B.Sc 3rd Year [VI Semester]: Paper 602: Unit VI Genetic Engineering & Techniques

A molecular genetic technique use for the direct manipulation, alteration or modification of genes or genome of organisms in order to manipulate the phenotypes is called genetic engineering."

Or in other words, we can say,

"Genetic engineering is a technique using which the genetic composition of an organism can be altered." The technique is often known as genetic manipulation, genetic modification or genetic alterations, broadly categorised as genetic engineering. By inserting a gene of interest or by deleting the unwanted DNA sequences from the genome, altered gene or DNA is constructed called as recombinant DNA which is transferred into the host genome using the vectors.

The first recombinant DNA was constructed by Paul Berg in 1972.

Using the genetic engineering technique genetically modified organism can be constructed which are economically very important. It is employed for the production of improved plant species, therapeutic drugs or proteins, prevention of inherited genetic disorders and construction of a genetically modified organism.

In the present article, we will discuss on genetic engineering and its applications. The content of the article is,

- What is genetic engineering
  - Definition
  - History
  - Types
  - Process
- Application of genetic engineering
- Limitations of genetic engineering
- Conclusion

What is genetic engineering?

Humans are manipulating the genetic material of many organisms since long. Using selective breeding and cross-hybridization, economically important plant species were created by humans. The purpose of developing the genetic engineering or genetic manipulating technique is to produce organisms or phenotypes which are useful to us. Genetic engineering techniques are used for,

- Construction of genetically modified plant species.

- Abiotic and biotic stress resistant plant species.
- Economically important plant species
- Commercially valuable organism
- For the production of therapeutic drugs
- Prevention of genetic abnormalities.

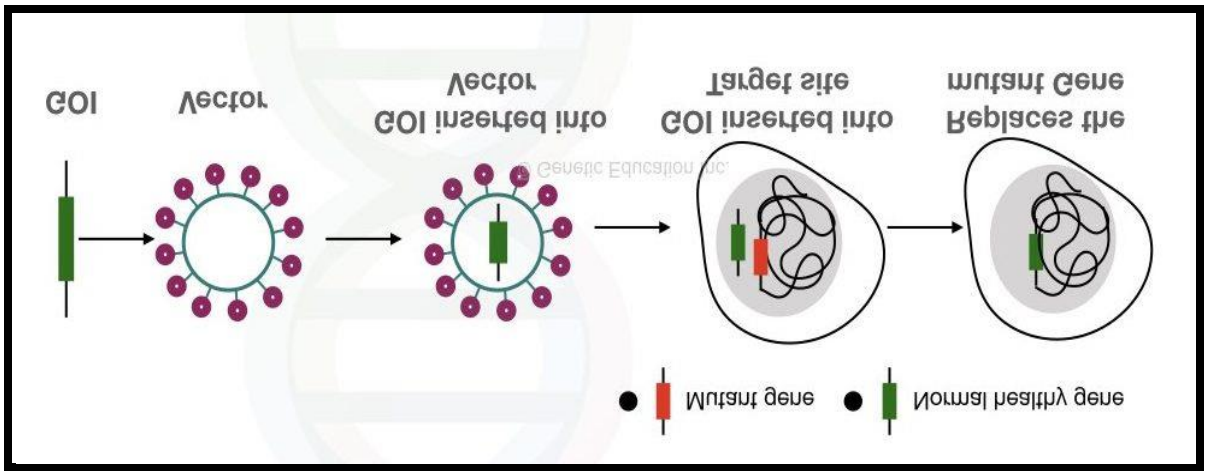
"In genetic engineering, two different cell's DNA are combined and inserted into the host genome via vector. "

Important components in any of the gene manipulation experiments are:

Gene of interest: **A DNA sequence which we want to insert in our target cells.**

Vector: **using the plasmid DNA like vectors the gene of interest is inserted into the host genome. Vectors are kind of vehicles which transfer the genetic material.**

Target cells: **target cells are the population of cells whose genome we wish to manipulate or change.**



