DNA Delivery into Host cells

The delivery of DNA into the host is required for generation of genetically modified organism. DNA delivery to host is a **three stage process**:-

- Attachment (DNA sticking to the host cell) DNA molecules must first come into contact and adhere to the host cell surface. This initial interaction depends heavily on electrostatic forces and the chemical nature of both the DNA and the host cell membrane.

-Internalization (Uptake):

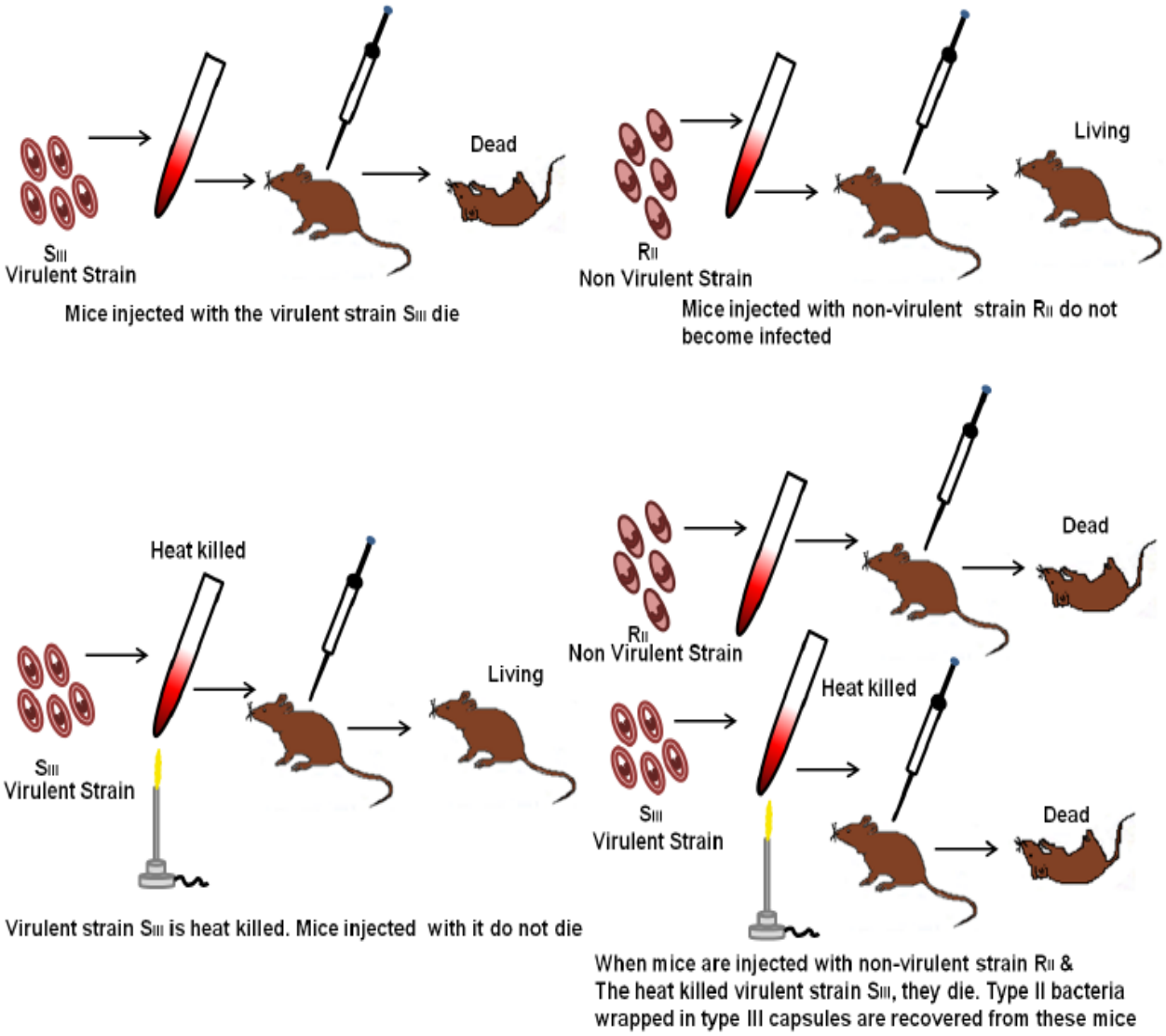
Once attached, the DNA must cross the cell membrane—and, in some organisms, additional barriers like the cell wall—to enter the cytoplasm. Different methods such as transformation, transfection, electroporation, and biolistics (gene gun) are often used to facilitate this step.

