TRANSFECTION METHODS, PROMOTERS AND EXPRESSION VECTORS - I

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Biotechnology

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INTRODUCTION TO TRANSFECTION

- Transfection is the process of artificially introducing nucleic acids (DNA or RNA) into cells, utilizing means of other than viral infection.

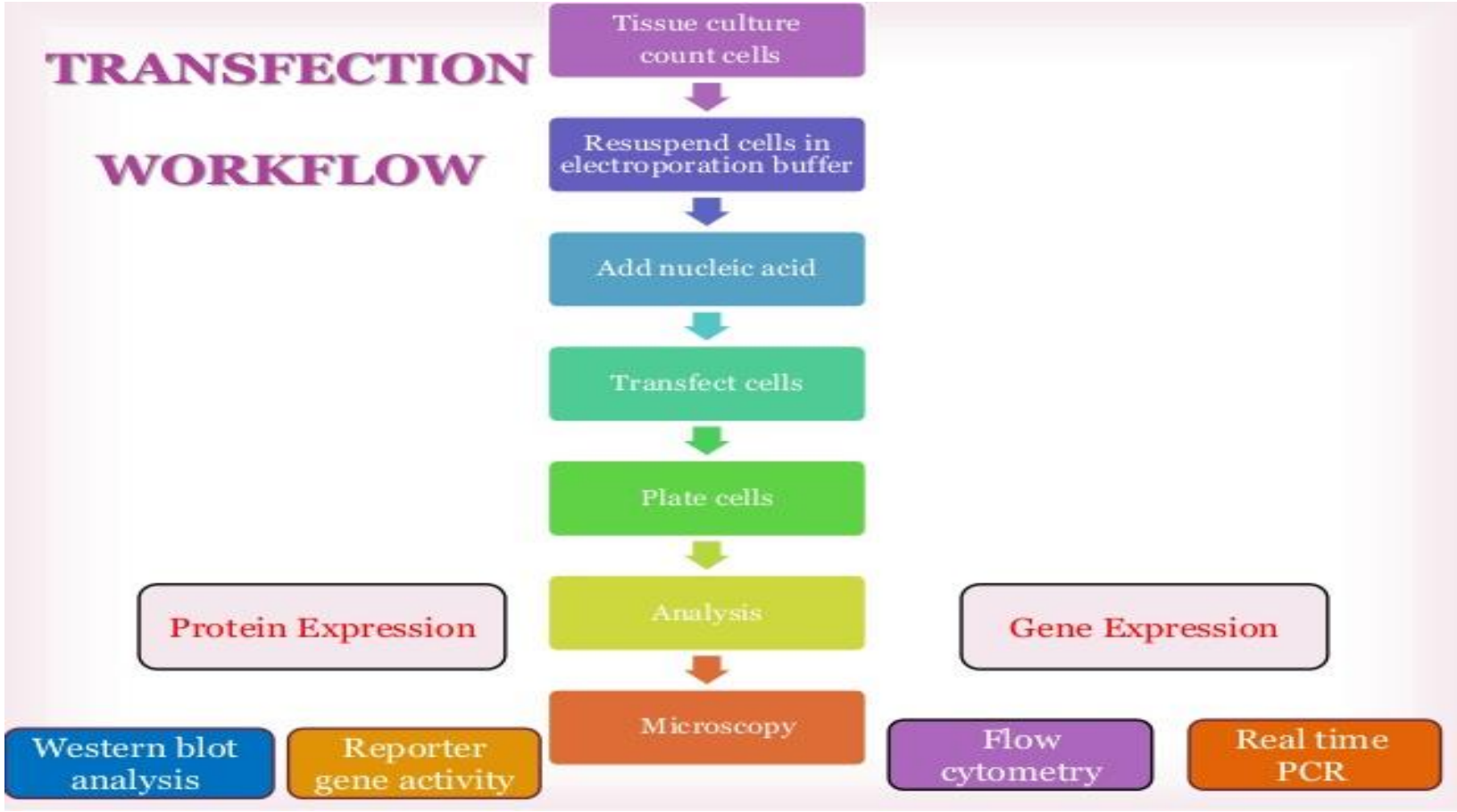
- Introduction of foreign DNA into cells either by physical (electroporation) or chemical (cationic lipid or calcium phosphate reagents) methods.