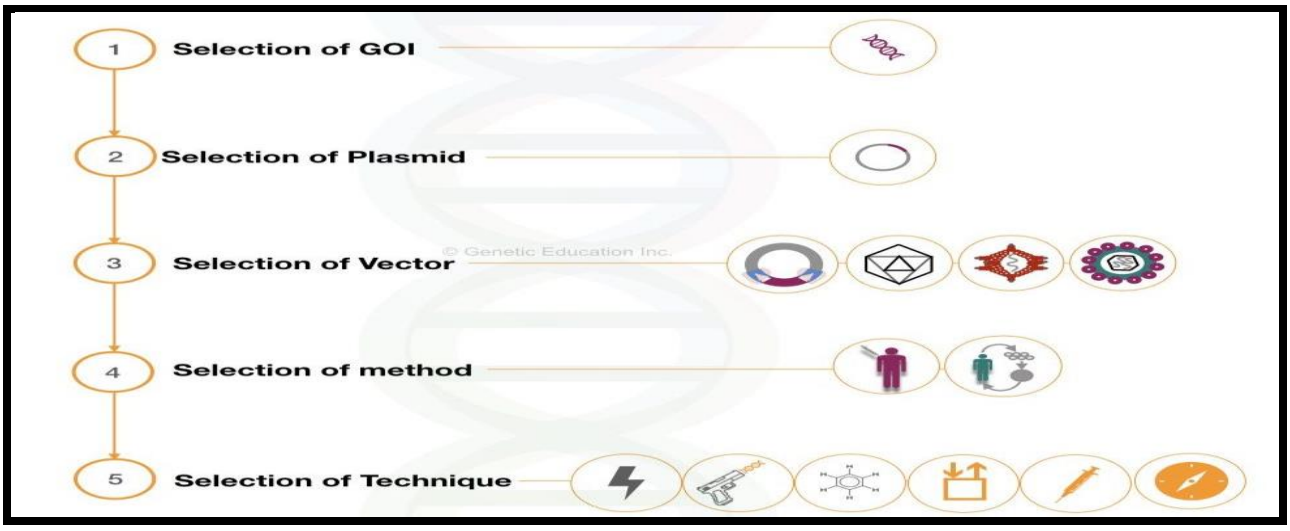
The general process of gene therapy.

➢ <u>History of genetic engineering:</u>

The term genetic engineering was first used by the science-fiction novelist, not by any scientist. In the year, 1951, Jack Williamson used the term "genetic engineering" for the first time in his novel "Dragon's island". Soon after that, the molecular structure of the DNA was discovered by Watson and Crick, although the genetic experiments were popular since the time of Mendel. The first recombinant DNA was constructed by Paul Berg in 1972. In the same year, Herbert Boyer and Stanley Cohen performed gene transfer experiments. In 1974, Rudolf Jaenisch had created genetically modified mice the first time in the history of genetics.

- **Type of genetic engineering techniques:**
  - ***Recombinant DNA-*** A recombinant DNA technology is a type of genetic engineering technique in which an artificial DNA molecule is constructed using some of the physical methods.For that, the gene of interest is inserted into the plasmid vector and used for gene transfer experiments.
  - ***Gene delivering-*** Gene delivering technique is employed for the insertion of a gene of interest into the host genome. Electrophoration, solicitation and viral vector-mediated gene transfer, liposome-mediated gene transfer, transposon-mediated gene transfer are some of the methods used for that.
  - ***Gene editing-*** A gene-editing technique is used to edit the genome in which undesired DNA sequence is removed or a new gene can be inserted into the host genome. CRISPR-CAS9, TALEN and ZFN are some known gene-editing tools used in gene therapy experiments. Read more: What is gene editing and CRISPR-CAS9?
- **Process of genetic engineering:**

  The genetic engineering technique is used for many different purposes thus we must have to decide first the purpose of the experiment. The entire process of genetic engineering can be divided into 5 broader steps:
  - Selecting and isolating the candidate gene
  - Selection and construction of plasmid
  - Gene transformation
  - Insertion of DNA into the host genome
  - Confirmation of insert



