Genetic Engineering

Dr. Sarmad Ghazi

Food Science Department

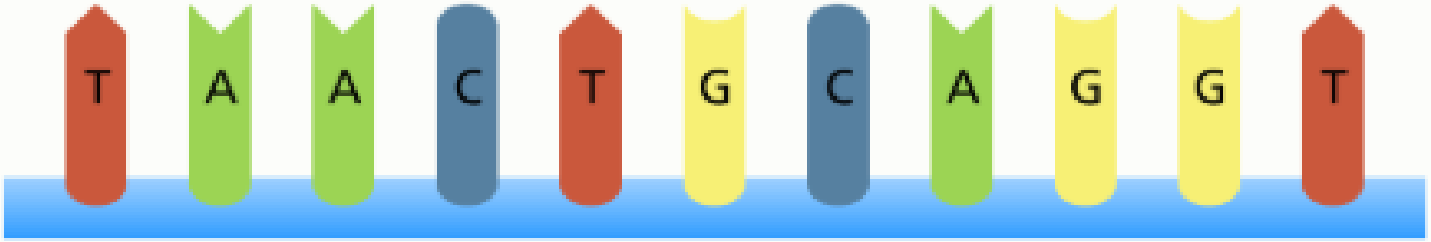
Agriculture College – Basrah University

What is a mutation?

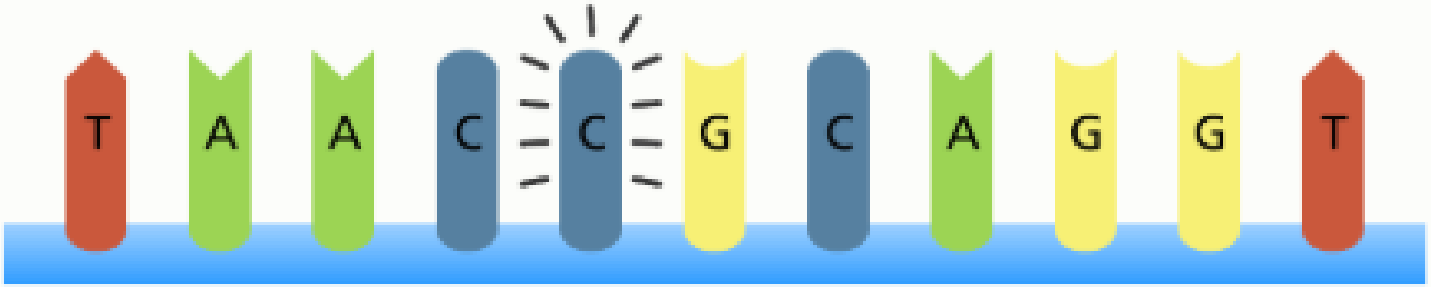
A mutation is a change that occurs in our DNA sequence, either due to mistakes when the DNA is copied or as the result of environmental factors such as UV light and cigarette smoke.

- Over a lifetime our DNA[?] can undergo changes or 'mutations[?]' in the sequence of bases[?], A, C, G and T.
- This results in changes in the proteins[?] that are made. This can be a bad or a good thing.
- Mutations can occur during DNA replication[?] if errors are made and not corrected in time.
- Mutations can also occur as the result of exposure to environmental factors such as smoking, sunlight and radiation.
- Often cells can recognize any potentially mutation-causing damage and repair it before it becomes a fixed mutation.
- Mutations contribute to genetic variation[?] within species[?].
- Mutations can also be inherited, particularly if they have a positive effect.
- For example, the disorder sickle cell anaemia[?] is caused by a mutation in the gene[?] that instructs the building of a protein called haemoglobin[?]. This causes the red blood cells[?] to become an abnormal, rigid, sickle shape. However, in African populations, having this mutation also protects against malaria[?].
- However, mutation can also disrupt normal gene activity and cause diseases, like cancer[?]
- Cancer is the most common human genetic disease; it is caused by mutations occurring in a number of growth-controlling genes. Sometimes faulty, cancer-causing genes can exist from birth, increasing a person's chance of getting cancer.

