Gene Delivery

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INTRODUCTION

Definition

Gene therapy is a process that involves introduction of genetic material into a person's cell to cure or prevent the disease condition.

- The method of gene transfer and its expression is termed as "transduction".
- A gene can be transferred to an individual using a carrier known as a "vector".

- Broadly cells can be divided into
  - Somatic (most cells of the body)
  - Germline (eggs or sperm)
- Either somatic cells or germ cells can manipulate genetic composition and serve the basis of gene therapy.
- Gene therapy offers promising approaches towards treating many incurable diseases.

Basic process of gene therapy

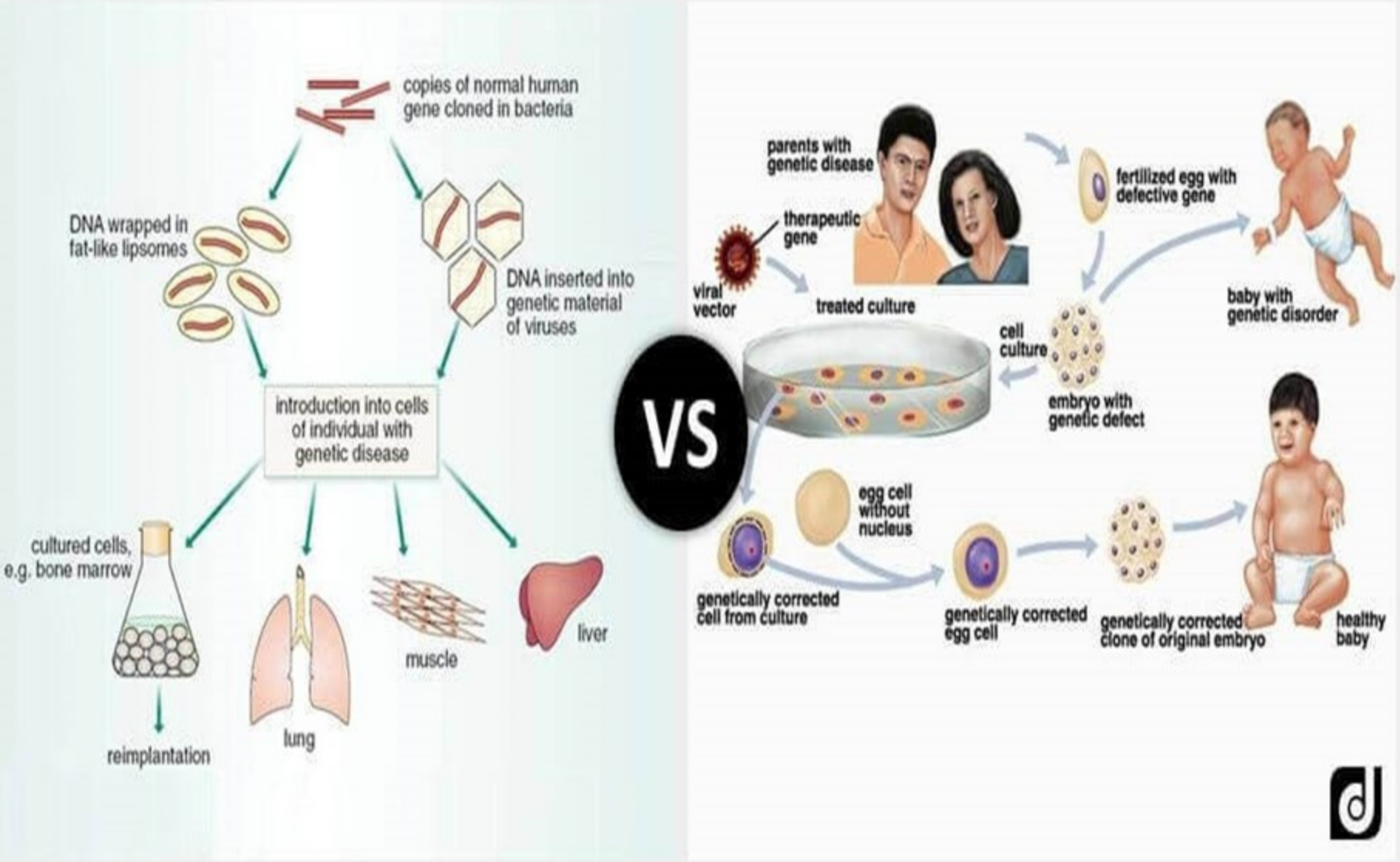
- Introducing a new specific gene into the body to combat a particular disease.
- Interchanging a disease causing gene with a healthy copy of same gene.
- Inactivating, or "knocking out," of an impaired gene that is not carrying normal function using engineered nucleases

Types of Gene Therapy

- Somatic gene therapy
  - *Ex vivo* therapy
  - *In vivo* therapy
- Germ line gene therapy

