Methods of Gene Transfer

Gene transfer is a process in which a gene from one DNA molecule known as donor is transferred to another DNA molecule known as recipient. After the DNA enters into the cell, crossing over occurs between the two DNA molecules which result into recombinant cell with the transferred gene. Gene transfer can be categorized in to two types depending upon the cell involved in the process. One is somatic gene transfer where somatic cells are involved and the change will not transfer to the next generation. Second is germ line transfer where germ cells (egg and sperm) are involved and the goal is to pass the change to the next generation. In nature, gene transfer takes place in prokaryotes and eukaryotes with different mechanisms.

There are several mechanisms for gene transfer in bacteria.

Transformation, the genetic alteration of a cell resulting from the introduction, uptake and expression of foreign genetic material (DNA or RNA). This process is relatively common in bacteria, but less so in eukaryotes. Transduction, the process in which bacterial DNA is moved from one bacterium to another by a virus (a bacteriophage, or phage). Bacterial conjugation, a process that involves the transfer of DNA via a plasmid from a donor cell to a recombinant recipient cell during cell-to-cell contact.

In plants gene transfer takes place with the help of Agrobacterium a gram-negative soil borne bacteria which infects the dicot plants and result into the crown gall disease.

In case of animal cell the term transfection is used and is the combination of trans and infection which involves the same process of uptake of genetic material and even proteins like antibodies. Transfection may be of two types:- Transient transfection In transient transfection, the transfected DNA is not integrated into host chromosome. DNA is transferred into a recipient cell in order to obtain a temporary but high level of expression of the target gene. Stable transfection Stable transfection is also called permanent transfection. By the stable transfection, the transferred DNA is integrated (inserted) into chromosomal DNA and the genetics of recipient cells is permanently changed. Apart from natural process of transferring the genetic material one could transfer the gene of interest using genetic engineering approaches/tools in either animal cells to treat the human diseases or in plant cell to improve the yield and quality in the area of agriculture and in bacterial cell to increase the production of specific

component which has industrial and medical importance. Methods of Gene transfer There are different methods for transferring the gene of interest in either bacterial, plant or animal cell. Broadly there are two types of gene transfer methods: 1. Direct

2.Vector mediated or indirect Direct method includes various chemical, physical or mechanical methods for gene transfer like,

1.Ca+ phosphate mediated transformation

2. Electroporation

3. Particle Bombardment

4. Microinjection

5. Lipid mediated method

6. Sonoporation

It is well known that cell membrane is made up of phospholipids bilayer which is amphiphatic in nature and separates cell from the environment and allows controlled transport of molecules inside and outside the cell. As we all know that DNA is non polar, larger in molecular weight and sensitive to nucleases degradation and cannot cross the plasma membrane easily. To facilitate the in vitro transfer of DNA into the cell various charged chemical compounds, physical methods like microinjection can be used. Due to the presence of these compounds and force generated due to physical and electrical methods, cell membrane assembly may get disrupted, or the pore size may increase temporally to allow the passage of DNA into the cell. Chemicals used to transfer DNA into the cell should have ideal properties like transport of DNA into the target cell without its degradation from nucleases and it should also promote its import into the nucleus. The commonly used methods for transformation are:-

1. **Calcium phosphate method**: This method is based on the principle of precipitation. In this method calcium phosphate and DNA gets precipitated which make them small and insoluble and due to this they are adsorbed on the cell surface easily. After adsorption on the cell surface, endocytosis takes place and release DNA into the cell that gets integrated into the cell genome. These types of integration can result into the stable or transient transformation. Endocytosis is a form of active transport in

which a cell transport molecules (such as proteins) into the cell (endo- + cytosis) by engulfing them in an energy-using process. Endocytosis includes pinocytosis (cell drinking) and phagocytosis (cell eating).

2. Electroporation:

Pulse electric field can be used to insert polar molecules like DNA, protein into the cell and the method was first demonstrated by Wong and Neumann in 1982. Electroporation is the next easy and effective technique based on the ability of plasma membrane to reassemble after the disturbance, as we all know that plasma membrane is made up of weak hydrophilic /hydrophobic interactions which temporally disrupts due to quick voltage shock and allow the passage of polar molecules. Using this property one can easily use this technique to introduce foreign material into plant, animal and bacterial cell.

The cell and DNA are suspended in the solution and high voltage shock is applied which temporarily disrupts the integrity of plasma membrane and make it permeable to allow the entry of polar and large molecules inside the cell. If two cells are in vicinity then they fuse to form hybrids. The membrane reseals leaving the cell intact soon afterwards. Sometimes if the applied voltage is very high then destruction of cell membrane may occur.