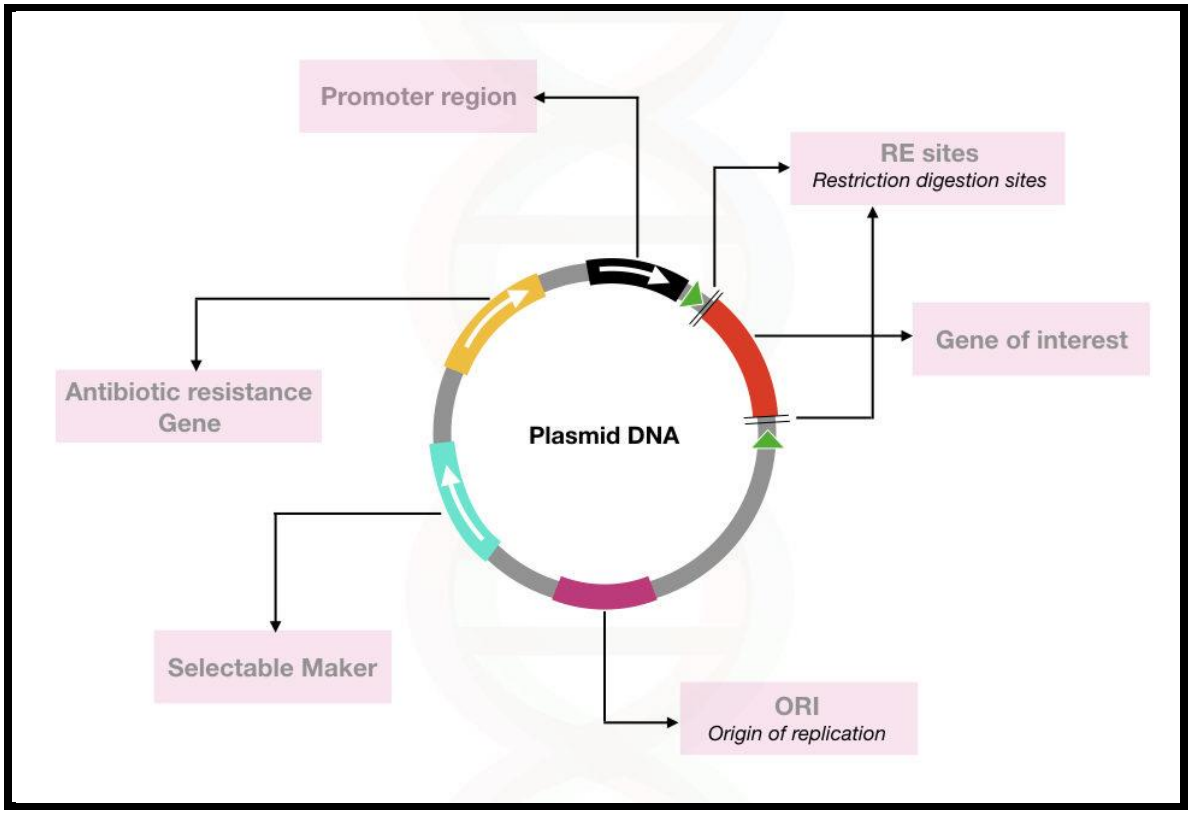
- **Selecting and isolating the candidate gene:**

The gene must contain a sequence of DNA which we want to study. The candidate gene does not have repeated DNA sequences and higher GC content. In addition to this, the gene of interest must not be too long- only a few kb genes can be successfully inserted. Longer the gene higher the chance of failure. The candidate gene must have a start and stop codon in it. Now, the gene of interest can be isolated from the rest of the DNA using either restriction digestion or polymerase chain reaction. The restriction endonucleases are the bacterial enzyme having the power to digest DNA sequence at a specific location. Using a specific type of restriction endonuclease we can cut and isolated our gene of interest. The restriction digestion method is explained in our previous article: What is restriction digestion? In the polymerase chain reaction, using the information of the sequence of a gene, the gene of interest or the candidate gene is amplified in the thermocycler. The machine, using the polymerase chain reaction amplifies millions of copies of a gene of our interest. Through the process of agarose gel electrophoresis, the amplified gene can be isolated. If the gene of interest is well studied, previously, then the information of a gene is accessible in the genetic library and we can use it for the artificial synthesis of a gene of our interest. (using the genetic library information, the gene can also be artificially synthesised). In the next step, perform DNA purification, if required, now our DNA is ready for the construction of plasmid.