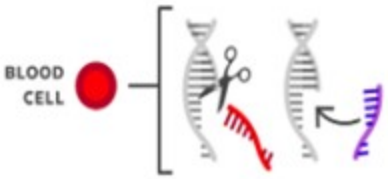
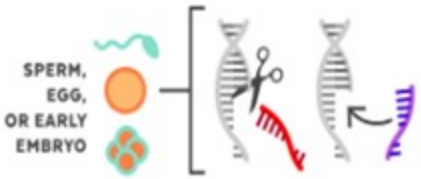
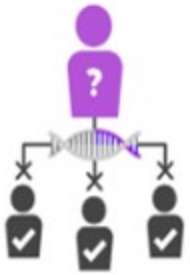
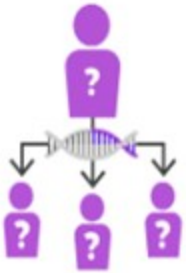
Differences Between Somatic & Germ line gene therapy

Somatic Gene Therapy	Germ Line Gene Therapy
Genes introduced into somatic cells	Therapeutic genes transferred into germ cells
Will not be inherited into next generations	Inherited into next generations
Techniques for somatic cell culture, introduction of healthy gene and re-implantation back to body are successfully developed	Many practical difficulties in introduction of healthy genes into germ cells
Not much ethical issues attached	Many ethical problems yet to be answered
Most often it may not be possible to achieve normal level of expression similar to that of normal gene	High frequency of insertional mutations are observed . Often lead to teratogenic consequences



Somatic Gene Therapy vs. Germline Gene Therapy

SOMATIC GENE EDITING VS. GERMLINE GENE EDITING

	SOMATIC GENE EDITING	GERMLINE GENE EDITING
EDIT	 Somatic therapies target genes in specific types of cells (blood cells, for example).	 Germline modifications are made so early in development that any change is copied into all of the new cells.
COPY	 The edited gene is contained only in the target cell type. No other types of cells are affected.	 The edited gene is copied in every cell, including sperm or eggs.
RISKS	 Any changes, including potential off-target effects, are limited to the treated individual.	 If the person has children, the edited gene is passed on to future generations.
NEXT GENERATION	The edited gene is not passed down to future generations.	
CONSENSUS	 Somatic cell therapies have been researched and tested for more than 20 years and are highly regulated.	 Human germline editing is new. Heritability of germline changes presents new legal and societal considerations.

Gene Therapy - Approaches

Non-classical gene therapy

- It involves the inhibition of expression of genes associated with the pathogenesis, or to correct a genetic defect and restore the normal gene expression.

Classical Gene Therapy

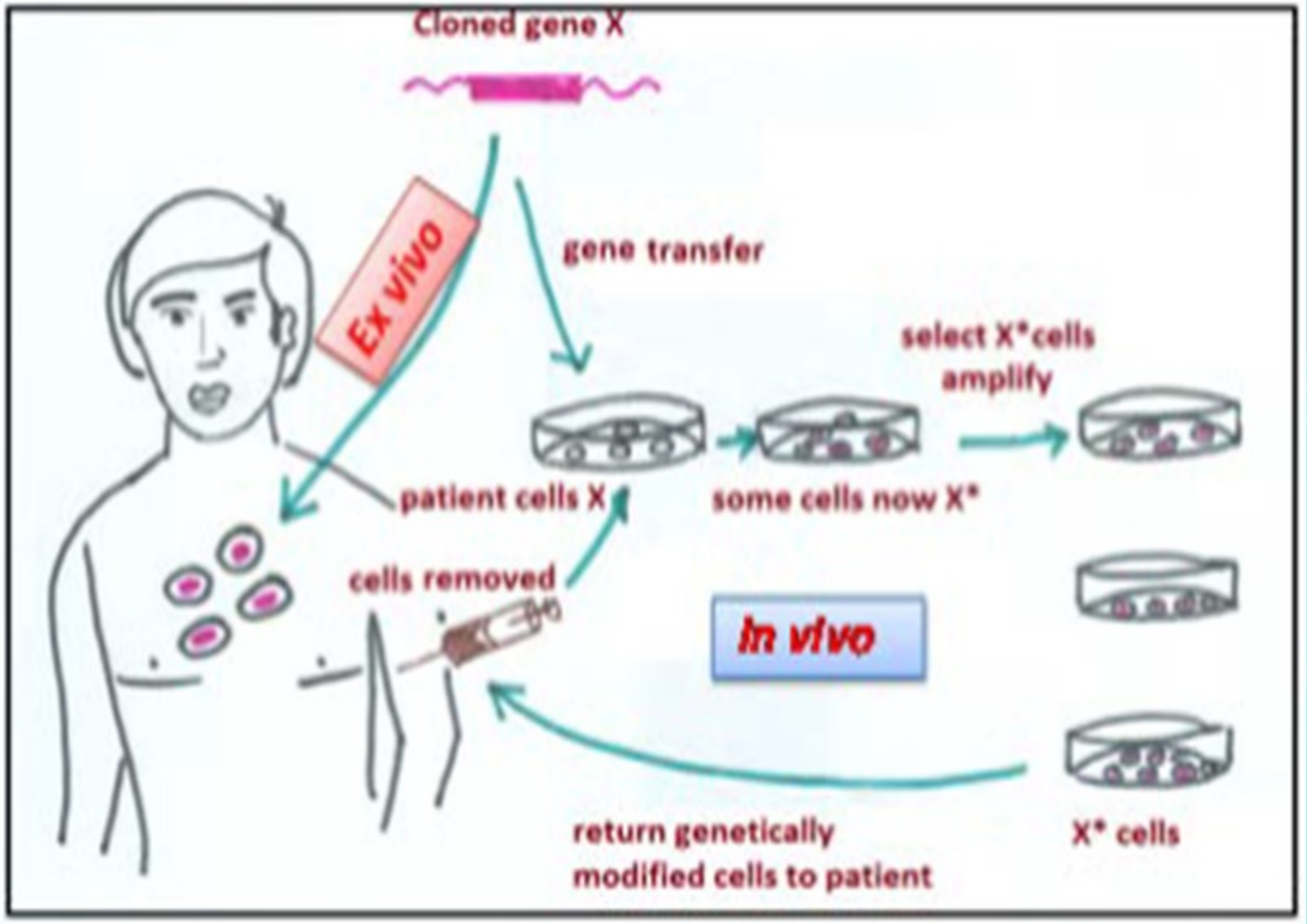
- It involves therapeutic gene delivery and their optimum expression once inside the target cell.
- The foreign genes carry out following functions
  - Produce a product (protein) that the patient lacks
  - Produces toxin so that diseased cell is killed
  - Activate cells of the immune system so as to help in killing of diseased cells

