Genetics and Biotechnology

Genetic Engineering:

The gene is the basic unit of heredity: apiece of deoxyribonucleic acid DNA (consist of Nucleotides) that codes for the production of single proteins. These proteins are usually enzymes, and because enzymes control the formation of many different substances, genes are able to exert a direct influence on the characterization of organisms. A single gene which codes for a particular characteristic in this way can be passed on from generation to generation.

Genetic engineering or modification is a means of introducing new genes into cells or modifying existing genes. The resulting cells will, therefore have new characteristics. Organisms which have been genetically engineering are often referred to as (GMO_s) [genetically modified organisms].

In the early 1970_s researchers found restriction enzymes in bacterial cells. These enzymes are part of the natural defense mechanism of bacteria and are released when any foreign nucleic acid enters the bacteria cell. For instance, when a virus invades a bacterium, it injects its own nucleic acid, either DNA (Deoxyribose + Nitrogen base + Phosphate group) or RNA (Ribose + Nitrogen base + Phosphate group), which "reprogrammes" the bacterial cells and uses the bacterial ribosomes to manufacture new virus particles. To defend itself, the bacterium produce restriction enzymes that cut the viral DNA or RNA into small fragments, which cannot reprogrammed the cell.

Genetic engineers have found these restriction enzymes useful for a number of reasons. Restriction enzymes are very specific and each recognizes and cuts only one particular nucleotide sequence in the DNA.

Another useful property of restriction enzymes in that some of them has the ability to produce a staggered cut. This means that fragments produced from a double strand of DNA have single-stranded "sticking ends" protruding (figure).