- **Selection and construction of plasmid**:

Selecting plasmid for the genetic engineering experiment is one of the crucial steps in the entire experiment. Before selection the plasmid, we must understand why the plasmid is used in the gene transfer experiments. The plasmid DNA is a circular, double-stranded cytoplasmic DNA of the bacteria that replicate independently. Scientists are using it as a vehicle for transferring the gene of interest to the target

location in the genome. It can efficiently transfer the gene of interest at the target location.

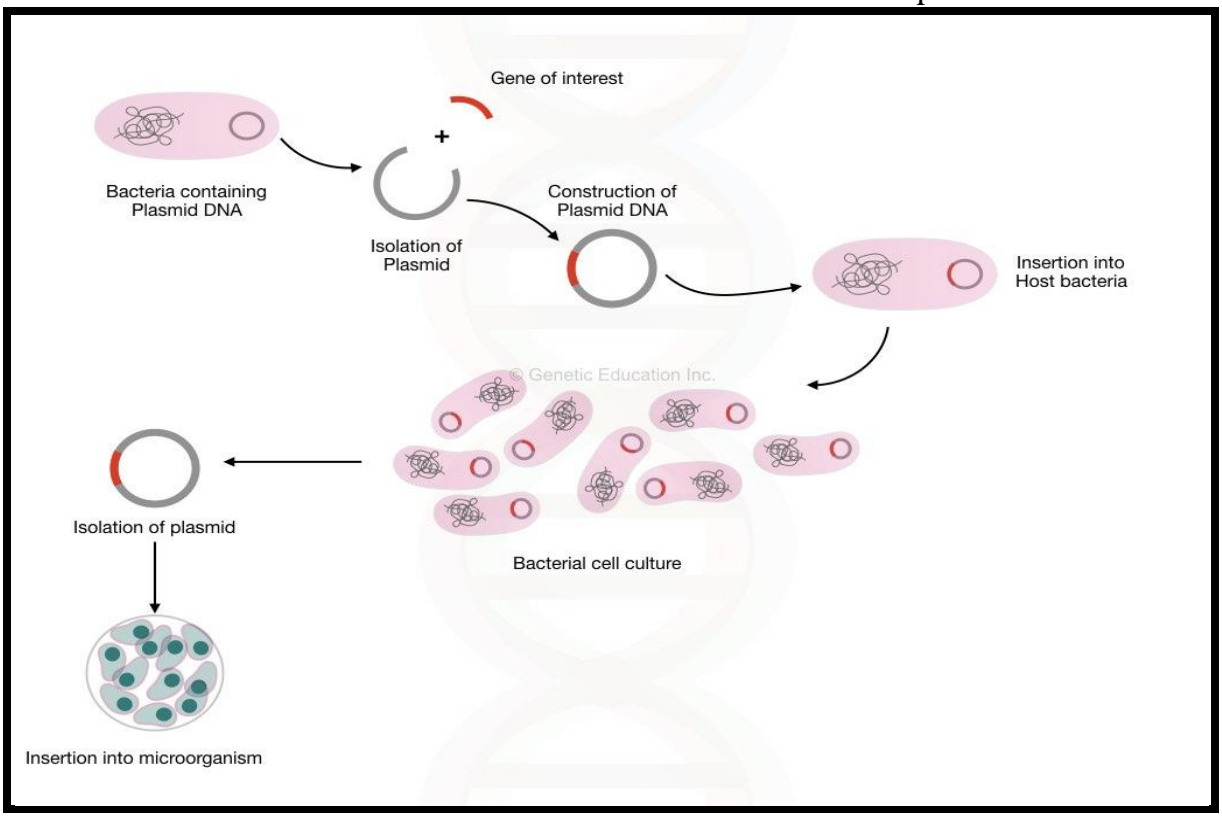


The general structure of the plasmid DNA used in recombinant DNA technology.