Somatic Gene Therapy

- There are mainly two approaches for the transfer of genes in gene therapy:

  1. Transfer of genes into patient cells outside the body (*ex vivo* gene therapy)

  2. Transfer of genes directly to cells inside the body (*in vivo* gene therapy)



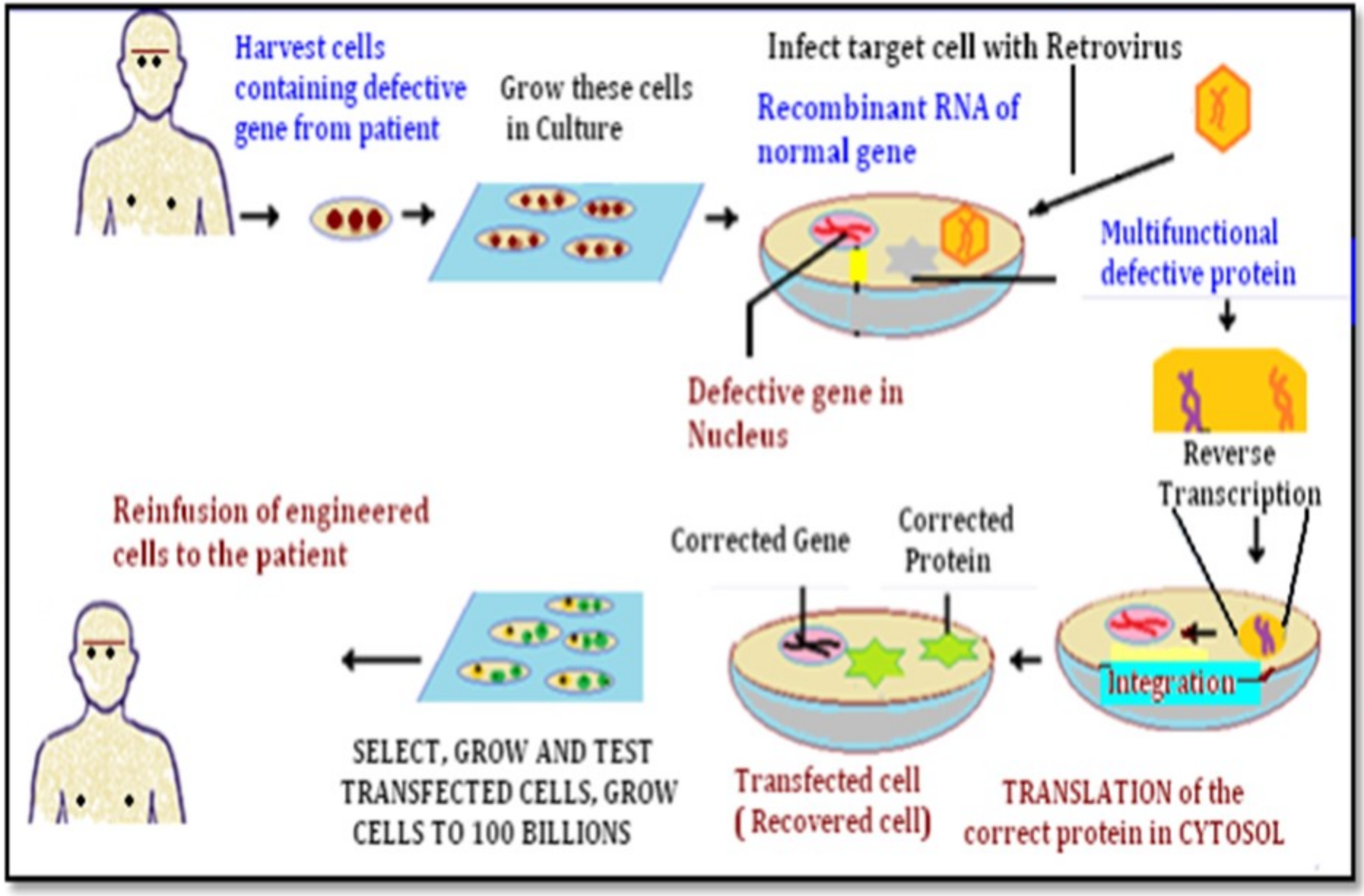
Reference: NPTEL module

Ex vivo Gene Therapy

- Cells are sourced from the patient to be treated
- Genes are transferred to the cells grown in culture
- Transformed cells are selected
- Selected Cells are multiplied
- Introduced into the patient

Ex vivo Gene Therapy

- Autologous cells prevents immune system rejection
- This approach can be applied to the tissues like hematopoietic cells and skin cells
- These can be removed from the body, genetically corrected outside the body and reintroduced into the patient body where they become engrafted and survive for a long period of time.



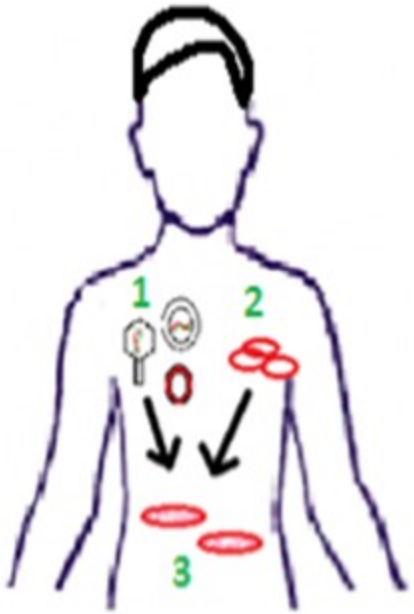
Reference: NPTEL module

In vivo Gene Therapy

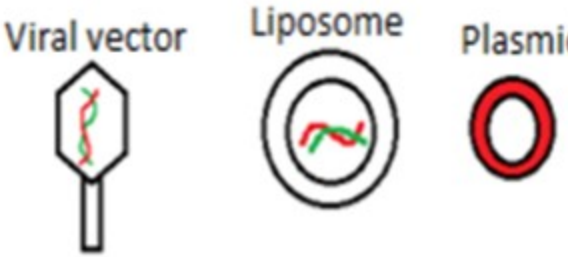
- *In vivo* method of gene transfer involves the transfer of cloned genes directly into the tissues of the patient.
- Advantageous incase of