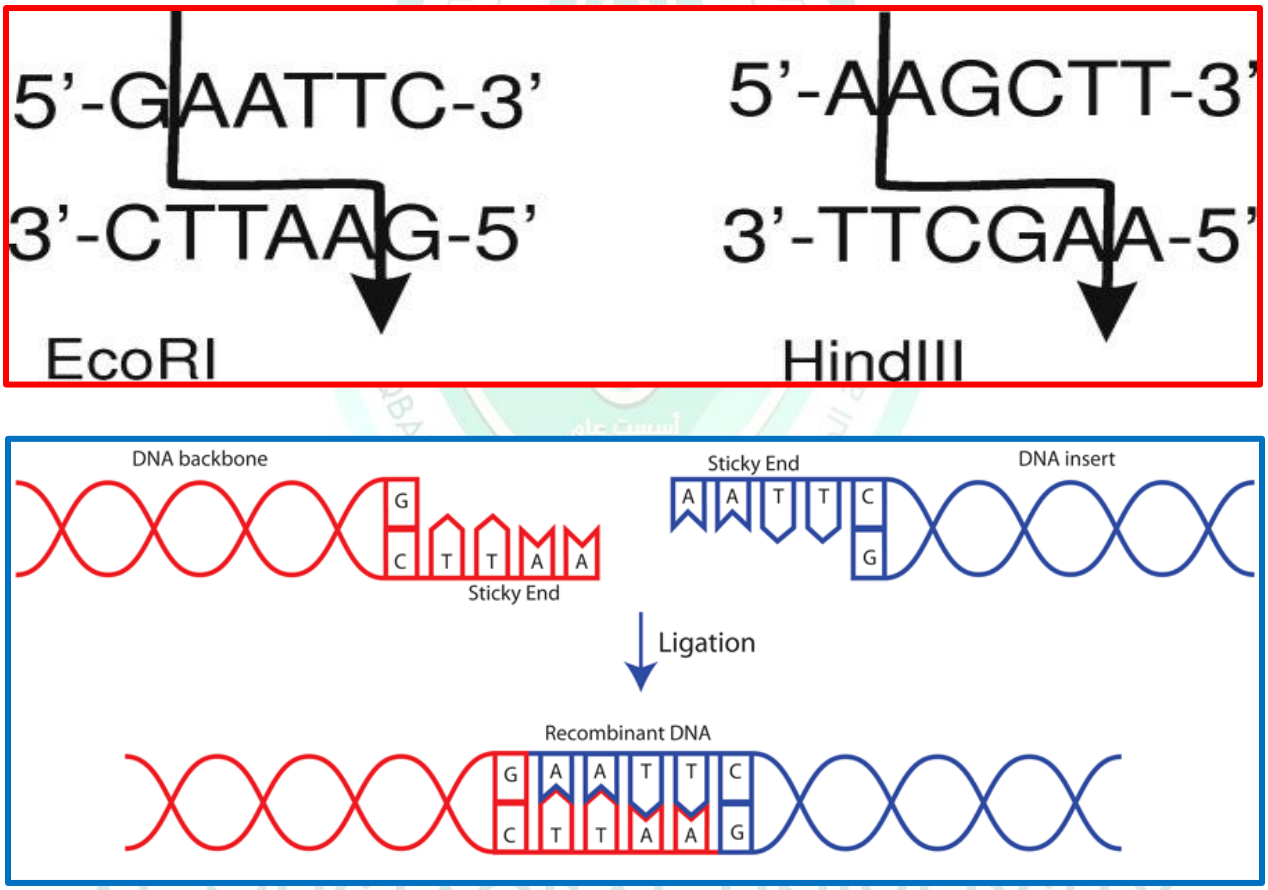


Figure : How "sticky ends" are produced

These single-stranded ends have a sequence of bases [purines (A and G) and pyrimidines (T, C and U)] that can recognize and pair with one another. For example, the restriction enzyme (EcoRl from *Escherichia coli* RY13) recognizes the sequence GAATTC and cuts the DNA between G and A so that the fragments have the

sequence A ATT on one end and TTAA on the other. The restriction enzyme Hind III from *Haemophilus influenza*. Rd, recognizes the sequence AAGCTT and cuts the DNA between the two A bases so that AGCT is on one fragment and TCGA on the other. If the fragments are brought together, provided that the conditions are right, they will join together again and can be resealed by using another enzyme called a DNA ligase. This allows DNA from different sources to be combined, forming recombinant DNA. This will happen provided that the DNA fragments are cut originally by the same restriction enzyme.

Mutations:

Microorganisms used in biotechnological processes were originally isolated from the natural environment but have subsequently been modified by the industrial geneticist into superior organisms for specific productivity. The success of strain selection and improvement programmes practiced by all biologically-based industries (e-g-brewing and antibiotics, etc.) is a direct result of the close cooperation between the technologist and geneticist. All properties of organisms depend on the sum of their genes. There are two types of genes. Structural and regulatory genes. Structural genes encode for amino acid sequences of protein which, as enzymes. In contrast, the regulatory genes control the expression of the structural genes by determining the rate of production of their protein products. In biotechnological processes the aim is primarily to obtain better organisms by using screening and selection techniques for optimize the conditions for production.

In most industrial genetics the basis for changing the organism's genome has been by mutation using physical mutants such as X-rays, U.V-rays and thymine starvation or chemical mutants such as Mitomycin c, Methyl methane sulphonate, 5-Bromo