Release (DNA entry into the cytoplasm or nucleus):

After entering the cell, the DNA must be released from any carrier or vesicle and reach its target site (cytoplasm or nucleus) for expression or integration.

Transformation

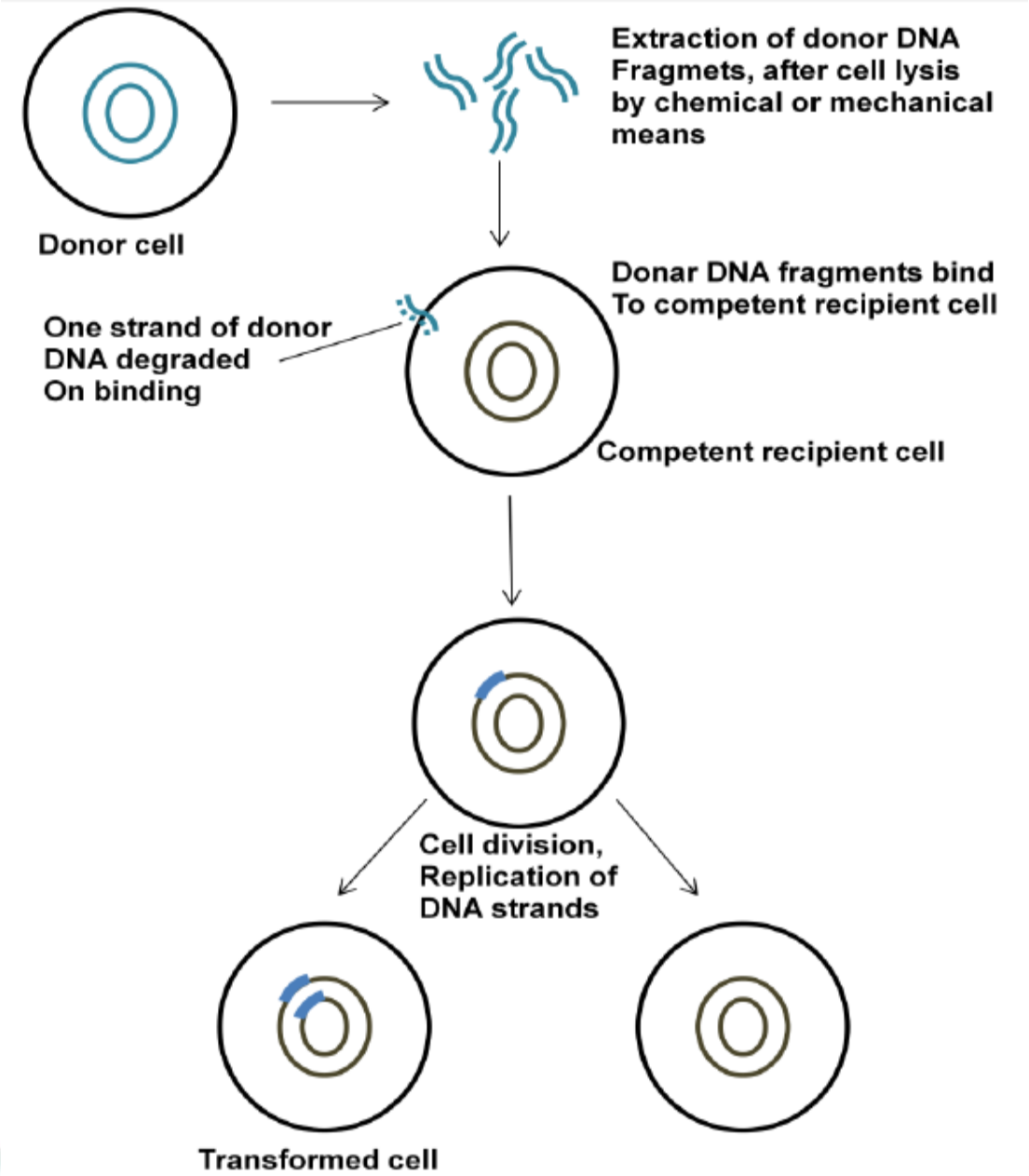
It is the natural process, through which bacterial population transfer the genetic material to acquire phenotypic features. The event of transformation was first time demonstrated by **Frederick Griffith** in 1928. The schematic presentation of the experiment is given in the next Figure. Griffith has used two different *Streptococcus pneumonia* strains, virulent (**S**, causes disease and death of mice) and a virulent (**R**, incapable of causing disease or death of mice). In a simple experiment he injected 4 different combination of bacterial mixture, (1) live S, (2) heat killed S, (3) live R, (4) mixture of live R and heat killed S in to the mice. The observation indicates that live S has killed the mice whereas mice were healthy with heat killed S or live R. Surprisingly, mice injected with mixture of live R with heat killed S were found dead, and bacteria isolated from these dead mice were virulent. Based on these observations, Griffith hypothesized the existence of a transforming agent (Protein, DNA) being transferred from heat killed virulent strain to a virulent strain and proposed the concept of transformation. Later, Oswald has proved that the transforming factor is DNA rather than protein.



