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LESSON PREPARED UNDER LMS E-CONTENT DEVELOPMENT

SUBJECT : BIOTECHNOLOGY

CLASS : III BSc. BIOTECHNOLOGY

SEMESTER / PAPER – SEMESTER – V / PAPER – VI

PAPER – RECOMBINANT DNA TECHNOLOGY

Lesson: Gene Transfer - Physical & Chemical Methods

e-Content Developer

Smt. G. Indravathi,
Assistant Professor
Department of Biotechnology
KVR Govt. College for Women (A)
Kurnool, Andhra Pradesh.

Gene Transfer - Physical & Chemical Methods

- Genetic material from one organism is selected and then artificially introduced to a host organism. If the foreign DNA integrates into the host genome, it gets replicated along with the genome and then express the foreign protein. Genes can be transferred in to the host cells by three methods

1. Transformation
2. Transfection
3. Transduction

Transformation:

Transformation is direct uptake of exogenous DNA via cell membrane and its insertion into the host DNA which is naturally seen in bacteria. In genetic engineering transformation requires different tools like vectors, restriction enzymes, ligases and other DNA modifying enzymes. During transformation host cells are made competent to take up the foreign DNA.

Transfection:

The process of foreign DNA uptake by host cell driven by mechanical or chemical factors is termed as transfection. Different methods of non-bacterial transformation are microinjection, electroporation, biolistics $CaCl_2$, liposome and PEG mediated transformation .

Transduction:

Transduction is the process of transfer of DNA molecule using viruses In this method phage vectors like Lambda, M13 etc. are used to carry and replicate foreign DNA inside the bacterial host system. The phage DNA inserts into the host chromosome by recombination.

Gene transfer techniques – Physical Methods

Due to amphipathic nature of the phospholipid bilayer of the plasma membrane, polar molecules such as DNA and protein are unable to freely pass through the membrane. The following physical or mechanical methods are employed to promote gene transfer

1. Electroporation
2. Microinjection
3. Particle Bombardment

Microprojectile/particle Bombardment (biolistics)

Biolistics is a method where genes are directly transferred in to the cells using high pressure Helium gas. Prof. John Stanford and colleagues at Cornell University (USA) were the first to develop the bombardment concept in 1987 and coined the term "biolistics". This technique is also called as Particle bombardment, Gene gun, Micro projectile bombardment and Particle acceleration. This method is commonly employed for genetic transformation of plants and in particular monocots.

It employs high-velocity micro projectiles to deliver substances into cells and tissues. Biolistics apparatus consists of a bombardment chamber which is connected to an outlet for vacuum creation. The bombardment chamber consists of a gas acceleration tube, plastic rupture disk below which macro carrier is loaded with micro carriers. These micro carriers consist of gold or tungsten micro pellets (0.6 – 1 mm in size) coated with

Figure: A biolistic microprojectile gun.

Source: http://en.wikipedia.org/wiki/Gene_gun (cc)

DNA. Prior to coating, DNA is precipitated with calcium chloride, spermidine and polyethylene glycol. A wide range of tissues such as cell suspensions, callus cultures, embryos, seedlings, leaves, floral tissues, apical and floral meristems could be used as target cells in this method. Biolistic apparatus is placed in Laminar flow while working to maintain sterile conditions. 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.