Preparation of plasmid:

Select the plasmid which suits your experiment. The plasmid must have the origin of replication, promoter region, antibiotic resistance gene and other important sequences. Using the restriction digestion method, an insertion site is introduced in the plasmid at which our gene of interest is ligated. Utilizing the T4 DNA ligase like power sealer, the DNA of our interest in inserted and ligated in the plasmid. Along with the plasmid, a selectable marker is also introduced in the plasmid DNA to identify the recombinant DNA. In addition to this, a promoter region and terminator sequences are also introduced in the plasmid for effective expression of a gene of our interest. A plasmid with our gene of interest and some other important sequences is called a recombinant DNA molecule. Now our recombinant DNA is ready for for the expression. If we are performing gene cloning than the plasmid is inserted in the bacterial host, for that generally *E.Coli* are commonly used. Once the bacteria starts dividing, our recombinant plasmid DNA is also replicated along with it.

Now we have the multiple copies of our plasmid DNA which are extracted using the plasmid DNA extraction kit and used for the transformation experiments.



The process of Genetic engineering.

- **Transformation into the host genome:**

Transporting the recombinant DNA into the recipient cell or the host genome is yet another tedious and difficult task. Various methods for recombinant DNA insertion is used for various cell types because a single method is not suitable for all cell types.

Various method for transformation: ***Using stress-*** bacteria easily uptake the plasmid DNA using some stress factor such as heat or electrical sock. ***Microinjection-*** a sharp needle is used for insertion of DNA directly into the nucleus of a cell, however, the method is less effective and required a higher level of expertise for that. ***Electroporation-*** one of the best method having a great success rate is an electrophoration method in which the recombinant DNA is inserted into the host genome by permeabilizing the cell with electrical current. *Sonication-* **sonication is yet another method sometimes used in the gene transfer experiment in which the recombinant DNA is inserted into the target cell using ultrasonic waves. The ultrasonic waves also increase the permeability of the cell.** *Liposome mediated gene transfer-* **Using an artificial cell-like outer coat known as a liposome- recombinant DNA can be inserted in the host genome.**

- ***Gene transfer using bacterial infection:***

This method is one of the popular methods and routinely used in plant genetic engineering experiments. Here, the plant species is infected with the transformed bacteria for inserting a gene of interest. *Agrobacterium tumifecian* is utilised to insert recombinant DNA into the plant cell. A gene of interest is inserted into the T-plasmid of the Agrobacterium. The plant cells are infected by this bacteria cell culture and the transformed cells are regenerated using the plant tissue culture methods. ***Chemical in gene transfer-*** Some metal ions, chemicals and solution of different chemicals are also used in the gene transfer experiments, however, the success rate is too low as compared with the other methods.

Confirmation of insert: Our work is still not completed. Now we have to conform, whether the recombinant DNA is inserted in our target cell or not. Various molecular genetic technologies are used for that. In the traditional culturing method, presence or absence of selectable marker is used to differentiate transformed cells from the untransformed cells. Although, it is not necessary for the PCR based detection method. The polymerase chain reaction-based detection method is widely accepted more trusted than other methods. DNA is extracted from the transformed cell and amplified using the primers complementary to our gene of interest or our recombinant DNA. If the recombinant DNA is present it surely amplified otherwise no amplification obtained. For the two factor conformation, one primer set complementary to recombinant DNA specific and one set of primer complementary to the selectable marker sequence are taken and multiplex PCR is performed. For conforming results, amplification must be obtained in both the reaction. But wait a minute! What happened if any mutation occurred during the experiment in our gene of interest? Because the PCR can only amplify the DNA.