Original sequence



Point mutation

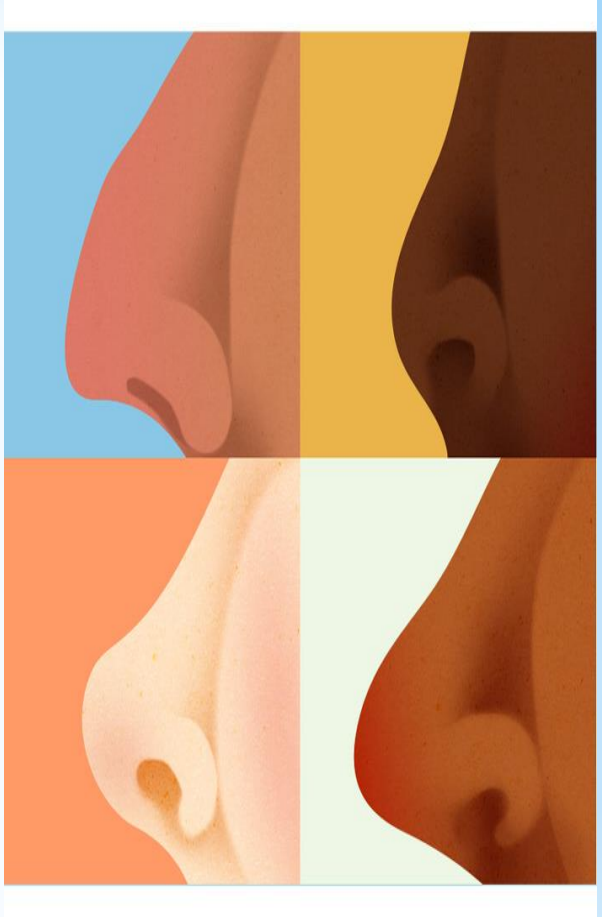


An illustration to show an example of a DNA mutation.

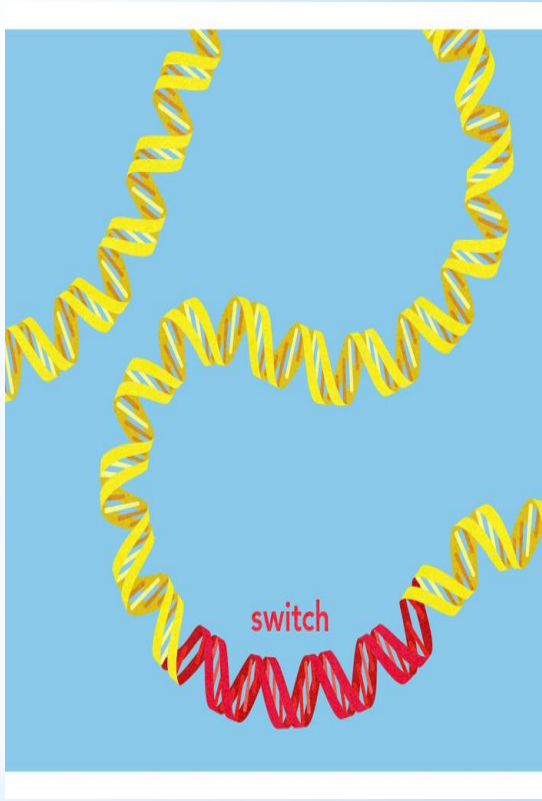
Image credit: Genome Research Limited

* *Mutation Generates New Alleles*
* The whole human family is one species with the same genes. Mutation creates slightly different versions of the same genes, called alleles. These small differences in DNA sequence make every individual unique. They account for the variation we see in human hair color, skin color, height, shape, behavior, and susceptibility to disease. Individuals in other species vary too, in both physical appearance and behavior.
* Genetic variation is useful because it helps populations change over time. Variations that help an organism survive and reproduce are passed on to the next generation. Variations that hinder survival and reproduction are eliminated from the population. This process of natural selection can lead to significant changes in the appearance, behavior, or physiology of individuals in a population, in just a few generations.
* Once new alleles arise, meiosis and sexual reproduction combine different alleles in new ways to increase genetic variation

* *Mutation vs. variation*
* It's useful to think of mutation as a process that creates genetic variation. We often refer to a mutation as a thing—the genetic variation itself. This approach can be useful when it comes to a gene associated with a disease: the disease allele carries a mutation, a DNA change that compromises the protein's function. However, this approach gives mutation a bad name.
* It’s important to remember that losing the function of a gene doesn’t always affect health. For example, most mammals have hundreds of genes that code for olfactory receptors, proteins that help us smell. Losing one of these genes probably doesn’t make all that much difference.
* In contrast to variations that cause disease, there are many more examples of variations that are neither good nor bad, but just different—like blood types and eye color. Just like with disease alleles, the process of mutation creates these more neutral variations. But with neutral variations, it can be impossible to tell which allele is the "normal" one that existed first and which is the "mutant"—and the distinction is often meaningless.