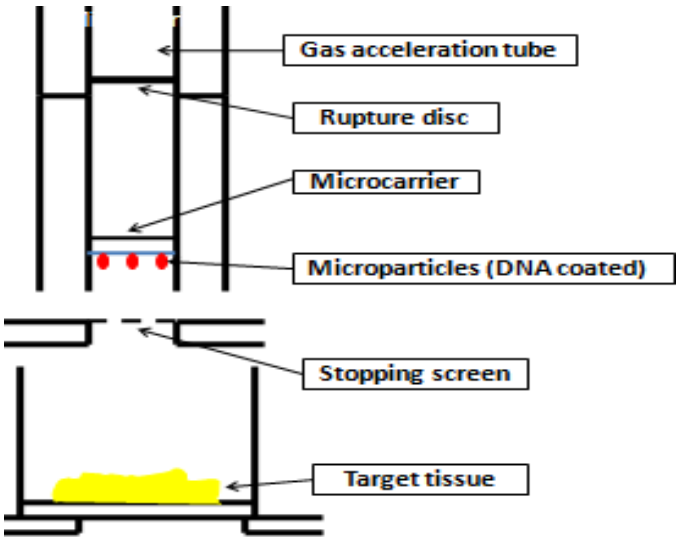


Figure-Working system of particle bombardment gun.

Source- https://nptel.ac.in/courses

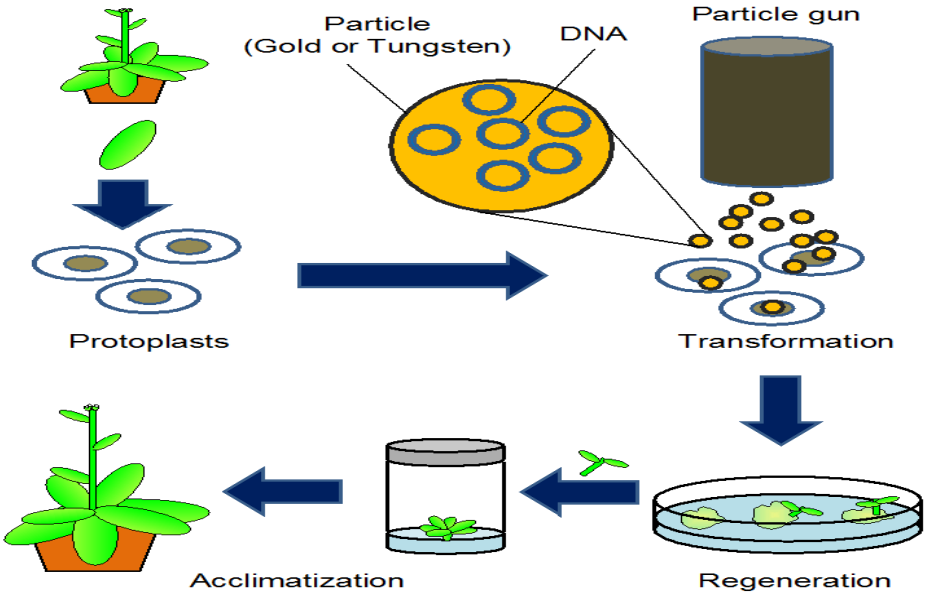


Figure-Illustration of microparticle bombardment method

Source-http://www.intechopen.com/books/transgenic-plants-advances-and-limitations/methods-to-transfer-foreign-genes-to-plants (cc)

Electroporation:

Electroporation uses electrical pulses to create transient holes in the cell membrane through which DNA is translocated across the cell membrane leading to stable or transient DNA expression. This method

was first demonstrated by Wong and Neumann in 1982 to study gene transfer in mouse cells Cells which are arrested at metaphase stage of cell cycle are especially suitable for electroporation as these cells have absence of nuclear envelope and an unusual permeability of the plasma membrane. Protoplasts, immature zygotic embryos and embryogenic calli are used for electroporation in plants.