We must need sequence information to detect the mutation. For that, the DNA sequencing method is used. DNA is extracted from the transformed cells and the gene of interest is amplified using the PCR. Now the PCR amplicons are used for DNA sequencing in which using the fluorescent chemistry the sequence of our gene of interest is orderly determined. Once all the parameter for determining the gene of interest fulfilled, our cells are now ready to inject in the host organism or for tissue culture experiments.

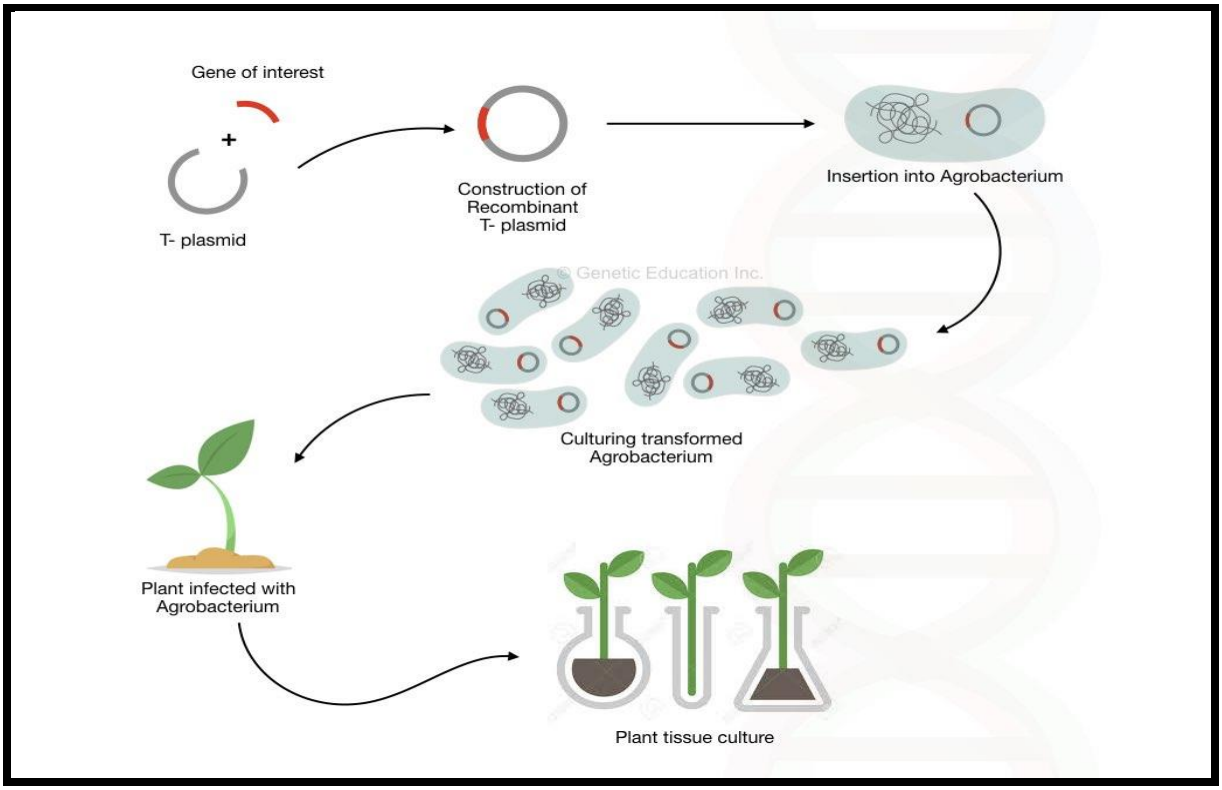
- **Applications of Genetic engineering:**

Now coming to the important point of this topic, ***"What is genetic engineering used for?"*** Genetic engineering has great industrial and agriculture value. It is practised in the medicine, genetic research, agriculture, crop improvement and for production of Therapeutic drugs. It is also used in the development of genetically modified organisms. Here we are discussing some of the important applications of genetic engineering. The recombinant DNA technology is used in the crop improvement and development of new economically important traits. Some of them are: Herbicide resistance Virus resistance Delayed fruit ripening Altered oil content Pollen control

Development of cold and drought-tolerant plant species. A classical example of it is the BT cotton- one of the types of genetically modified species give resistance to the plant against bacillus thuringiensis.