Factors that influence efficiency of transfection by electroporation are applied electric field strength, electric pulse length, temperature, DNA conformation, DNA concentration, and ionic composition of transfection medium. In plant cells, fusion of protoplast is done by using this technique. This method is widely used for the introduction of transgene into bacterial, plant and animal cells.

Electroporation can be used for more efficient gene transfer in a wide range of tissues like skin, muscle, lung, kidney, liver, artery, brain, cornea etc. and it avoids the vector-specific immune-responses that are achieved with recombinant viral vectors and thus are promising in clinical applications.

3. Biolistics (Gene gun, or microparticle bombardment): The gene gun method was developed by Klein at Cornell University in 1987 (Klein, John Sanford). The method is widely used for gene transfer in plants because in plants outside the plasma membrane cell wall is present, which makes plant cells more rigid and impermeable to foreign particles. Most commonly gene transfer in plant cells is done by particle bombardment method to achieve maximum results. For dicots Agrobacterium-mediated gene transfer method is widely used but for monocots and plants with less regeneration capacity gene-gun method is applicable. Some bacteria also have an outer membrane in addition to plasma membrane which makes them impermeable for uptake of foreign DNA by electroporation or other method, in such cases biolistic approach can be used for gene transfer in bacteria also. The method is based on the principle of conservation of momentum and uses the passage of helium gas through the cylinder with a range of velocities required for optimal transformation of various cell types.

The apparatus of Biolistic consists of a bombardment chamber which is connected to an outlet for vacuum creation and consists of a plastic rupture disk below which macro carrier is loaded with micro carriers. These micro carriers consist of gold or tungsten micro pellets coated with DNA for transformation. To maintain the sterile condition the apparatus is placed in Laminar flow. The target cells/tissue is placed in the apparatus and a stopping screen is placed between the target cells and micro carrier assembly. The passage of high-pressure helium ruptures the plastic rupture disk propelling the macro carrier and micro carriers. The stopping screen prevents the passage of macro projectiles but allows the DNA coated micro pellets to pass through it thereby delivering DNA into the target cells. The efficiency of the gene gun transfer depends on the following factors: cell type, cell growth condition, culture medium, gene gun ammunition type, gene gun settings and the experimental experience. The stopping screen prevents the passage of macro projectiles but allows the DNA coated micro pellets to pass through it thereby, delivering DNA into the target cells.

4. Microinjection: DNA microinjection was first proposed by Dr. Marshall A. Barber in the early part of nineteenth century. This method is widely used for gene transfection in mammals. It involves delivery of foreign DNA into a living cell (e.g. a cell, egg, oocyte, embryos of animals) through a fine glass micropipette. The introduced DNA

may lead to the over or under expression of certain genes. It is used to identify the characteristic function of dominant genes.

Procedure: The delivery of foreign DNA is done under a powerful microscope using a glass micropipette tip of 0.5 mm diameter. Cells to be microinjected are placed in a container. A holding pipette is placed in the field of view of the microscope that sucks and holds a target cell at the tip. The tip of micropipette is injected through the membrane of the cell to deliver the contents of the needle into the cytoplasm and then the empty needle is taken out.

5. Sonoporation: It involves the use of ultrasound for temporary permeabilization of the cell membrane allowing the uptake of DNA, drugs or other therapeutic compounds from the extracellular environment. This method leaves the compound trapped inside the cell after ultrasound exposure treatment. It employs the acoustic cavitations of micro bubbles for enhancing the delivery of large molecules like DNA. The micro bubbles form complex with DNA followed by injection and ultrasound treatment to deliver DNA into the target cells. Unlike other methods of transfection, sonoporation combines the capability to enhance gene and drug transfer.

6. Lipid-mediated method: The word liposome derives from two Greek words: lipo ("fat") and soma("body"); it is so named because its composition is primarily of phospholipids. Liposome's are most often composed of phospholipids, especially phosphatidylcholine, but may also include other lipids, such as egg phosphatidylethanolamine, so long as they are compatible with lipid bilayer structure. The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection. A liposome has an aqueous solution core surrounded by a hydrophobic membrane, in the form of a lipid bilayer; hydrophilic solutes dissolved in the core cannot readily pass through the bilayer. Hydrophobic chemicals associate with the bilayer. To deliver the molecules to a site of action, the lipid bilayer can fuse with other bilayer such as the cell membrane, thus delivering the liposome contents; this is a complex and nonspontaneous event, however. By preparing liposomes in a solution of DNA or drugs can be delivered across the lipid bilayer. In addition to gene and drug delivery applications, liposomes can be used as carriers for the delivery of dyes to textiles, pesticides to plants, enzymes and nutritional supplements to foods, and cosmetics to the skin.