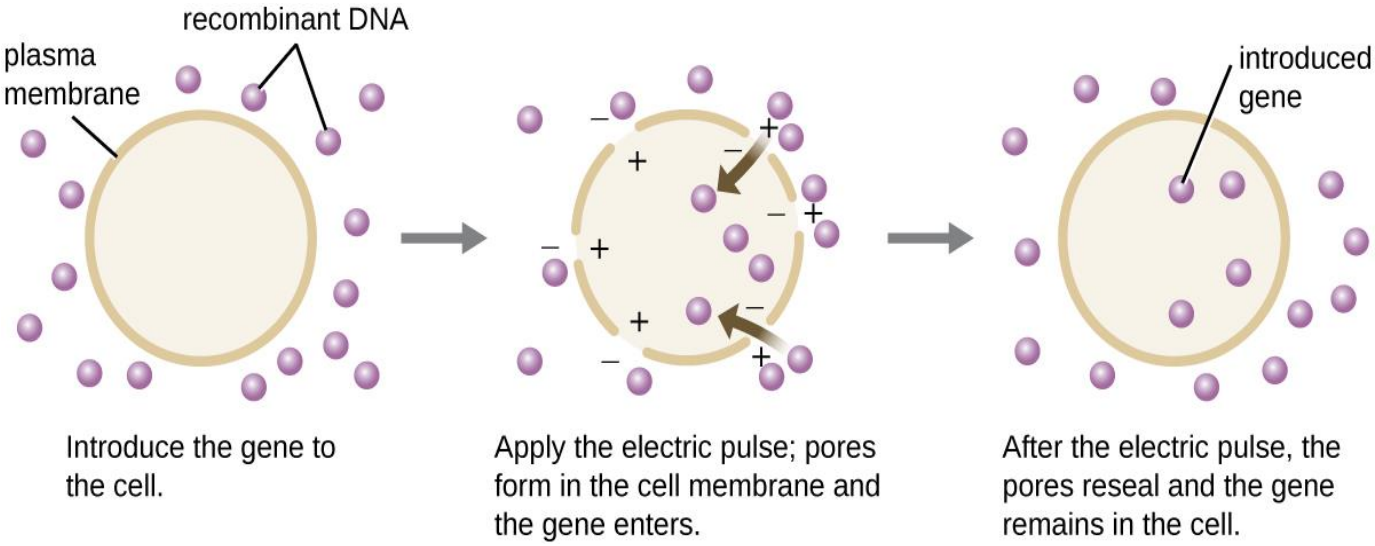


Figure: Formation of transient pores in cell membrane by electric impulses

Sourcehttps://bio.libretexts.org/TextMaps/Map%3A_Microbiology_(OpenStax)/12%3A_Modern_Applications_of_Microbial_Genetics/12.1%3A_Microbes_and_the_Tools_of_Genetic_Engineering-CC BY-SA-NC

A suspension of cells with plasmid DNA is taken in an electroporation cuvette placed between electrodes and electrical pulses are applied. The basis of electroporation is the relatively weak hydrophobic/hydrophilic interaction of the phospholipids bilayer and ability to spontaneously reassemble after disturbance. A quick voltage shock may cause the temporary disruption of areas of the membrane and allow the passage of polar molecules. The membrane reseals leaving the cell intact soon afterwards. Electroporation is usually done by two methods:

- High voltage for short time (1000 – 10000 V/cm for few micro seconds),
- Low voltage for long time (250-750 V/cm for few milli seconds).

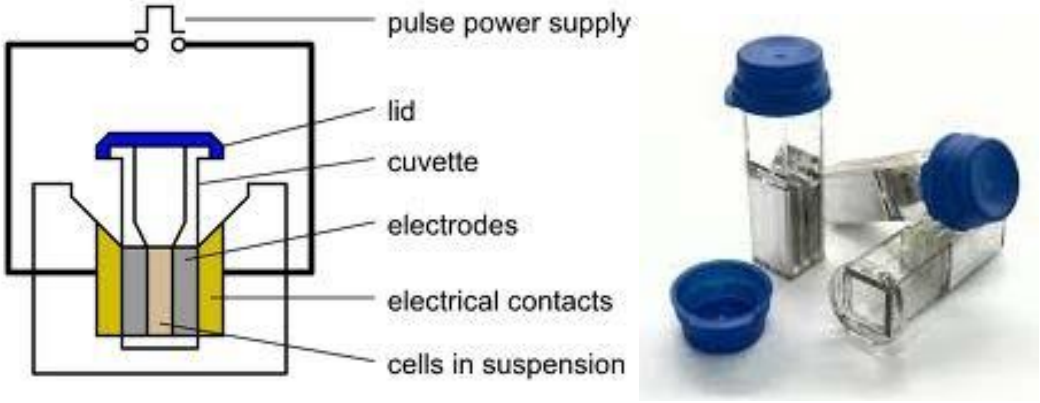


Figure: Electroporation Device and Cuvvetes

Source:http://commons.wikimedia.org/wiki/File:Electroporation_Diagram.png(cc)
http://commons.wikimedia.org/wiki/File:Electroporation_Cuvettes.jpg (cc)