Discovery of Transformation

Mechanism of Transformation-

Transformation is the process by which cell free DNA is taken up by another bacterium. The principle steps of transformation are given in the next Figure. The DNA from donor bacteria binds to the competent recipient cell and DNA enters into the cell. The DNA enters into the recipient cell through an uncharacterized mechanism. The DNA is integrated into the chromosomal DNA through a homologous recombination. Naturally transformation is common between closely related species only.



Principle steps in transformation

Electroporation

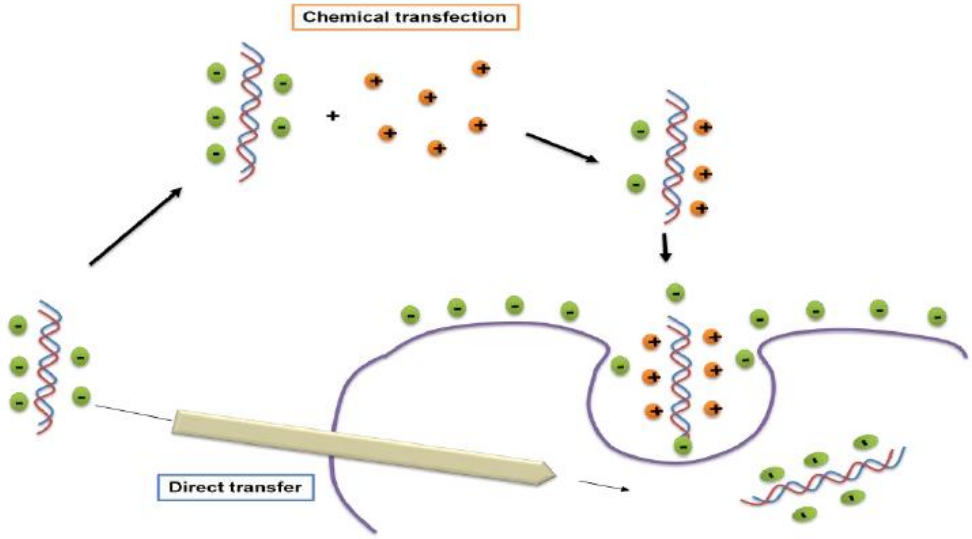
Plasma membrane is composed of lipid and protein. These macromolecules give a partial conductance to the cell membrane. When a high electric pulse is given to the cell, the charge run across the membrane and partially disturbs the arrangement of lipid molecule. As a result, it makes formation of pore and allow easy passage of macromolecule especially

charged molecule like DNA into the cell. After the electroporation, cell is allowed to recover from the damage and it forms colony on the selective solid media.