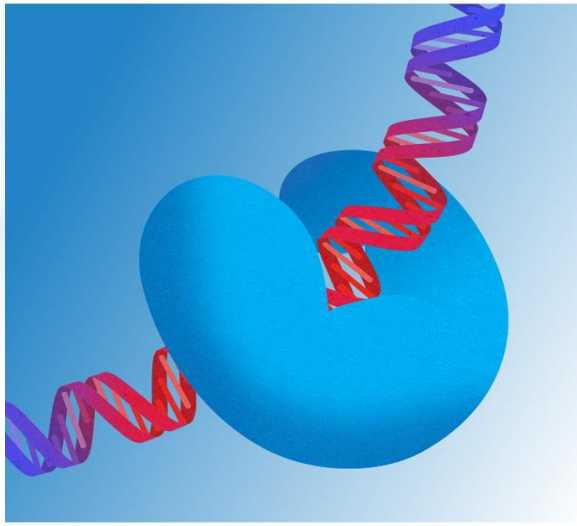


* *Proteins and switches*
* Mutation creates variations in protein-coding portions of genes that can affect the protein itself. But even more often, it creates variations in the "switches" that control when and where a protein is active and how much protein is made.
* Lactase is an enzyme that helps infants break down lactose, a sugar in milk. Normally the gene that codes for lactase is active in babies and then turned off at about age four. When people who don't make lactase consume milk, they experience gas, nausea, and discomfort. But some people have a variation in a genetic switch that keeps the lactase gene active. This variation is called "lactase persistence," and people who have it can keep milk in their diets even as adults.



* Other drivers of mutation: Environmental agents
* Radiation, chemicals, byproducts of cellular metabolism, free radicals, ultraviolet rays from the sun—these agents damage thousands of nucleotides in each of our cells every day. They affect the nucleotides themselves: converting one base to another, knocking a base off its backbone, or even causing a

* *DNA Repair*
* Most of the time, mutation is reversed. DNA repair machines are constantly at work in our cells, fixing mismatched nucleotides and splicing broken DNA strands back together. Yet some DNA changes remain. If a cell accumulates too many changes—if its DNA is so damaged that repair machinery cannot fix it—it either stops dividing or it self-destructs. If any of these processes go wrong, the cell could become cancerous.
* When we put on sun screen, we are protecting ourselves against mutation in somatic cells—the cells that make up the body and are not involved in reproduction. Only when DNA changes are carried in egg and sperm cells are they passed to the next generation. Believe it or not, a certain amount of sloppiness is built into the system. Without mutation there would be no variation, and without variation there would be no evolution.



Mutagen

* In genetics, a mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level. As many mutations can cause cancer, mutagens are therefore also likely to be carcinogens, although not always necessarily so. All mutagens have characteristic mutational signatures with some chemicals becoming mutagenic through cellular processes. Not all mutations are caused by mutagens: so-called "spontaneous mutations" occur due to spontaneous hydrolysis, errors in DNA replication, repair and recombination.

* Mutagens may be of physical, chemical or biological origin. They may act directly on the DNA, causing direct damage to the DNA, and most often result in replication error. Some however may act on the replication mechanism and chromosomal partition. Many mutagens are not mutagenic by themselves, but can form mutagenic metabolites through cellular processes,

*Types

* **Physical mutagens**

* Ionizing radiations such as X-rays, gamma rays and alpha particles cause DNA breakage and other damages. The most common lab sources include cobalt-60 and cesium-137.
* Ultraviolet radiations with wavelength above 260 nm are absorbed strongly by bases, producing pyrimidine dimers, which can cause error in replication if left uncorrected.
* Radioactive decay, such as ^{14}C in DNA which decays into nitrogen.