  - Tissues whose individual cells cannot be cultured *in vitro* in sufficient numbers Eg: Brain cells)
  - Re-implantation of the cultured cells in the patient is not efficient.
- Examples of Vectors: Liposomes and certain viral

In vivo Gene Therapy

- Modified recombinant vectors are used as vectors in this therapy.
- These cultured cells will be called as vector-producing cells (VPCs)).
- The VPCs transfer the gene to surrounding disease cells.
- The efficiency of gene transfer and expression determines the success of this approach.



1. THERAPEUTIC GENES ARE INSERTED INTO VIRAL DNA, LIPOSOME OR AS PLASMID DNA



2. GENETICALLY ALTERED DNA IS INSERTED INTO PATIENTS BODY BY DIRECT TISSUE INJECTION

3. THE INSERTED DNA IS INCORPORATED INTO THE SPECIFIC CELL AND PRODUCES THE NEEDED PROTEIN ENCODED BY THE INSERTED GENE

Reference: NPTEL module

In vivo Vs Ex vivo Gene Therapy

Ex vivo Gene Therapy	*In vivo* Gene Therapy
More invasive	Less invasive
Technically complex	Technical simple
No vectors introduced directly	Vectors introduced directly
Safety check possible	Safety check not possible
Close control possible	Decreased control over target cells