DNA Delivery in mammalian cells

Mammalian cell membrane surface chemistry, intracellular comparatmentization and uptake mechanism is different from the prokaryotic cells or yeast. Hence specialized methods have been developed to suit mammalian cells. There are 4 major strategies to deliver the DNA in mammalian cells:

1-Chemical transfection techniques-The principle behind the chemical transfection technique is to coat or complex the DNA with a polymeric compound to a reasonable size precipitate (Figure below). It facilitates the interaction of the precipitate with the plasma membrane and uptake through endocytosis. There are multiple chemical compounds have been discovered which can be able to make complex and deliver DNA into the mammalian cell.

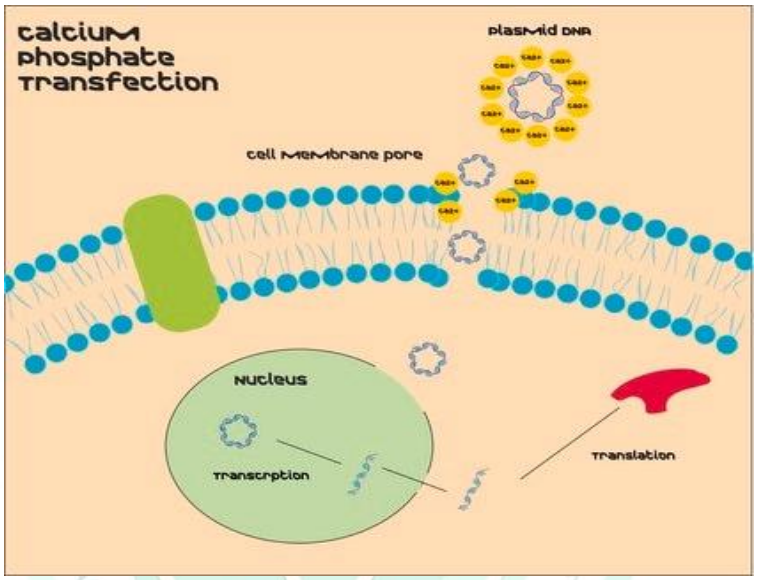


Mechanism of chemical method mediated DNA delivery in animal cells

- **Calcium Phosphate method-**

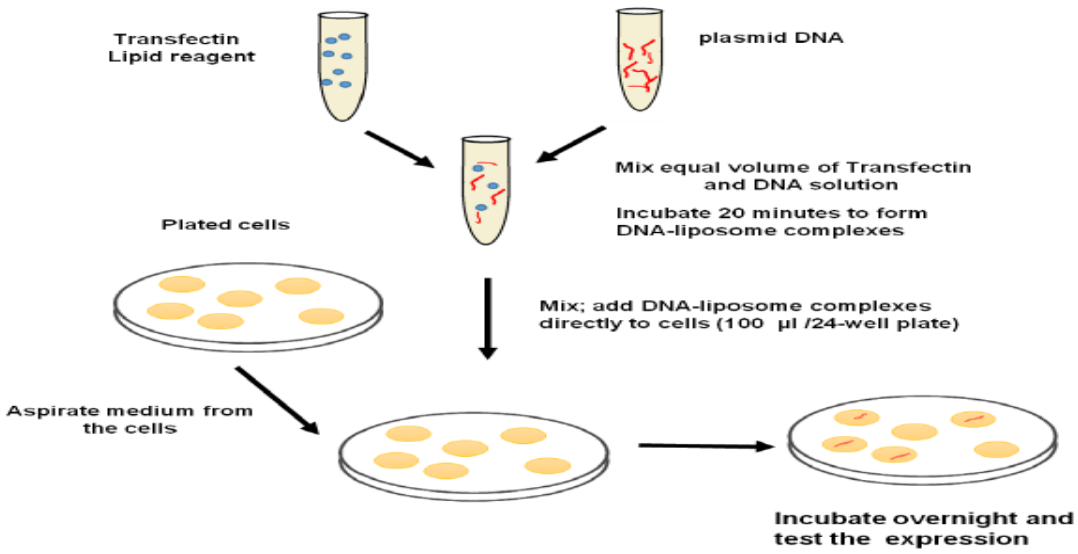
In this method, DNA is mixed with calcium chloride in phosphate buffer and incubated for 20mins. Afterwards, transfection mixture is added to the plate in dropwise fashion. DNA-calcium phosphate complex forms a precipitate and deposit on the cells as a uniform layer. The particulate matter is taken up by endocytosis into the internal storage of the cell. The