Microinjection:

This technique was first proposed by Dr. Marshall A. Barber in the early 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 **microcapillary injection** pipette. The introduced DNA may lead to expression of certain genes. 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 and a holding pipette is placed in the field of view of the microscope to hold the target cell by suction pressure. Then the tip of injection micropipette containing the DNA insert is injected into the cytoplasm/ nucleus of the cell through the cell membrane.

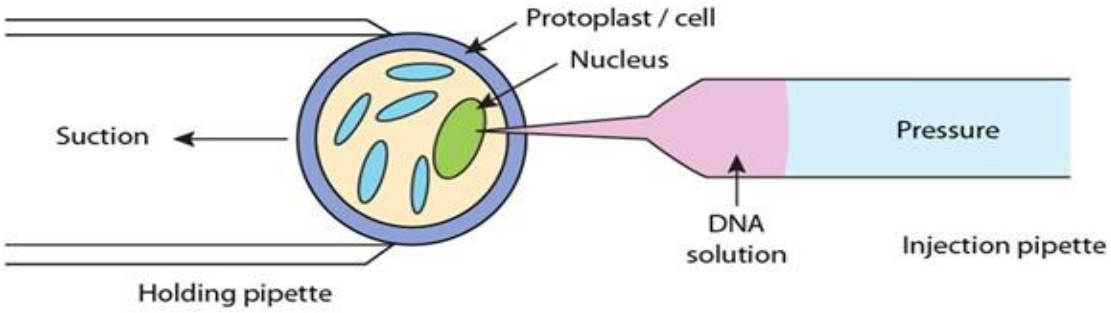


Figure: Illustration of microprojectile method

Source: http://nptel.ac.in/courses/102103016/module3/lec24/3.html (cc)

Conclusion

Physical methods for transferring of genes are more effective for single/ multiple targeted cells. This method carries modest risk of transfection reagents. Further through these methods scientist observed results very quickly and in short span of time. However they also have some drawbacks. For example,it is very difficult for the genes to be transported into the nucleus because of little access in passing through the membrane or enzymatic degradation of the naked DNA or RNA, which resulted low transfection efficiency and low clinical applications. On the other hand, they present damage to cells; difficulty has been seen in large-scale manipulation, labor-intensive protocols and very high cost of the instruments, which only available in well develop labs.

GENE TRANSFER TECHNIQUES: CHEMICAL METHODS

5-2.1. Introduction

Cell membrane is an assembly of amphipathic molecules that separate cells from their environment. They are selectively permeable and allow only the controlled exchange of materials between the cell and its surroundings. DNA is a large size, polyanionic, hydrophilic molecule and sensitive to nuclease degradation in biological matrices. They cannot easily cross the physical barrier of membrane and need support to enter the cells.

The necessary assistance is provided by various charged chemical compounds which facilitate DNA transfer directly to the cell. These synthetic compounds (Chemical+ DNA) when introduced near the surroundings of target cells ,it causes disturbances in the cell membranes, widens the pore size and allows the passage of the DNA into the cell.
The commonly used methods of chemical transfection use the following,

1. Calcium phosphate
2. DEAE dextran
3. Cationic Lipid
4. Other polymers - poly-L-lysine (PLL), polyphosphoester, chitosan, dendrimers