DNA reactive chemicals

* A large number of chemicals may interact directly with DNA. However, many such as PAHs, aromatic amines, benzene are not necessarily mutagenic by themselves, but through metabolic processes in cells they produce mutagenic compounds.
* Reactive oxygen species (ROS) – These may be superoxide, hydroxyl radicals and hydrogen peroxide, and large number of these highly reactive species are generated by normal cellular processes, for example as a by-products of mitochondrial electron transport, or lipid peroxidation.
* Deaminating agents, for example nitrous acid which can cause transition mutations by converting cytosine to uracil.
* Polycyclic aromatic hydrocarbon (PAH), when activated to diol-epoxides can bind to DNA and form adducts.
* Alkylating agents such as ethylnitrosourea. The compounds transfer methyl or ethyl group to bases or the backbone phosphate groups.
* Aromatic amines and amides have been associated with carcinogenesis since 1895 when German physician Ludwig Rehn observed high incidence of bladder cancer among workers in German synthetic aromatic amine dye industry. 2-Acetylaminofluorene, originally used as a pesticide but may also be found in cooked meat, may cause cancer of the bladder, liver, ear, intestine, thyroid and breast.
* Alkaloid from plants, such as those from Vinca species,[34] may be converted by metabolic processes into the active mutagen or carcinogen.
* Bromine and some compounds that contain bromine in their chemical structure.[35]
* Sodium azide, an azide salt that is a common reagent in organic synthesis and a component in many car airbag systems
* Psoralen combined with ultraviolet radiation causes DNA cross-linking and hence chromosome breakage.
* Benzene, an industrial solvent and precursor in the production of drugs, plastics, synthetic rubber and dyes.

* **Base analogs**

* Base analog, which can substitute for DNA bases during replication and cause transition mutations.
* **Intercalating agents**
* Intercalating agents, such as ethidium bromide and proflavine, are molecules that may insert between bases in DNA, causing frameshift mutation during replication. Some such as daunorubicin may block transcription and replication, making them highly toxic to proliferating cells.
* **Metals**
* Many metals, such as arsenic, cadmium, chromium, nickel and their compounds may be mutagenic, but they may act, however, via a number of different mechanisms.[36] Arsenic, chromium, iron, and nickel

* **Biological agents**
* Transposon, a section of DNA that undergoes autonomous fragment relocation/multiplication. Its insertion into chromosomal DNA disrupts functional elements of the genes.
* Virus – Virus DNA may be inserted into the genome and disrupts genetic function. Infectious agents have been suggested to cause cancer as early as 1908
* Bacteria – some bacteria such as *Helicobacter pylori* cause inflammation during which oxidative species are produced, causing DNA damage and reducing efficiency of DNA repair systems, thereby increasing mutation.

Traditional Genetic Improvement Strategies

* **Mutation and Selection**

* In nature, mutations (changes in the chromosome of an organism) occur spontaneously at very low rates (one mutational event in every 10^6 to 10^7 cells per generation. These mutations occur at random throughout the chromosome, and a spontaneous mutation in a metabolic pathway of interest for food fermentations would be an extremely rare event. The mutation rate can be dramatically increased by exposure of microorganisms to mutagenic agents, such as ultraviolet light or various chemicals, which induce changes in the deoxyribonucleic acid (DNA) of host cells. Mutation rates can be increased to one mutational event in every 10^1 or 10^2 cells per generation for auxotrophic mutants, and one in 10^3 to 10^5 for the isolation of improved secondary metabolite producers. A method of selection is critical for effective screening of mutants as several thousand individual isolates may need to be evaluated to find one strain with improved activity in the property of interest.
* Mutation and selection techniques have been used to improve the metabolic properties of microbial starter cultures used for food fermentations; however, there are severe limitations with this method. Mutagenic agents cause random mutations, thus specificity and precision are not possible. Potentially deleterious undetected mutations can occur, since selection systems may be geared for only the mutation of interest. Additionally, traditional mutation procedures are extremely costly and time-consuming and there is no opportunity to expand the gene pool. In spite of these limitations, mutation and selection techniques have been used extensively to improve industrially important microorganisms and, in some cases, yields of greater than 100-times the normal production level of bacterial secondary metabolites have been achieved.