- In transfection, the introduced nucleic acid may exist in the cells transiently, such that it only expressed for a limited period of time and does not replicate, or it may be stable and integrated into the genome of the recipient, replicating when the host genome replicates.

TRANSFECTION TERMINOLOGY

- The terminology used for various gene delivery systems has evolved to keep pace with technology advances in the field and further refined to distinguish various methods and cell types.
- Cell that have incorporated the foreign DNA are called transfectants.
- **Stable transfectants:** Cells that have integrated foreign DNA in their genome.
- **Transient transfectants:** Foreign DNA does not integrate in the genomebut genes are expressed for limited time (24-96 hours).

TRANSFECTION WORKFLOW

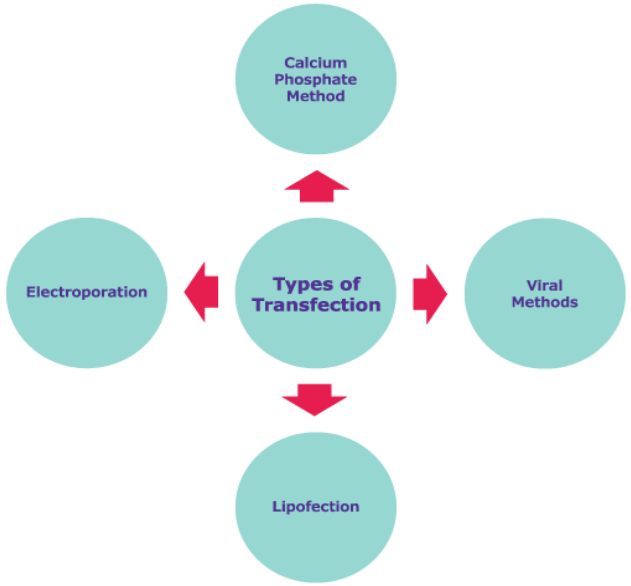


FACTORS INFLENCING TRANSFECTION EFFICIENCY

- Cell type
- Cell health
- Confluency
- Serum
- Time
- DNA quality & quantity

COMMON TRANSFECTION METHODS

i. Calcium phosphate Precipitation
ii. DEAE – Dextran mediated transfection
iii. Lipofection
iv. Electroporation
v. Retroviral infection
vi. Microinjection



CALCIUM PHOSPHATE PRECIPITATION

- This method is popular transfection method since its introduction in the early 1970s by Graham and Ven der Eb, 1973.
- This technique is easy and effected with many types of cultured cells
- It can be used for both transient and stable transfection of variety of cultured cell types.
- **Principle:**

i. DNA is mixed with calcium chloride
ii. Addition to buffered saline/phosphate solution and incubating at room temperature
iii. Formation of DNA- calcium phosphate coprecipitation which adhere to surface of cells
iv. Uptake presumably by endocytosis or phagocytosis.

METHOD USED

- **Steps –**

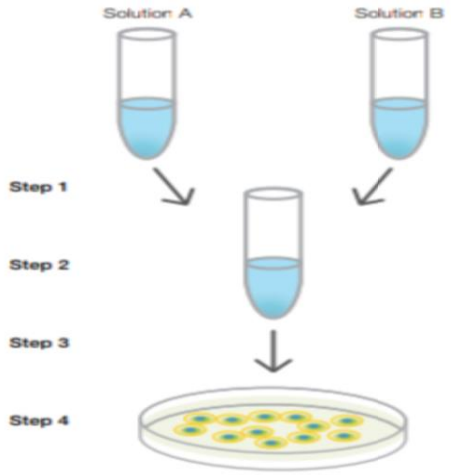
i. Solutions used are DNA in calcium solution and 2x Hanks buffered saline solution

ii. Add solution DNA in calcium to hanks buffered saline solution

iii. Incubate 20-30 min . Apply the solution to a subconfluent cell culture

iv. Incubate 2-12 hr. Replace the solution with complete growth medium.

v. Assay for transient gene expression or begin selection for stable transformation time.