DNA is then escapes from the precipitate and reach to nucleus through an unknown mechanism. This method suited to the cell growing in monolayer or in suspension but not for cells growing in clumps. But the technique is inconsistent and the successful transfection depends on DNA-phosphate complex particle size and which is very difficult to control.

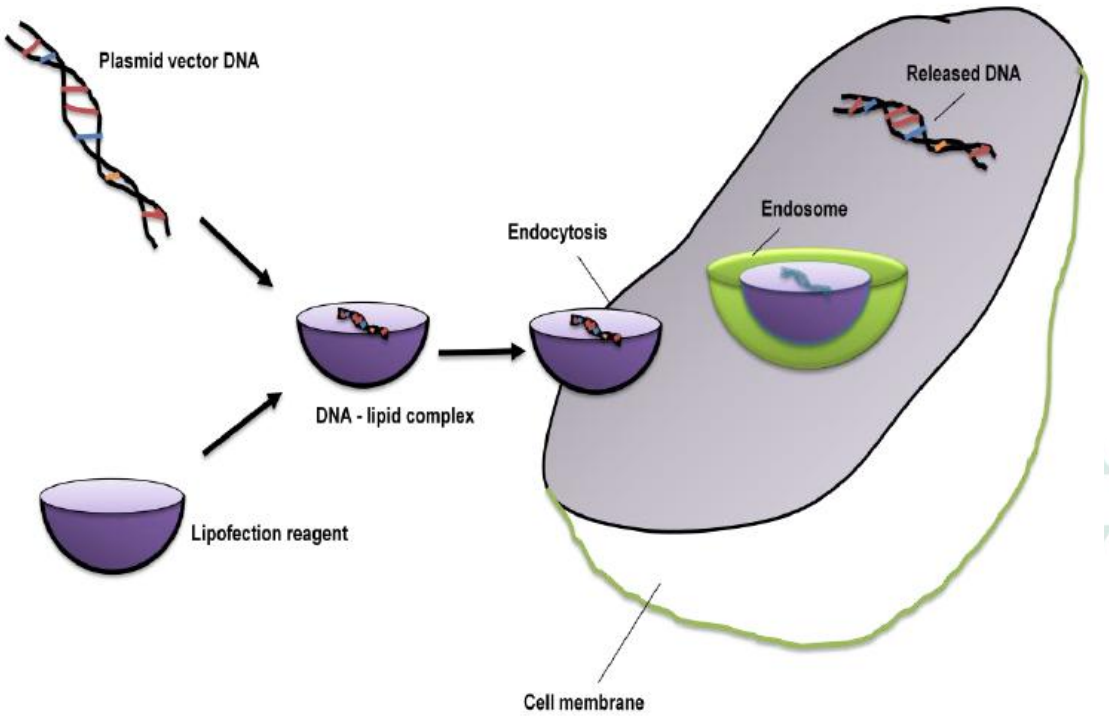


- **Polyplexes method-**

The disadvantage of calcium phosphate method is severe physical damage to the cellular integrity due to particulate matter settling on the cell. It results in reduced cellular viability and cytotoxicity to the cell. An alternate method was evolved where DNA was complexed with chemical agent to form soluble precipitate (polyplexes) through electrostatic interaction with DNA (Figure below). A number of polycationic carbohydrate (DEAE-Dextran), positively charged cationic lipids (transfectin), polyamines (polyethylenimines) etc. are used for this purpose. The soluble aggregates of DNA with polycationic complex is readily been taken up by the cell and it reaches to the nucleus for expression (Figure below).