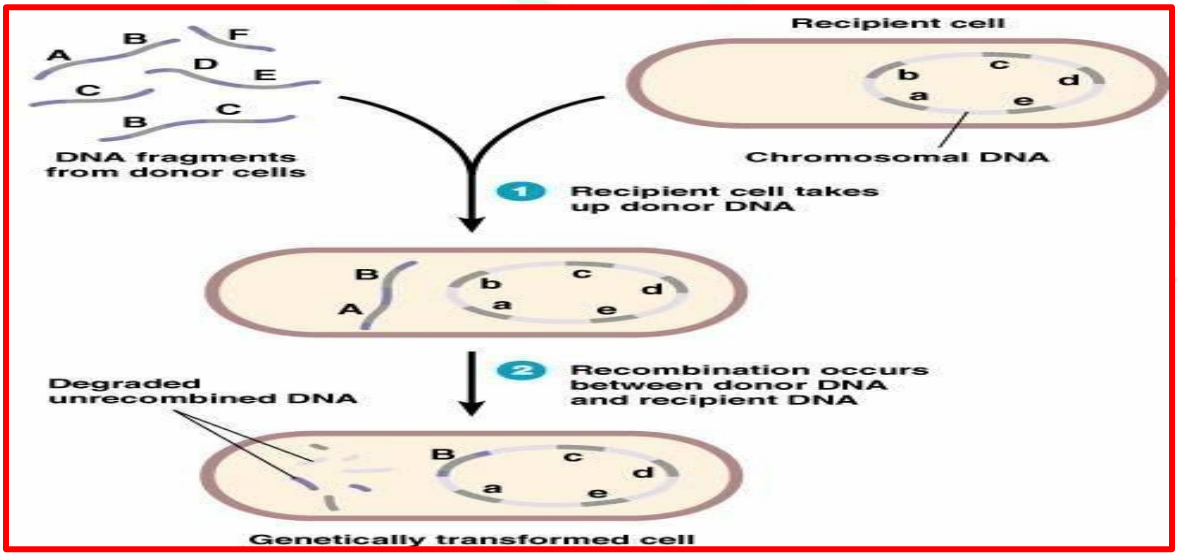
uracil, Ethidium bromide and Nitrosoguanidine. These methods of mutation depend on the type and dose of mutants and the time of mutation. However, such methods normally lead only to the loss of undesired characters or increased production due to loss of control functions.

Natural Para-sexual modes of gene (DNA) transfer:

Three natural processes are known to transfer of genetic material (DNA) from one organism to another.

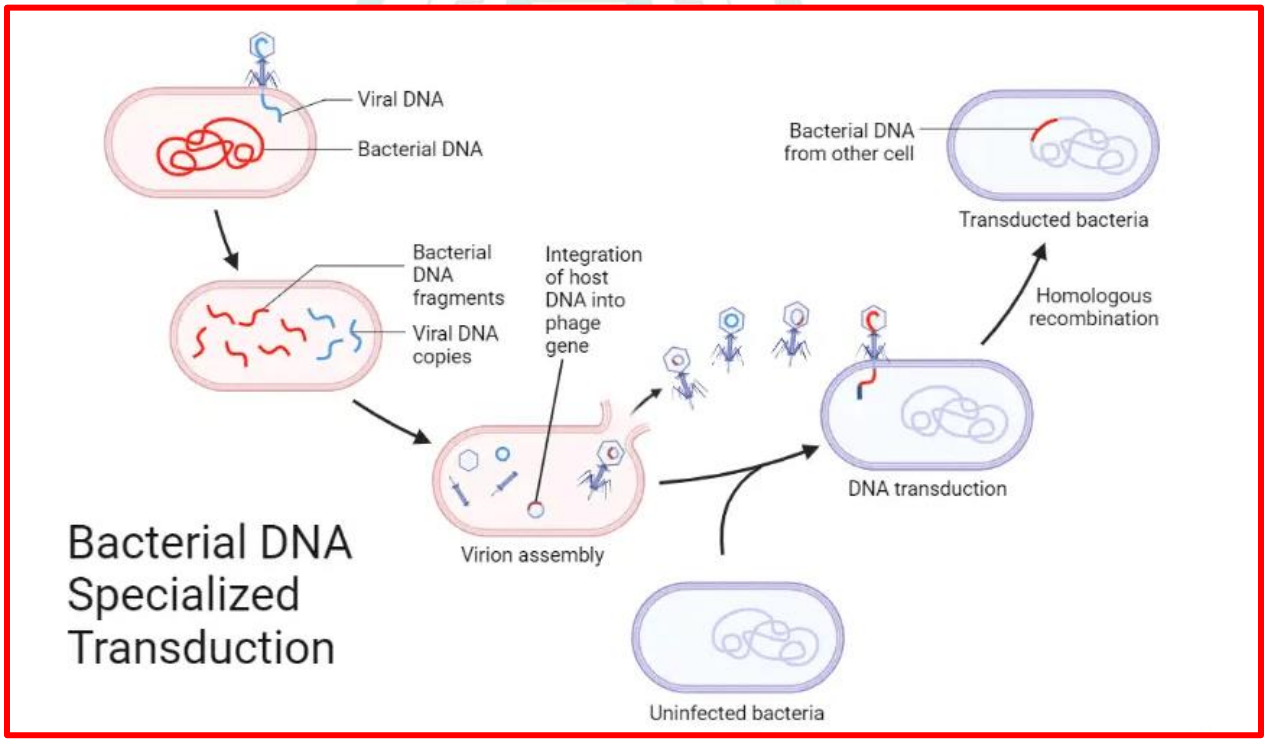
1- Transformation:

The direct uptake of DNA by microorganism from its environment or the method is to transfer the extracted DNA from one cell into a suitable host organism (Figure). The exact method of transformation depends on the host (recipient cell). *E.coli* is made competent (capable of taking up purified DNA by treating it with cold shock $CaCl_2$), heat shock many other bacteria including *Streptomyces* spp., can only be transformed if converted to protoplast by removing their cell walls.



2- Transduction:

The DNA is transferred from one organisms to another by way of carrier or vector system, this para-sexual method means of gene transfer or transduction is a process by which bacterial DNA fragments are introduced into natural bacteriophage and the random fragments of bacterial DNA (No.1) are introduced into fresh cells (No.2) by infection with the phages carrying the DNA fragments of bacteria. (No.1)

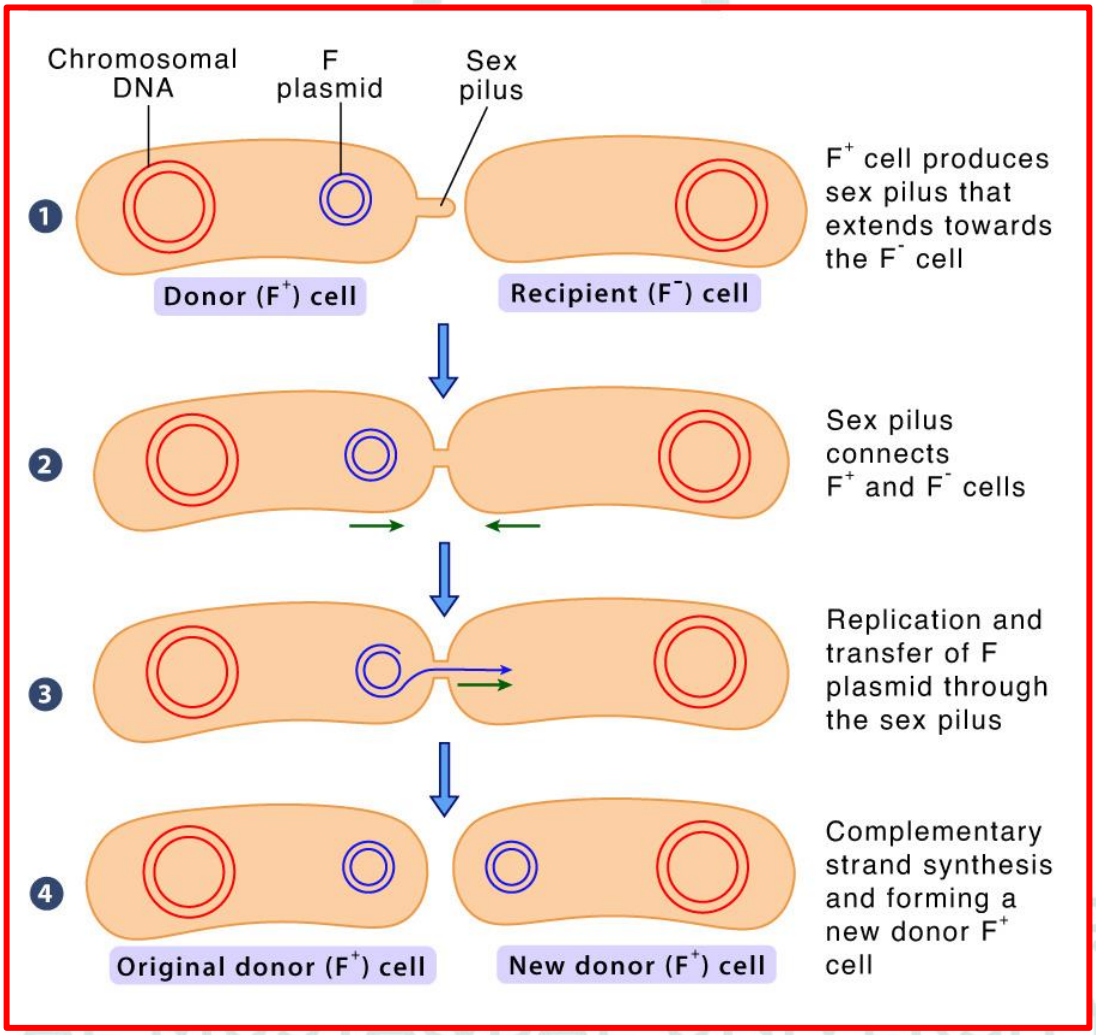


3- Conjugation:

The conjugation method is essentially a sexual process in which there is donor called male, and a recipient called female.

One of the natural plasmide of bacteria (F^+ cell) . F plasmid-carried genes that are capable to transfer to another cell (F^- cell) through sex pilus. A cell with a free F- plasmid is called an F^+ cell; a cell without one is called an F^-cell. F-plasmid acts as a sex factor in bacteria such as *E.coli.*

This method used to development many type of bacteria such as *E.coli, shigella, salmonella* and *pseudomonas species*. These natural processes are essential and provide in nature means of mixing the gene pool to allow new genes to enter an organism. Sexual conjugation is the main natural method for transferring nucleus information.



Protoplast fusion:

Plant and most microbial cells are characterized by having a distinct outer wall which gives the shape characteristic to the cell or organism and in some time this cell wall prevent the transformation of DNA between the cells. For some years now it has been possible, using special techniques, in particular hydrolytic enzymes such as proteases and cellulases, to remove the cell wall and produce the protoplasts. These protoplasts are extremely fragile but can be maintained in isolation for variable periods of time. Isolated protoplast cannot propagate themselves