1. **Calcium Phosphate method-**

This technique was first applied by Graham and Van Der Eb in 1973 for the analysis of the infectivity of adenoviral DNA. In this method, DNA is mixed with calcium chloride in phosphate buffer and incubated for a period of time. Later this transfection mixture is added to the plate containing target cells in dropwise fashion. DNA-calcium phosphate complex forms a precipitate and deposit on the cells as a uniform layer. This precipitate is engulfed by cells through endocytosis and the DNA gets integrated into the cell genome resulting in stable or permanent transfection. This method is mainly used in the production of recombinant viral vectors. It is also used in transformation of mammalian cell lines. This method is suited to the cells growing in monolayer or in suspension but not for cells growing in clumps.

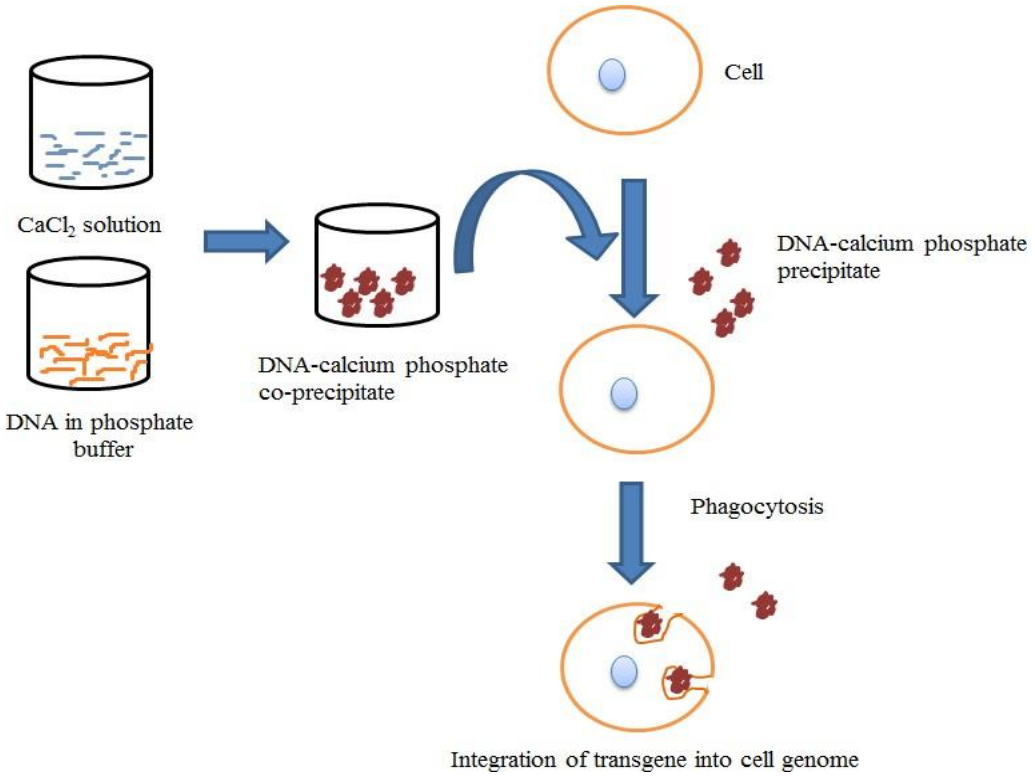
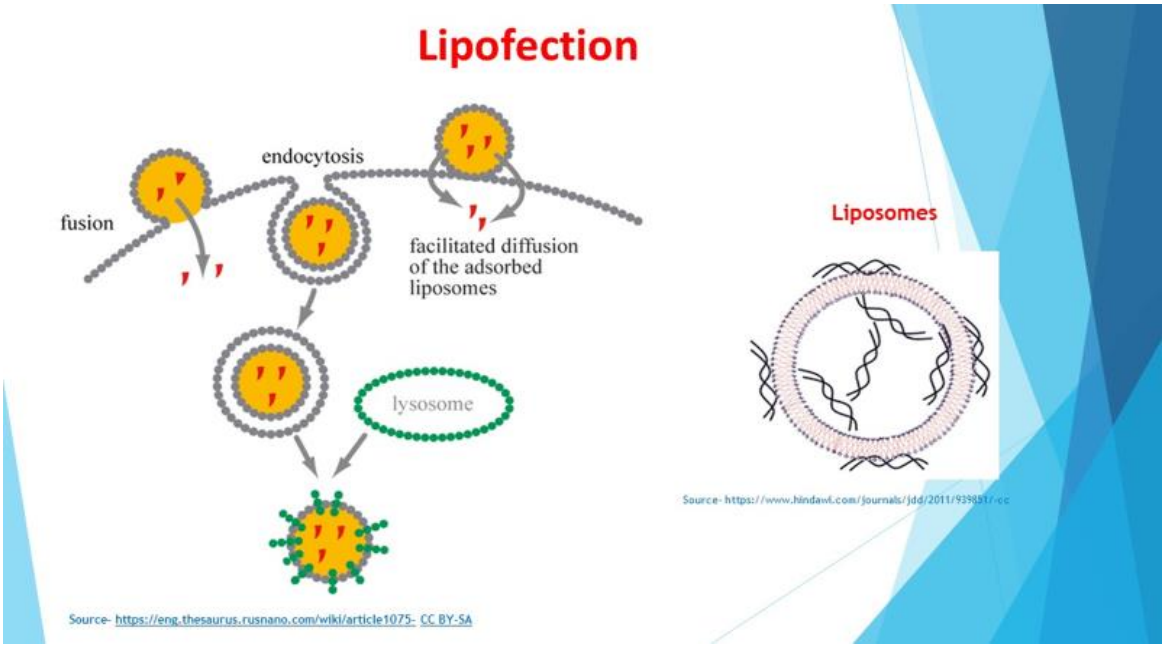


