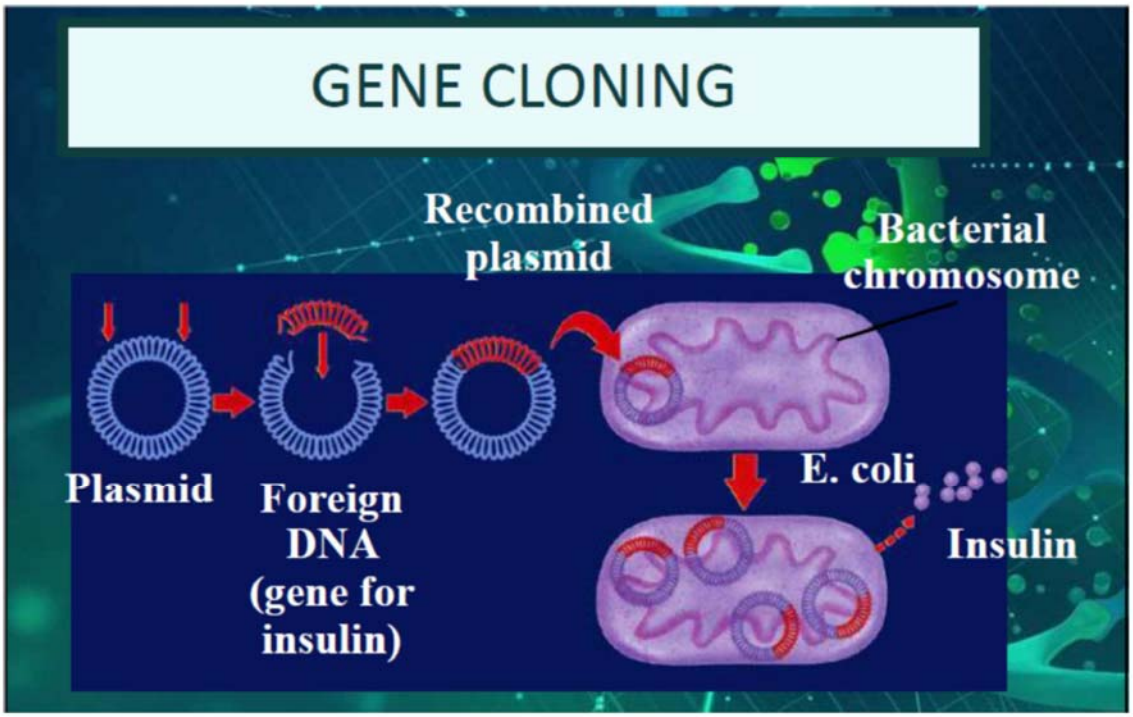
Lecture No. 5

Genetic Engineering

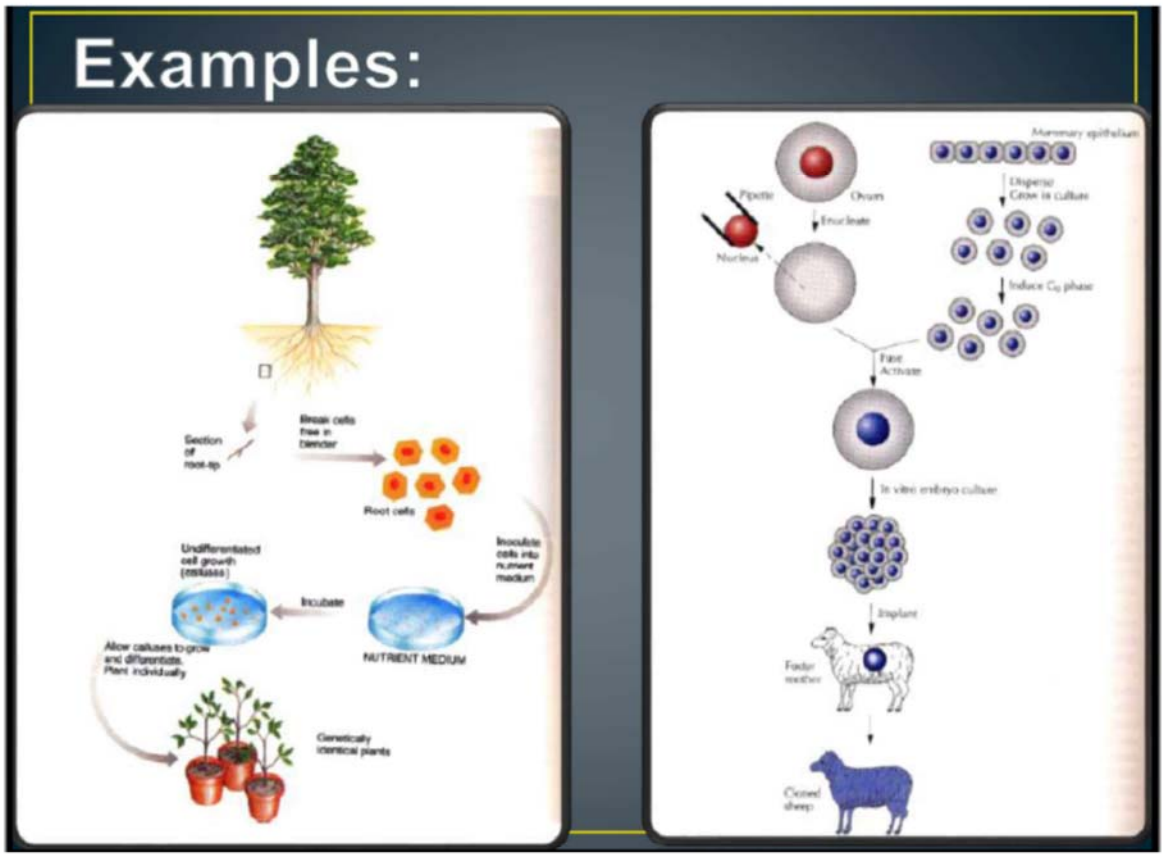
Genetic engineering (recombinant DNA technology) is the direct transfer of DNA from one organism to another. Or Genetic engineering, is manipulation/alteration of structure of gene to create a desired characteristic in an organism. It is also called genetic modification. It involves the introduction of foreign DNA or synthetic genes into the organism of interest or by altering the sequence of a gene to convert it to a different gene or deletion of an undesirable gene. The resultant DNA is called recombinant DNA. Thus it is also called recombinant DNA Technology.