* Natural Gene Transfer Methods

* The discovery of natural gene transfer systems in bacteria has greatly facilitated the understanding of the genetics of microbial starter cultures and in some cases has been used for strain improvement. Genetic exchange in bacteria can occur naturally by three different mechanisms: transduction, conjugation, and transformation.

* **Transduction**
* Transduction involves genetic exchange mediated by a bacterial virus (bacteriophage). The bacteriophage acquires a portion of the chromosome or plasmid from the host strains and transfers it to a recipient during subsequent viral infection. Although transduction has been exploited for the development of a highly efficient gene transfer system in the gram-negative organism *Escherichia coli*, it has not been used extensively for improving microorganisms used in food fermentations. In general, transduction efficiencies are low and gene transfer is not always possible between unrelated strains, limiting the usefulness of the technique for strain improvement. In addition, bacteriophage have not been isolated and are not well characterized for most strains.

Conjugation

- Conjugation, or bacterial mating, is a natural gene transfer system that requires close physical contact between donors and recipients and is responsible for the dissemination of plasmids in nature. Numerous genera of bacteria harbor plasmid DNA. In most cases, these plasmids are cryptic (the functions encoded are not known), but in some cases important metabolic traits are encoded by plasmid DNA. If these plasmids are also self-transmissible or mobilizable, they can be transferred to recipient strains. Once introduced into a new strain, the properties encoded by the plasmid can be expressed in the recipient. The lactic acid bacteria naturally contain from one to more than ten distinct plasmids, and metabolically important traits, including lactose-fermenting ability, bacteriophage resistance, and bacteriocin production, have been linked to plasmid DNA. Conjugation has been used to transfer these plasmids into recipient strains for the construction of genetically improved commercial dairy starter cultures.
- There are some limitations in the application of conjugation for strain improvement. To exploit the use of conjugative improvement requires an understanding of plasmid biology and, in many cases, few conjugative plasmids encoding genes of interest have been identified or sufficiently characterized. Conjugation efficiencies vary widely and not all strains are able to serve as recipients for conjugation. Moreover, there is no opportunity to expand the gene pool beyond those plasmids already present in the species.

Transformation

Certain microorganisms are able to take up naked DNA present in the surrounding medium. This process is called transformation and this gene transfer process is limited to strains that are naturally competent. Competence-dependent transformation is limited to a few, primarily pathogenic, genera, and has not been used extensively for genetic improvement of microbial starter cultures. For many species of bacteria, the thick peptidoglycan layer present in gram-positive cell walls is considered a potential barrier to DNA uptake. Methods have been developed for enzymatic removal of the cell wall to create protoplasts. In the presence of polyethylene glycol, DNA uptake by protoplasts is facilitated. If maintained under osmotically stabilized conditions, transformed protoplasts regenerate cell walls and express the transformed DNA. Protoplast transformation procedures have been developed for some of the lactic acid bacteria; however, the procedures are tedious and time-consuming, and frequently parameters must be optimized for each strain. Transformation efficiencies are often low and highly variable, limiting the application of the technique for strain improvement.

*Vector Systems

* A vector can be defined as a vehicle for transferring DNA from one strain to another. Plasmids are frequently used for this purpose because they are small autonomously replicating circular DNA forms that are stable and relatively easy to isolate, characterize, and manipulate in the laboratory. Native plasmids do not naturally possess all of the desirable features of a vector (e.g., multiple cloning sites, selectable marker(s), ability to replicate in several hosts, and so forth). Therefore, genetic engineering is frequently used to construct multifunctional cloning vectors. Although antibiotic resistance markers greatly facilitate genetic engineering in microbial systems, vectors derived solely from food-grade organisms may be critical in obtaining regulatory approval for use of the organisms, as antibiotic resistance determinants may not be acceptable in food systems.
* An alternative vector strategy involves the development of linear fragments of DNA that are capable of integrating into the host chromosome via homologous recombination. Although transformation frequencies are very low, the advantage of the integrative vector is that transformed genetic information is targeted to the chromosome where it will be more stably maintained. Insertion sequences (IS elements) naturally present in the chromosome that can transpose chromosomal DNA to plasmids could be used as an alternative strategy for developing integrative vectors for some strains of lactic acid bacteria.