but requiring first to regenerate a cell wall be for regaining reproductive capacity. The fusion of protoplast can be enhanced by treatment with the chemical polyethylene glycol (PEG), which under optimum conditions can lead to extremely high frequencies of recombinant formation which can be increased still further by ultraviolet irradiation of the parental protoplast preparation. Protoplast fusion can used in *Bacillus* sp. and Streptomyces sp. and can also occur with plant or human or animal cell types.

Plasmids:

Many of the properties of bacteria which make them interesting for the biotechnologist are encoded by genes on bacterial plasmids. Plasmids are circular DNA molecules which can be stably inherited in bacterial cells without being linked to the main bacterial chromosome. Plasmids are also used in genetic engineering to clone genes of interest. Plasmids exist in bacterial cells in the form of double stranded circular DNA molecules (closed circules) which are twisted to form super coils.

Plasmid genes which are particularly interesting to the biotechnologist include those coding for nitrogen fixation, the degradation of organic compounds and virulence factors of pathogenic bacteria.

Gene cloning (recombinant DNA technology):

The reproduction of foreign genes in bacteria or other cells is called gene cloning, the foreign gene has first to be inserted into the DNA of a culture of bacterial or other cells. The recombinant DNA is replicated as the cells divide and produces many copies of the gene (Fig.).

Plasmids are circular strands of DNA which are found in many bacteria, and are separate from bacterial chromosome. They are often used to insert foreign genes into cells. Viruses also used. When genetic engineering techniques are applied to microbial cells, not all the cells will contain recombinant DNA, it is important that the genetic engineer can select out those cells which have been successfully transformed (i.e which contain the recombinant DNA) . This is usually done by adding a "marker gene" as well as the gene that is required. These marker genes are often for antibiotic resistance such as tetracycline resistance. The genetic engineer can then

the transformed organism by growing them on a medium containing tetracycline, so that all the untransformed bacteria will die.