Figure - A schematic representation of transfection by Calcium Phosphate Precipitation.

Source- https://nptel.ac.in/courses

Lipofection

Liposomes are artificial phospholipid vesicles used for the delivery of a variety of molecules into the cells. Mostly they are be multi-lamellar or unilamellar vesicles with a size range of 0.1 to 10 micrometer or 20-25 nanometers respectively. These lipid vesicles when fused with the cell membrane of the target cell releases the foreign DNA in to the cell . Good transfection efficiency depends on the preparation of liposome and encapsulating DNA. Liposome prepared with the cationic or neutral lipid facilitates DNA binding to form complex (lipoplex) and allow uptake of these complexes by endocytosis. The most commonly used liposomes in gene transfer are cationic liposomes which are positively charged liposomes which associate with the negatively charged DNA molecules by electrostatic interactions forming a stable complex.This method is applicable to a wide variety of cells, and found to transfect large size DNA fragments. By tagging the lipid bilayer of liposomes with ligands, lipofection can be used to target specific organ in the animal or a site within an organ.



DEAE-Dextran (Di Ethyl Amino Ethyl Dextran) mediated DNA transfer:

This method was first reported by Vaheri and Pagano in 1965. DEAE-dextran is a soluble polycationic carbohydrate that promotes interactions between DNA and

endocytotic machinery of the cell. In this method, the negatively charged DNA and positively charged DEAE – dextran form aggregates through electrostatic interaction and form apolyplex. These complexes, when added to the cells, bind to the negatively charged plasma membrane and get internalized through endocytosis. Further, DNA delivery can be improved by osmotic shock using DMSO or glycerol.

Advantages

- This method is simple and inexpensive, applied to a wide range of cell types. This method is used for transient transfection.
- More sensitive

Disadvantages

- Toxic to cells at high concentrations
- Transfection efficiency varies with cell type
- Can only be used for transient transfection but not for stable transfection
- Typically produces less than 10% delivery in primary cells.

Another polycationic chemical, the detergent Polybrene, has been used for the transfection of Chinese hamster ovary (CHO) cells, which are not amenable to calcium phosphate transfection.

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