* Recombinant DNA Technology

* Steps in Recombinant DNA Technology:

* Basic steps involved in rec DNA technology (or genetic engineering) are given below (Fig. 1):

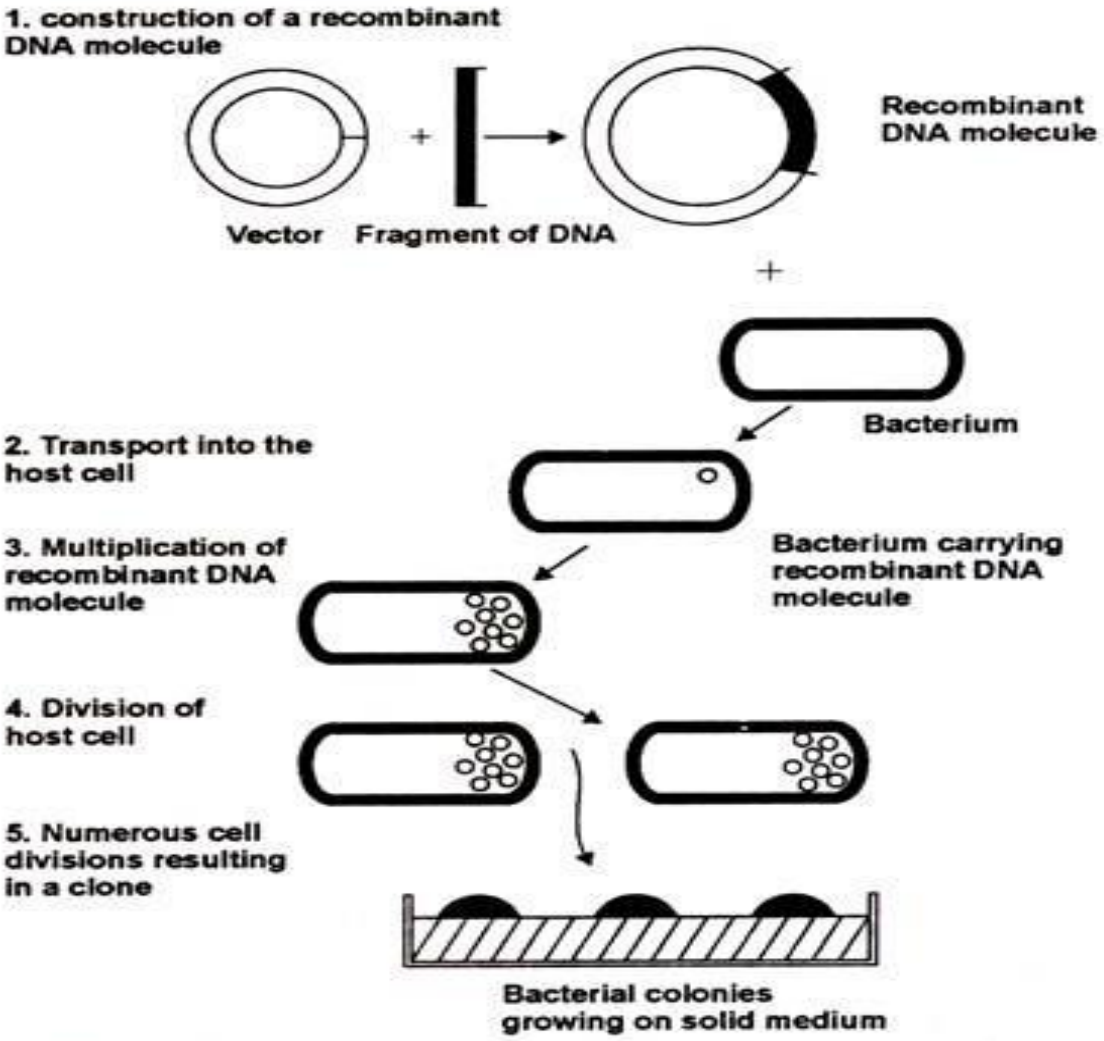


Fig. 1. The basic steps of rec DNA Technology using the bacterial plasmid as cloning vector.

* i. Selection and isolation of DNA insert
* ii. Selection of suitable cloning vector
* iii. Introduction of DNA-insert into vector to form rec DNA molecule
* iv. rec DNA molecule is introduced into a suitable host.
* v. Selection of transformed host cells.
* vi. Expression and multiplication of DNA-insert in the host.