Transfection of animal cell with tranfectin (polyplexes)



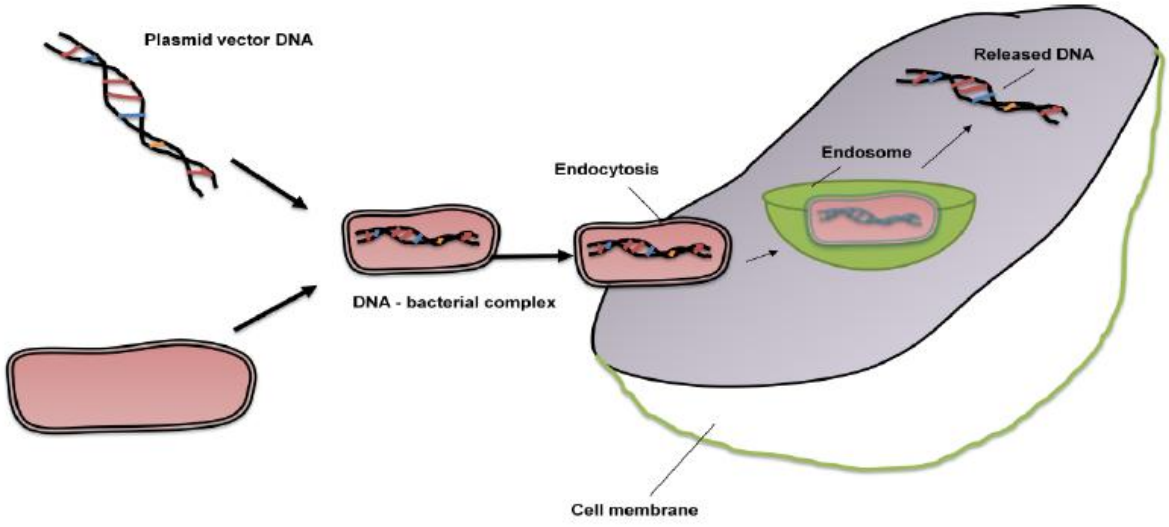
Proposed mechanism of DNA-lipid complexes in mammalian cells

- **Liposome and lipoplex method-**

Another approach of DNA transfection in animal cell is to pack the DNA in a lipid vesicle or liposome. In this approach, DNA containing vesicle will be fused with the cell membrane and deliver the DNA to the target cell. Preparation of liposome and encapsulating DNA was a crucial step to achieve good transfection efficiency. Liposome prepared with the cationic or neutral lipid facilitates DNA binding to form complex (lipoplex) and allow uptake of these complexes by endocytosis. The lipoplex method was applicable to a wide variety of cells, and found to transfect large size DNA as well. Another advantage of liposome/lipoplex is that with the addition of ligand in the lipid bilayer, it can be used to target specific organ in the animal or a site within an organ.

2-Bactofection-

This mode of gene transfer is very popular in plant where agrobacterium tumefactions is used. In animal cell, bacteria are actively being taken up by the host cell through phagocytosis and entrapped in a membranous vesicle known as phagosome. Then bacteria get escape from phagosome and get lysed to release the DNA into the cytosol. In alternate mechanism, bacteria get lysed inside the phagosome and DNA is released into the cytosol. The bacterial species used in this methods are salmonella, shigella etc. Most of the strain used to deliver the DNA are attenuated so that they should not harm host cell (Figure below).