Process of developing genetically modified plant species:

A gene of interest is isolated from the organism using restriction digestion or amplified using the polymerase chain reaction. Recombinant DNA is constructed by inserting a gene of interest into the plasmid, here the T- plasmid is used. In the next step, the T- plasmid is inserted into the agrobacterium. In the last step, the plant species is infected with the transformed bacterial cells and cultured. The entire process of it is shown in the figure below,



Agrobacterium-mediated gene transfer in plant species.

GMF- genetically modified food is another best application of genetic engineering in which economically important food products are constructed using recombinant DNA technology.

The classical example of it is Flavr Savr tomato, a genetically modified tomato species made up of the antisense RNA technology.

It has great economic values as the GM- tomato can easily be transported from one region to another region of the country.

Another important application of genetic engineering is genetically modified or genetically engineered food.

The quality of some of the food products such as cotton, corn and soybeans are improved using the present recombinant DNA technology.

The aim of developing genetically modified crop or plant species is to make them economical important, nutritious, protein-rich, disease and stress resistance.

Even, using genetic engineering and tissue culture techniques insecticides resistance plant species in tobacco, potato, corn and cotton are developed.

In addition to this, some modified plants capable of generating their own fertilizers can also be created using the present genetic modification technique.

Transgenic model organisms are developed to test different parameters- the function of certain genes can be determined by designing the transgenic microorganism and animal models.

Harmful pathogens and insecticidal pasts can be destroyed using genetically modified microorganism capable of degrading toxics.

- Medicinal applications:

Low-cost drugs, hormones, enzymes and vaccines are created using the genetic engineering tools.

The anti-blood-clotting factor is a great example of it in which plasminogen activating enzyme which is capable of dissolving the blood clot is artificially designed and used in the patients with coronary artery disease or heart attack.

Other examples are two other therapeutic proteins somatostatin and lymphokines which are used against several disease condition and synthesised artificially.

Insulin is yet a classic example of therapeutic protein designed using genetic engineering technology.

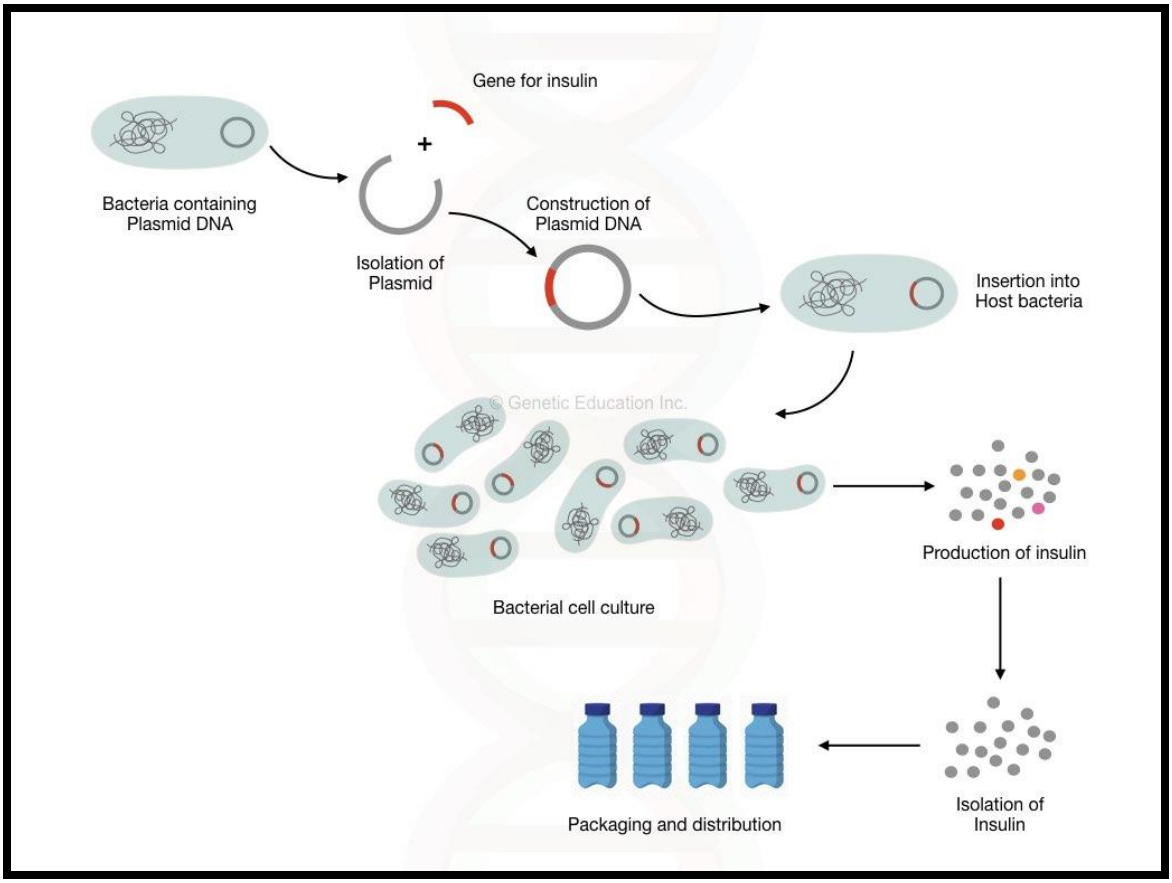
A gene for insulin is isolated by restriction digestion or through PCR and inserted int the plasmid.