* **(i) Selection and isolation of DNA insert:**
* First step in rec DNA technology is the selection of a DNA segment of interest which is to be cloned. This desired DNA segment is then isolated enzymatically. This DNA segment of interest is termed as DNA insert or foreign DNA or target DNA or cloned DNA.
* **(ii) Selection of suitable cloning vector:**
* A cloning vector is a self-replicating DNA molecule, into which the DNA insert is to be integrated. A suitable cloning vector is selected in the next step of rec DNA technology. Most commonly used vectors are plasmids and bacteriophages.
* **(iii) Introduction of DNA-insert into vector to form recDNA molecule:**
* The target DNA or the DNA insert which has been extracted and cleaved enzymatically by the selective restriction endonuclease enzymes [in step (i)] are now ligated (joined) by the enzyme ligase to vector DNA to form a rec DNA molecule which is often called as cloning-vector-insert DNA construct.

* **(iv) rec DNA molecule is introduced into a suitable host:**
* Suitable host cells are selected and the rec DNA molecule so formed [in step (iii)] is introduced into these host cells. This process of entry of rec DNA into the host cell is called transformation. Usually selected hosts are bacterial cells like E. coli, however yeast, fungi may also be utilized.

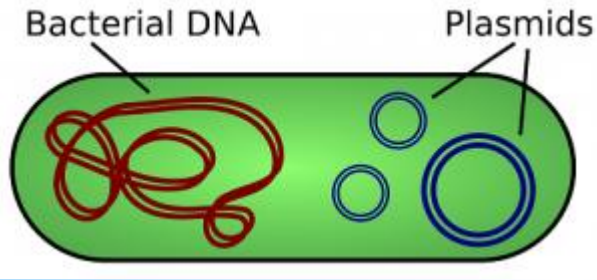
* **(v) Selection of transformed host cells:**
* Transformed cells (or recombinant cells) are those host cells which have taken up the recDNA molecule. In this step the transformed cells are separated from the non-transformed cells by using various methods making use of marker genes.

* **(vi) Expression and Multiplication of DNA insert in the host:**
* Finally, it is to be ensured that the foreign DNA inserted into the vector DNA is expressing the desired character in the host cells. Also, the transformed host cells are multiplied to obtain sufficient number of copies. If needed, such genes may also be transferred and expressed into another organism.

* **Important biological tools for rec DNA technology are:**
* **(A) Enzymes:**
* a. Restriction Endonucleases
* b. Exonucleases
* c. DNA ligases
* d. DNA polymerase
* (B) Cloning Vector
* (C) Host organism
* (D) DNA insert or foreign DNA
* (E) Linker and adaptor sequences.

*plasmid / plasmids

* A plasmid is a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA. Plasmids naturally exist in bacterial cells, and they also occur in some eukaryotes. Often, the genes carried in plasmids provide bacteria with genetic advantages, such as antibiotic resistance. Plasmids have a wide range of lengths, from roughly one thousand DNA base pairs to hundreds of thousands of base pairs. When a bacterium divides, all of the plasmids contained within the cell are copied such that each daughter cell receives a copy of each plasmid. Bacteria can also transfer plasmids to one another through a process called conjugation.
* Scientists have taken advantage of plasmids to use them as tools to clone, transfer, and manipulate genes. Plasmids that are used experimentally for these purposes are called vectors. Researchers can insert DNA fragments or genes into a plasmid vector, creating a so-called recombinant plasmid. This plasmid can be introduced into a bacterium by way of the process called transformation. Then, because bacteria divide rapidly, they can be used as factories to copy DNA fragments in large quantities.