History of Genetic Engineering

The term "Genetic Engineering" was first coined by Jack Williamson in his 1941 science fiction novel Dragon's Island. It was firstly accomplished in early 1970s. Since 1976 the technology has been commercialized of genetically modified food, feed-stock, and medicine Insulin, growth hormone, erythropoietin, factor IX, interferons, interleukins, tissue plasminogen activator, Vaccine production, Glo fish to help detect environmental pollutants, Flavr Savr tomato, Golden Rice, GM potato, soya, and Bt corn, the birth of the first cloned animal (the sheep: Dolly).

The gene that is transferred into a new host is known as transgene. The organisms developed after successful gene transfer are known as transgenics. The organism which carry the stably integrated foreign gene is called transformed organism.



Transformation was first demonstrated in 1928 by British bacteriologist Frederick Griffith in *Diplococcus pneumonia*. In 1944 this "transforming principle" was identified as being genetic by Oswald Avery, Colin MacLeod, and Maclyn McCarty.

Commonly there are three types of introducing genes between microorganisms, Transformation, Transduction and conjugation.

Transformation: is the process by which genetic makeup of an organism is altered by the insertion of new gene (or exogenous DNA) into its genome. This is usually done using vectors such as plasmids.

In contrast, recombinant DNA techniques, popularly termed gene cloning or genetic engineering, offer potentially unlimited opportunities for creating new combinations of genes that at the moment do not exist under natural conditions.

Genetic engineering has been also defined as the formation of new combinations of heritable material by the insertion of nucleic acid molecules, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur, but in which they are capable of continued propagation.

Genetic engineering has been gaining importance over the last few years, It will become more important in the current century as genetic diseases become more prevalent and agricultural area is reduced Genetic engineering plays significant role in the production of medicines Microorganisms and plant based substances are now being manipulated to produce large amount of useful drugs, vaccines, enzymes and hormones at low costs

Examples of genetically engineered (transgenic) organisms include:

Animals: resistant to diseases, tumor mice, Human therapeutics from recombinant DNA technology such as hormones, growth factors and antibodies.

Bacteria: Insulin, interferon, enzymes and hormones.

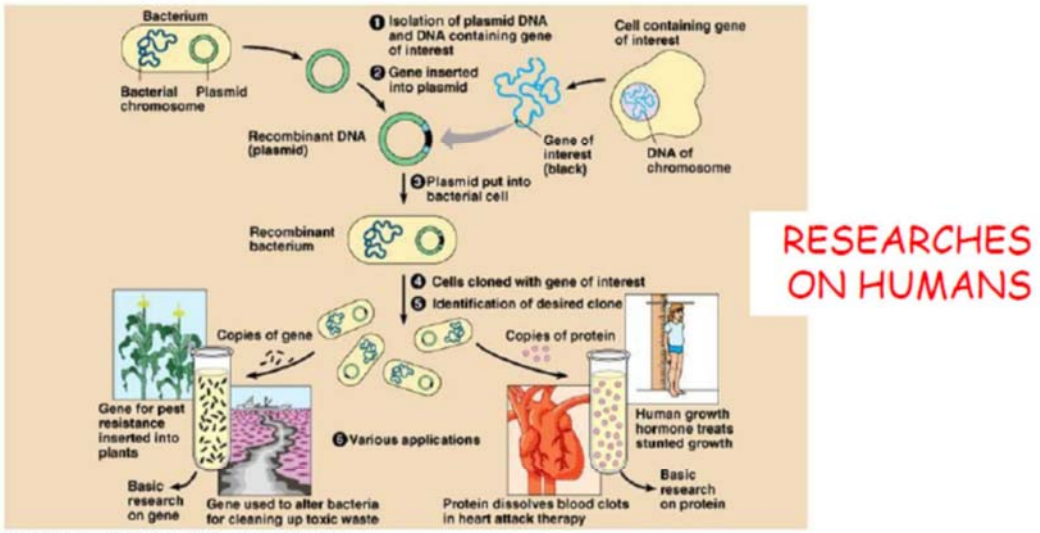
Plants: resistance to some insects, tolerate herbicides, and crops with modified oil content.