The recombinant plasmid DNA is now inserted into the bacterial or yeast cell in which the plasmid is multiplying.

As the microorganism starts dividing it starts making artificial insulin.

A large amount of insulin produced using the same technique at an industrial scale.

The detailed outline of insulin production is shown in the figure below,



Production of insulin using genetic engineering technology. The commercial production of insulin started after the FDA approval in 1982. **Recombinant vaccines:** Vaccines against smallpox, herpes simplex virus and hepatitis are produced using the genetic engineering technique. The vaccines are the inactivated viral particles used to induce an immune response against that pathogen, however, the chance of contamination is high in it. Using the recombinant DNA technology scientists has created a unique type of vaccines which only contains the DNA for viral coat protein thus the pathogen can never be activated again. The main advantage of it is that it is safer, contamination-free and more reactive.

- **Genetic engineering in gene therapy:**

Using the gene therapy or gene transfer technique, inherited genetic disorders can be cured. Cystic fibrosis, Duchenne muscular dystrophy and sickle cell anaemia like gene therapies are now under final clinical trial phase and ready to use on patients.

In the gene therapy, a faulty, non-function or mutated gene is replaced with the wild type one using the same technique as explained above.

We have covered amazing articles on gene therapy, read it here:

1. Gene Therapy: Types, Vectors [Viral and Non-Viral], Process, Applications and Limitations.
2. What is Gene Therapy? and How Does it Work?
3. Naked DNA Mediated Gene Therapy
4. Sleeping Beauty Transposon System: The Future of Gene Therapy

In addition to this, the genetic engineering technology is likewise used in the production of biofuel, disease, bio alcohol and other essential products.

- **Limitations of genetic engineering:**

There are ethical issues associated with the use of gene therapy and genetically engineered products.

Also, to provide an economic value to the food product or any GM product, the nutritional values are compromised.

Because of the adverse effect of it, new resistant pathogenic strains are evolved faster.

Also, the side effects of gene therapy and the use of viruses in it are harmful to the target organism.

The technology is costlier as gene therapy cost up to 50,000 USD.

- **Conclusion:**

Playing with the embryo or fetus is against the natural law, people strongly believe in it, thus genetically modified food and plant products are always becoming a centre of controversy.

However, using genetic engineering tools such as gene therapy and gene transfer technique, inherited disorders and cancer like lethal diseases can be prevented. Positive use of genetic engineering techniques can change the fate of mankind.