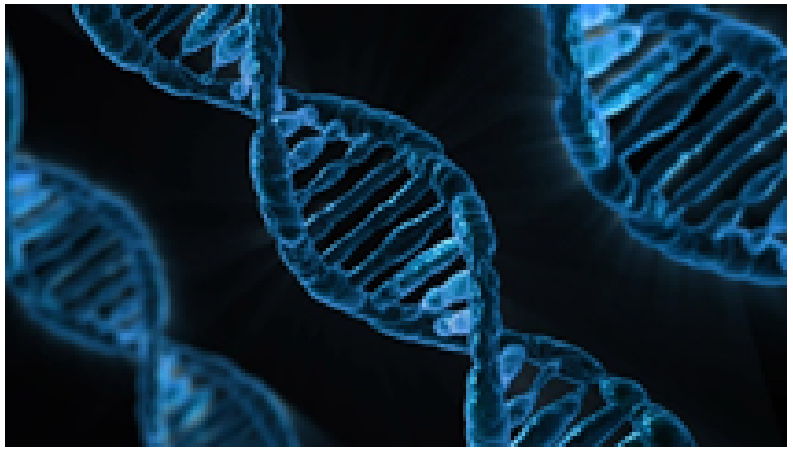
* Applications of Genetic Engineering
*
* (i) Transfer of nitrogen fixing genes (nif genes) from leguminous plants into cereals.
* (ii) Transfer of resistance against pathogens and pests from wild plants to crop plants.
* (iii) Improvement in quality and quantity of seed proteins.
* (iv) Transfer of genes for animal proteins to crop plants.
* (v) Elimination of unwanted genes for susceptibility to different diseases from cytoplasmic male sterile lines in crop like maize, where cytoplasmic male sterility and susceptibility are located in mitochondrial plasmid.
* (vi) Improvement of photosynthetic efficiency by reassembling nuclear and chloroplast genes and by the possible conversion of C_3plants into C_4 plants.
* (vii) Development of cell lines which may produce nutritious food in bioreactors.

* A number of other genes can be combined with crops to produce desirable properties such as:
* Herbicide-, drought-, freeze- or disease-resistance
* Higher yield
* Faster growth
* Improved nutrition
* Longer shelf life

What is genetic engineering?

Genetic engineering is the direct modification of an organism's genome, which is the list of specific traits (genes) stored in the DNA.

Changing the genome enables engineers to give desirable properties to different organisms.



Organisms created by genetic engineering are called genetically modified organisms (GMOs).

GMO Bacteria

Bacteria are the most common GMOs because their simple structure permits easy manipulation of their DNA.

One of the most interesting uses for genetically modified bacteria is the production of hydrocarbons (plastics and fuels) usually only found in fossil fuels.

- Cyanobacteria have been modified to produce plastic (polyethylene) and fuel (butanol) as byproducts of photosynthesis
- E. Coli bacteria have been modified to produce diesel fuel

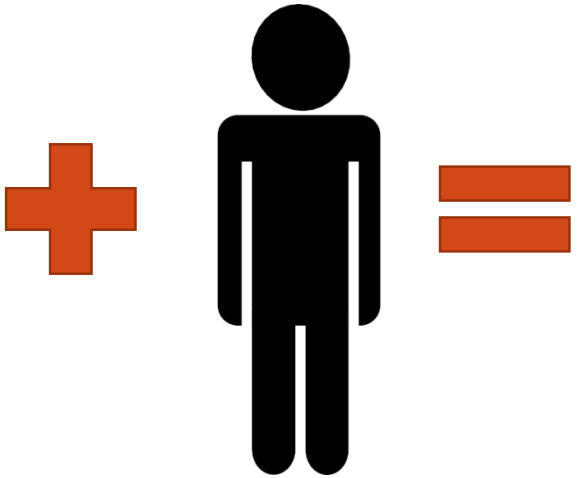
Fast-Growing Salmon

Genes from two other fish cause this salmon to continually produce growth hormones

Less Smelly Cows

Modifying bacteria responsible for methane production in cattle results in 25% less-flatulent cows

Could Spiderman Be Real?



Web-Producing Goats

Spider genes in goats enable the production of spider silk in goat milk

* Vocabulary/Definitions
* *DNA:* Acronym for deoxyribonucleic acid, which is a molecule that contains an organism's complete genetic information.
* *gene:* The molecular unit of an organism that contains information for a specific trait (specific DNA sequence).
* *genome:* An entire set of genes for an organism.
* *GMO:* Acronym for genetically modified organism.
* *nucleotide:* The building block of DNA.
* *plasmid:* The circular DNA structure used by bacteria.
* *protein:* Large biomolecules used by an organism for a number of purposes; in this context, to express a desired trait.
* *recombinant DNA:* DNA to which a section has been removed and replaced (recombined) with a new sequence.
* *restriction enzyme:* An enzyme that "cuts" DNA when specific base pair sequences are present.
* *trait:* A distinguishing characteristic.