

Genetic engineering

By

M.SC Jaafar Hamid

M.SC Nidaa Fadel

Genetic engineering is the process of manipulating genes, usually outside the organism's normal reproductive process.

Genetic modification of an organism can be achieved through a number of methods, most notably traditional breeding and recombinant technologies. It means altering the genes in a living organism to produce a Genetically Modified Organism (GMO) with a new genotype. Genetic engineering often involves the isolation, manipulation and reintroduction of DNA into cells or model organisms, usually to express a protein.

The aim is to introduce new characteristics such as increasing the yield of a crop species, introducing a novel trait, or producing a new protein or enzyme. Examples include the production of human insulin through the use of modified bacteria, the production of erythropoietin in Chinese Hamster Ovary cells, and the production of new types of experimental mice such as the OncoMouse (cancer mouse) for research, through genetic redesign.

How to perform genetic engineering

Genetic engineering is done in several ways, which basically consists of 4 steps:

1- Isolating the desired gene: Isolation is done by identifying the desired gene to be introduced into the cells through prior information about the genes, which is obtained either by making libraries of complementary DNA or gDNA, and then these genes are duplicated using polymerase chain reaction.

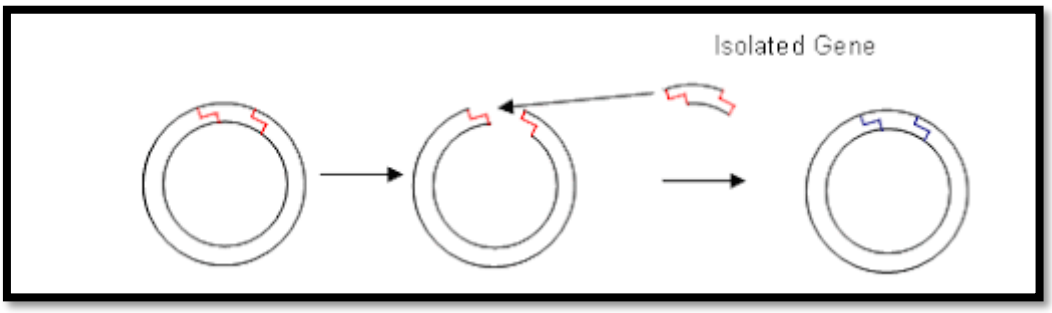
2- Inserting or loading the desired gene into a suitable carrier such as a plasmid. Other carriers such as viral carriers or liposomes can also be used.

3- Inserting the carrier into the cells of the organism to be modified, and this is done in several ways, including a DNA gun.

4- Isolating and separating cells or organisms that have been successfully genetically modified from normal ones. This is done in several ways, including: using a DNA probe to detect the introduced gene, or using discriminatory parameters (in English: Selectable Marker) to detect a resistance trait present with the carrier that is distinguished by its resistance to a specific trait, such as discriminatory parameters that confer resistance to a specific antibiotic.

Gene isolation

First, the gene to be introduced into the genetically modified organism is selected and isolated. Most of the genes currently transferred to plants provide some type of protection against insects or resilience to pesticides and most of the genes used in animals are genes for growth hormones. Once selected, the gene is isolated and this usually requires multiplexing the gene using polymerase chain reaction (PCR). If the selected gene or the genome of the donor organism is well studied, then it can be introduced into the genetic library. If the DNA sequence is known but copies of the gene are not available, it can be created artificially. Once the gene is isolated, it is inserted into a bacterial plasmid.



Processing of genetic complexes

The gene to be introduced into the genetically modified organism must be combined with the rest of the genetic elements in order for it to work effectively. The gene can be modified at this stage as well in order to obtain better expression or effectiveness. In addition to the gene to be inserted, most DNA structures contain a promoter and a covering region, such as the discriminating parameter gene. The promoter region initiates transcription of the gene and can be used to control the location and level of expression of the gene, while the

stop region terminates transcription. Discriminating parameters in most cases confer antibiotic resistance to the organism in which they are expressed and are crucial for determining which cells will develop a new gene. DNA recombinants are constructed using recombinant DNA techniques such as limited digestion, DNA ligation, and molecular cloning.