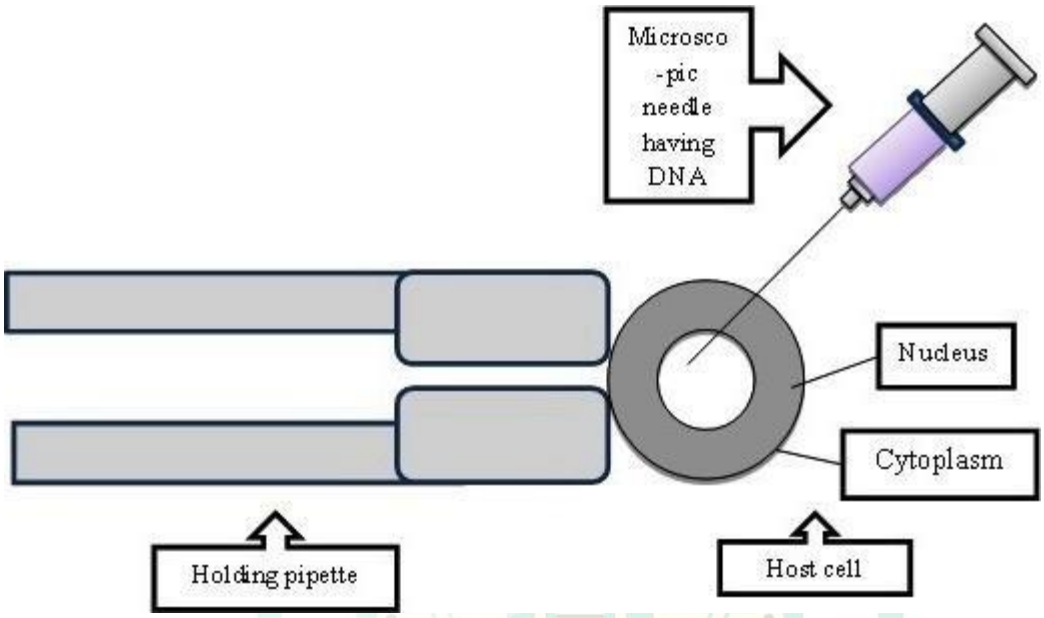
Proposed mechanism of transfection of mammalian cell with bacteri

3-Transduction (Virus mediated)

Viral particle has a natural tendency to attack and deliver the DNA into the eukaryotic cells. As discussed previously, cloning gene of interest in to the viral vectors is an innovative way to deliver the DNA into the host cell. If the recombination sequences are available, the delivered DNA is integrated into the host and replicate. Virus has essential components for expression of proteins required for DNA replication, RNA polymerase and other ligand for attachment onto the host cell. In addition, it has additional structural components to regulate infection cycle. The virus vector contains cassettes to perform all these functions then it is fully sufficient to propagate independently. Few virus strains may cause disease if their propagation will be uncontrolled. A mechanism has been devised to keep a check on the uncontrolled propagation of virus in cell. Few crucial structural blocks are placed on another helper plasmid, in this case virus propagate only if helper plasmid has been supplied along with the viral vector. This particular arrangement is made with the virus strains which can cause disease after integrating into the genome such as lentivirus.

4-DNA Microinjection-

is a technique of delivering foreign DNA into a living cell (a cell, egg, oocyte, embryos of animals) through a glass micropipette. One end of a glass micropipette is heated until the glass becomes somewhat liquefied. It is quickly stretched which forms a very fine tip at the heated end. The tip of the pipette attains to about 0.5 mm diameter which resembles an injection needle. The process of delivering foreign DNA is done under a powerful microscope. Cells to be microinjected are placed in a container. A holding pipette is placed in the field of view of the microscope. The holding pipette holds a target cell at the tip when gently sucked. The tip of the micropipette is injected through the membrane of the cell. Contents of the needle are delivered into the cytoplasm and the empty needle is taken out.



5-Gene gun bombardment-

Gene gun bombardment is a method for the physical introduction of DNA into plant cells containing cell walls. The gene gun is utilized to bombard the plant cell wall with many DNA coated metal particles by using compressed helium as the propellant. The metal particles commonly used for gene gun bombardment include gold, tungsten, palladium, rhodium, platinum, and iridium. They are coated with DNA, accelerated by helium gas, and bombard the plant cells. The metal particles (0.45–1.5 μm in diameter) punch holes in and pass through the cell wall and enter the plant cells, leaving the DNA cargo inside the cells. The DNA coated metal particles randomly bombard the cells, no targeting is currently possible with this method. For example, the metal particles are localized everywhere in the cell including the nucleus and cytoplasm. Once the DNA cargo diffuses from the surface of the metal carrier, it has the opportunity to influence the intracellular genetic process.