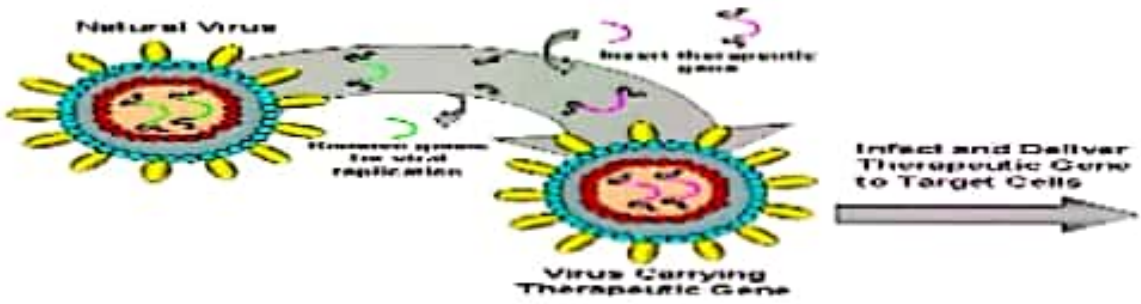
GENE TRANSFER TECHNOLOGY IN ANIMALS

VIRAL AND NON-VIRAL METHOD



INTRODUCTION

Gene transfer is to transfer a gene and DNA segments from one organism to another.

This area of genetic manipulation makes important contributions to domesticated animals in relation to immunology, vaccines, aging, and cancer.

Gene transfer represents a relatively new possibility for the treatment of rare genetic disorders and common multifactorial diseases by changing the expression of a person's genes.

Gene transfer method in animals

There are four major strategies for gene transfer to animal cells, two of which are considered :-

biological mechanisms using virus (transduction)

Virus (transduction); the transferred gene represents part of the viral genome.

or bacterial that invade animal cells (bactofection)

Bacteria (bactofection); the gene will be transferred as a plasmid.

Biochemical and physical methods which do not involve infection thus termed transfection.

Non viral methods (Bio chemical and Physical methods)

Biochemical methods

Calcium phosphate method;

involves the formation of a fine DNA/calcium phosphate co-precipitate which first settles on the cells and then internalized by endocytosis.

The precipitate must be formed freshly at the time of transfection.

The DNA escapes and reaches the nucleus and can be then expressed.

Since the cells must be coated by the calcium complex, monolayers of cells must be used for maximum efficiency. However, this method gives only 1-2% transfection efficiency.

DEAE-dextran mediated (diethylaminoethyloethyl-dextran)

There are three points that DEAE-dextran mediated transfection differs from calcium phosphate coprecipitation.

(1) It is used for transient transfection.

(2) (2) It works more efficiently with cell lines of BSC-1, CV-1 and COS, etc.

(3) (3) It is more sensitive.

protocols :-

(1) Harvest exponentially growing cells by trypsinization and transfer then into 60-mm tissue culture dished at a density of 105 cells/dish.

(2) Add 5 ml complete growth medium.

(3) Incubate 24 hours at 37°C with 5% CO_2.

(4) Prepare DNA/DEAE-dextran/TBS-D solution by mixing 2 mg of superoiled plasmid DNA into 1 mg/ml DEAE-dextran in TBS-D.

(5) Remove medium and wash tree times with PBS and twice with TBS-D.

DEAE-dextran mediated cont.

(6) Add DNA/DEAE-dextran/TBS-D solution 250 ml.

(7) Incubate 60 min at 37°C with 5% CO_2.

(8) Remove DNA/DEAE-dextran/TBS-D solution.

(9) Wash with TBS-D three time and PBS twice.

(10) Add 5 ml medium supplemented with serum and chloroquine (0.1 mM).

(11) Incubate 4 hours at 37°C with 5% CO_2.

(12) Remove medium.

(13)Wash with serum-free medium three times.

(14) Add to cells 5 ml of medium supplement with serum, and incubate 48 hours at 37°C with 5% CO_2.

(15) Harvest the cells after the 48 hours transfer-

ction.

(16) Analyze RNA or DNA by hybridization, or analyze expressed protein by radiommunoassay, immunoblotting, immuniprecipitation, or by enxzy moatic activity in cell extract.

Lipid mediated method

This method can be used for both transient and stable transfection, and it can be used for adherent cells, primary cell lines, and suspension cultures.

For the following protocol, the Lipofectamine Reagent from Invitrogen Corporation will be used. Lipofectamine Reagent is a 3:1 (w/w)

Liposome formulation of the plycationic lipid 2,3-dioleyloxy-N-[2(sperminecardox-ido)ethyl]-N,N-dimethyl-1-propanaminium trifluoro-acetate (DOSPA) and the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) in membrane-filtered water.

Lipid mediated method protocol :-

1) Put about 40,000 cells per well of a 24-well plate in 0.5 ml of the appropriate complete growth medium (add 10% serum if it needs).

(2) Incubate the cells at 37°C in a CO_2 incubator until the cells are 50-80% confluent (about 20 hours, depending on the cells).

(3) Dilute 3 mg DNA into 25 ml medium without serum for each well and mix.

(4) Dilute 3 ml Lipofectamine Reagent into 25 ml medium without serum for each well and mix.

(5) Combine diluted DNA (Step 3) and Lipofect-amine Reagent (Step 4) and incubate at room temperature for 30 min. In this step the DNA-liposome complexes are formed.

(6) Replace the medium in the cells with 0.2 ml transfection medium without serum.

Lipid mediated method cont.

(7) Add 0.15 ml medium without serum to the tube containing the complexes for each well.

(8) Incubate the cells with the complexes for about 10 hours at 37°C in a CO_2 incubator. The incubating time will be flexible by the cell type.

(9) Add 0.4 ml growth medium containing double the 2× normal concentration of the serum without removing the transfection mixture.

(10) Replace the medium with fresh, complete medium at 20 hours following the start of transfection if continued cell growth is required.

(11) Assay cell extracts for transient gene expression at 24-72 hours after transfection, depending on the cell type and promoter activity.

(12) To obtain stable transfectants, passage the cells 1:10 into the selective medium after 72 hours of transfection for the reporter gene transfected.

Physical method of gene transfer or transfection in animal (Non viral methods)

In this method, naked DNA is deposited directly into the cell by exploiting a physical force.

This includes

Microinjection

particle bombardment

ultrasound

electroporation

Which ever method used, the result is called **transformation** which is a change in the recipient cell's genome caused by the acquired transgene.