ADVANTAGES & DISADVANTAGES

- **ADVANTAGES-**

i. Easily available

ii. Inexpensive

iii. Can be used for transient and stable transfection

iv. Can be applied to wide range of cell types

v. Calcium phosphate appears to provide protection against intracellular and serum nuclease

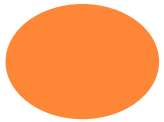
- **Disadvantages-**

i. Low efficiency

ii. It is not suited for in vivo gene transfer to whole animal

iii. Toxicity, especially to primary cells

iv. Size and quality of precipitate are crucial to the success of transfection.



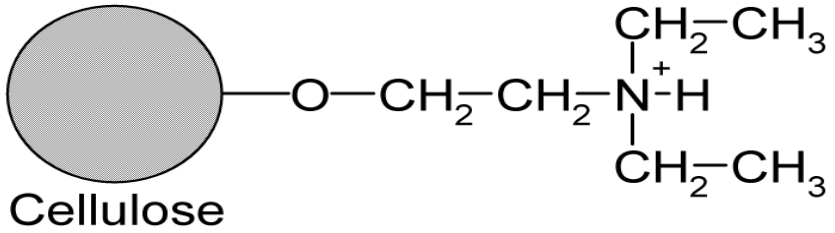
DEAE-DEXTRAN- MEDIATED TRANSFECTION

- DEAE- dextran was the first non-viral transfection method verified by Vaheri and Pagno in 1965.
- DEAE –Dextran is a cationic polymer that tightly associated with negatively charges nucleic acids.
- **Principle –**

i. DNA is mixed with DEAE-dextran

ii. DNA/polymer complex comes into contact with the negatively charged membrane due to excess of positive charge contributed by polymer entry in to the cytoplasm via endocytosis or osmotic shock induced by DMSO or glycerol.

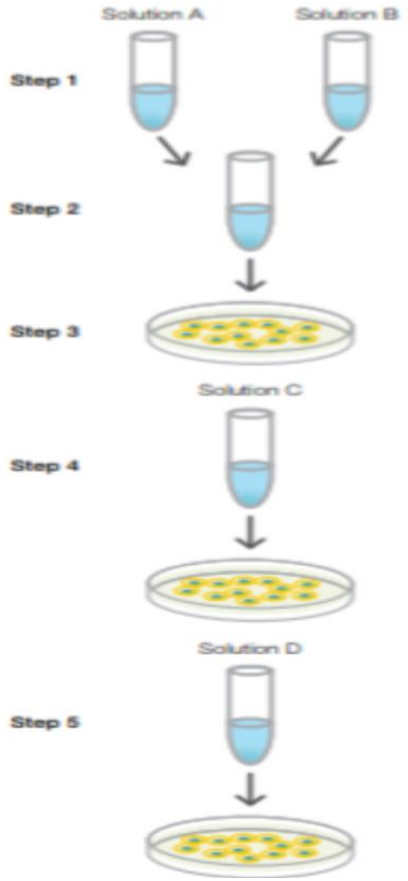
iii. Uptake presumably by endocytosis.



METHOD USED

- **STEPS-**

i. Mix nucleic acid with DEAE
ii. Add the nucleic acid-DEAE to cells
iii. Induce the uptake
iv. Wash cells
v. Assay cells



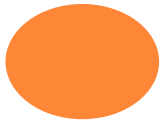
ADVANTAGES & DISADVANTAGES

- **Advantages:**

i. Inexpensive
ii. Easy to perform and quick
iii. Can be applied to wide range of cell types
iv. Low cost

- **Disadvantage:**

i. High concentration of DEAE-Dextran can be toxic to the cell
ii. Transfection efficiency will vary with cell type.
iii. Can be used for transient transfection
iv. Typically produces less than 10% delivery in primary cells.