Genetic engineering Vs traditional breeding?

Even if they share the same goal which is to improve an organism's traits, there are some key differences between them. While genetic engineering manually moves genes from one organism to another, traditional breeding moves genes through mating, or crossing, the organisms in hopes of obtaining offspring with the desired combination of traits. Genetic engineering, also called transformation, works by physically removing a gene from one organism and inserting it into another, giving it the ability to express the trait encoded by that gene.

APPLICATIONS OF GENETIC ENGINEERING

DNA Recombination



Genetic Engineering stages

1- Isolation and purification of nucleic acids (Desirable gene):

After disruption of the cells (Physically or chemically), the nucleic acids must be separated from other cellular components using a variety of techniques including centrifugation, electrophoresis, adsorption and various forms of precipitation

Fragmentation of DNA molecules: DNA can be cut using mechanical or enzymatic methods. non-specific mechanical shearing will generate random DNA fragments. In contrast, when specific restriction endonuclease enzymes are used it is possible to recognize and cleave specific target base sequences in double-stranded (ds) DNA.

Splicing DNA: DNA fragments can be joined together in vitro by the action of specific DNA ligases. The DNA ligase that is widely used was encoded by phage T4.

2- The vector or carrier system:

Two broad categories of expression vector molecules have been developed as vehicles for gene transfer, plasmids (small units of DNA distinct from

chromosomes) and bacteriophages (or bacterial viruses). Vector molecules should be capable of entering the host cell and replicating within it. Ideally, the vector should be small, easily prepared and must contain at least one site where integration of foreign DNA will not destroy an essential function.

Vectors: are Small DNA molecule capable of self-replication. Carrier of DNA fragment. E.g. Plasmid, Phage, Hybrid vector, Artificial Chromosomes.

Desirable (typical) vector: Self replication, multiple copies, Replication origin site, cloning site, Selectable marker gene, Small size, Low molecular weight, simply isolated & purified. Easily inserted into host cell and has Control elements – promoter, operator, ribosome binding site.

A- Plasmids: Extra chromosomal DNA molecules, Self-replicating, Double stranded, Short sequence of DNA, Circular DNA molecules, Found mostly in prokaryotes. Characteristics a. Minimum amount of DNA. B. Two suitable markers for identification C. Single restriction site. D. More restriction enzyme. E. Size range 1kg – 200kg. F. Relaxed replication control. G. Restriction endonuclease enzyme.

Types of plasmid

1. **Fertility plasmids:** - can perform conjugation between cells through producing conjugation bridge.
2. **Resistance plasmids:** - contain genes that build a resistance against antibiotics or poisons.
3. **Col plasmids**: - contain genes that code for proteins (Colicine) that can kill bacteria.

4- Degradation plasmids: has the ability to degrade large complexed hydrocarbons

5- Antibiotic producing plasmids: have the ability to produce antibiotics

Examples of plasmid vectors, pBR322, pBR327, pBR325, pBR328, pUC8, pUC9, pUC12, pUC13.

B- Phage, Cloning large DNA fragmance , Linear Phage molecule. , Efficient than plasmid, Used in storage of recombinant DNA, Commonly used *E coli.* e.g.:- λ phage M13 Phage.

C- Bacteriophage vectors Cloning Vectors. It infects bacteria. Commonly used *E coli* phages:- λ phage, M13 Phage.

D- Hybrid vector: Component from both plasmid & phage chromosomes. Such as Cosmid, Phagmed.

Cosmids Combine parts of the lambda chromosome with parts of plasmids. Contain the cos sites of λ and plasmid origin of replication. Behave both as plasmids and as phages. Cosmids can carry up to 50 kb of inserted DNA.

E- Artificial chromosome: Linear or Circular, 1 0r 2 copies per cell, Different types Bacterial Artificial Chromosome (BAC) Yeast Artificial Chromosome (YAC) P1 derived artificial chromosome (PAC) Mammalian Artificial Chromosome (MAC) Human Artificial Chromosome.

F- Shuttle vectors: can replicate in two different organisms, e.g. bacteria and yeast, or mammalian cells and bacteria. They have the appropriate origins of replication. Hence one can clone a gene in bacteria, maybe modify it or mutate it in bacteria, and test its function by introducing it into yeast or animal cells.

3- Host organism:

The new recombinant DNA can now be introduced into the host cell and if acceptable the new DNA will be cloned with the propagation of the host cell.

4- Selection markers screening:

Selecting for Transformants cells by growing on selective media (containing antibiotic) to select for cells that took up plasmid the transformants are grown on plates containing X-Gal and IPTG. 14. Drug Resistance Gene Transferred by Plasmid leading to make the host cell resistant Strain