Target genes

The conventional form of genetic engineering requires the random introduction of new genetic material into the host genome. Other techniques allow new genetic material to be introduced into a specific location in the host genome or to produce mutations at the desired genetic location capable of inactivating native genes. Gene targeting techniques use homologous recombination to target desired target changes and generally require the use of discriminatory parameters. Gene targeting frequencies can be greatly improved using engineered nucleases such as zinc finger nucleases and engineered homing nucleases or those made with downstream effectors. Engineered nucleases, in addition to improved gene targeting, are used to introduce mutations into the original genes that generate a defective gene.

Transformation

Metamorphosis (inheritance)

About 1 percent of bacteria can exploit foreign DNA naturally, but this DNA can also be induced in other bacteria. Stressing bacteria, for example using a thermal or electrical shock, can cause the cell membrane to become permeable to DNA, which may combine with the cell's genome or exist as extrachromosomal DNA. DNA is usually introduced into animal cells using microscopic insemination, where it can be injected into the nuclear envelope of cells directly into the nucleus or through the use of viral vectors. DNA is usually introduced into plants using Agrobacterium intermediate or bioplasmic recombination.

In Agrobacterium-Medium recombination, the plasmid construct must also contain vector DNA. Agrobacterium naturally inserts DNA from a tumor-inducing plasmid into any susceptible plant genome that it infects, causing crown gall disease. The vector DNA region of this plasmid is responsible for DNA insertion. The genes to be introduced are cloned into a binary vector which will contain vector DNA and can be grown in both E. coli and Agrobacterium. Once the

binary vector is constructed, the plasmid is transformed into an Agrobacterium that does not contain any plasmids and the plant cells are infected. The Agrobacterium will then be introduced into the genetic material of the plant cells.

During the biolistics process, gold or tungsten particles are coated with DNA and then released into the cells of a younger plant or plant embryo. Some genetic material will enter the cell and transport these molecules. This method can be used in plants that are not susceptible to Agrobacterium infection and also allows the transformation of plant plastids. There is another transformation method used to transform plant and animal cells and it is called electroporation. Electroporation requires subjecting plant or animal cells to an electrical shock that may cause the cell membrane to become permeable to the plasmid DNA. In some cases the electroporated cells will combine with DNA in their genome. Depending on the combination of cells and DNA, the transformation efficiency of both bioassay and electroporation is less than that of Agrobacterium medium transformation and microscopic inoculation.

Election

Not all cells in an organism are transformed when new genetic material is introduced. In most cases, discriminating parameters will be used to differentiate between transformed and non-transformed cells. If the cell is successfully transformed using DNA, it will also contain the indicator gene. By growing cells in the presence of an antibiotic or chemical that selects or marks cells that present that gene, it becomes possible to separate transgenic events from non-transgenic ones. Another screening method requires the use of a DNA probe, which will attach only to the introduced gene. A number of strategies have been developed that allow the selected indicator to be removed from the mature transgenic plant.

Renewal

Whenever a single cell is transformed within the genetic material, the organism must grow again from that cell. Since bacteria consist of a single cell and are reproduced by cloning, regeneration becomes unnecessary in this case. This is achieved in plants by the use of tissue culture. Each type of plant has different demands for successful regeneration using tissue culture. If successful, the adult plant that results will contain a transgene in every cell. It must be ensured that the DNA introduced into the animals is present in the embryonic stem cells. When the sequences are produced, the presence of the gene can be confirmed

by testing. All first generation lines will be heterozygous for the introduced gene and these lines must mate to produce a homozygous animal.

Confirmation

Additional testing using polymerase chain reaction, Southern blot, and biological tests is needed to confirm that the gene has been expressed and is functioning successfully. The strains of the organism are also examined to confirm that the morphology can be inherited and that it follows a Mendelian pattern of inheritance.