LIPOFECTION

- Lipofection is a technique used to inject genetic material into a cell by means of liposomes .
- Liposomes are vesicles that can easily merge with the cell membrane since they are both made of phospholipid bilayer.
- **Principle :**

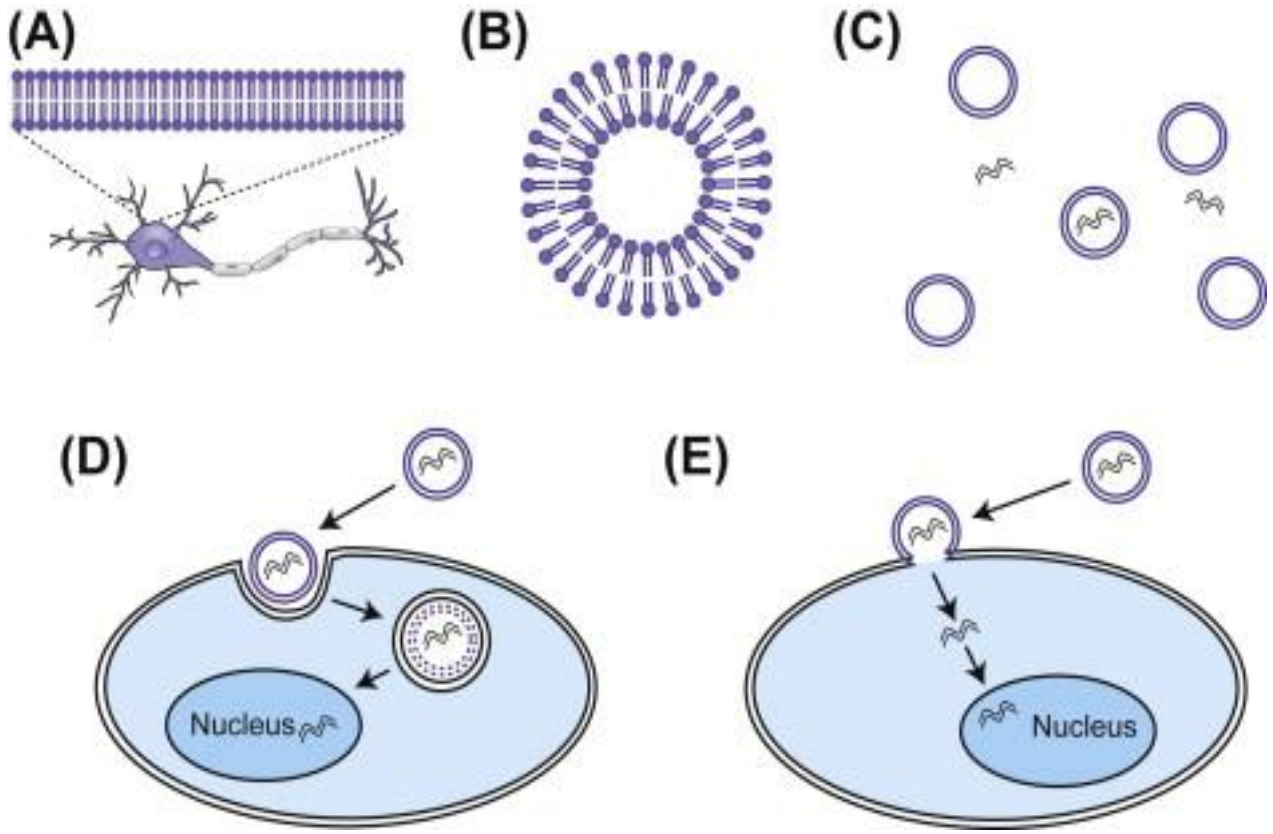
i. Generally uses a positively charged lipid to form a structure with negatively charged genetic material.

ii. Fusion of liposomes/nucleic acid transfection complex with negatively charged cell membrane takes place .

iii. Transfection complex enter the cell through endocytosis.

iv. Once inside the cell, the complex diffuse through cytoplasm and enter the nucleus for gene expression.

LIPOFECTION MECHANISM



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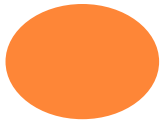

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