Vectors in Gene Delivery

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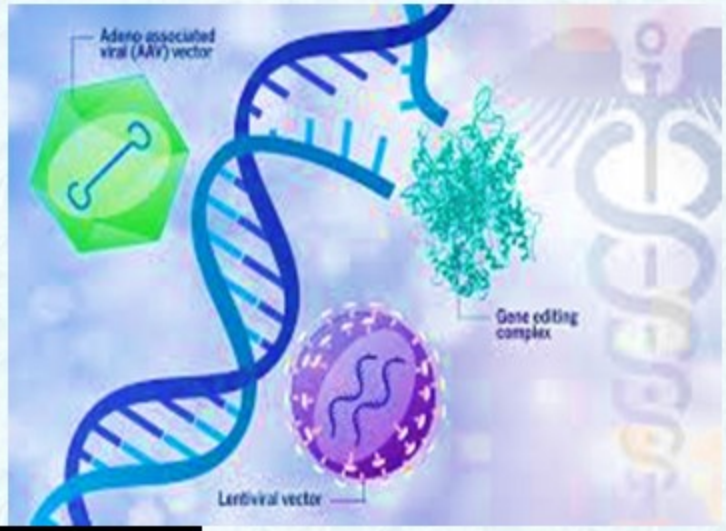
- Viral vectors
- Non viral vectors

INTRODUCTION

- A gene can be transferred to an individual using a carrier known as a "vector".
- The vector is very important as it carries the therapeutic gene to the target site.
- The efficiency of the gene therapy also depends on the efficiency of the carrier in taking the therapeutic gene to the target area.


Adeno-associated viral (AAV) vector
Gene editing complex
Lentiviral vector

Ideal properties

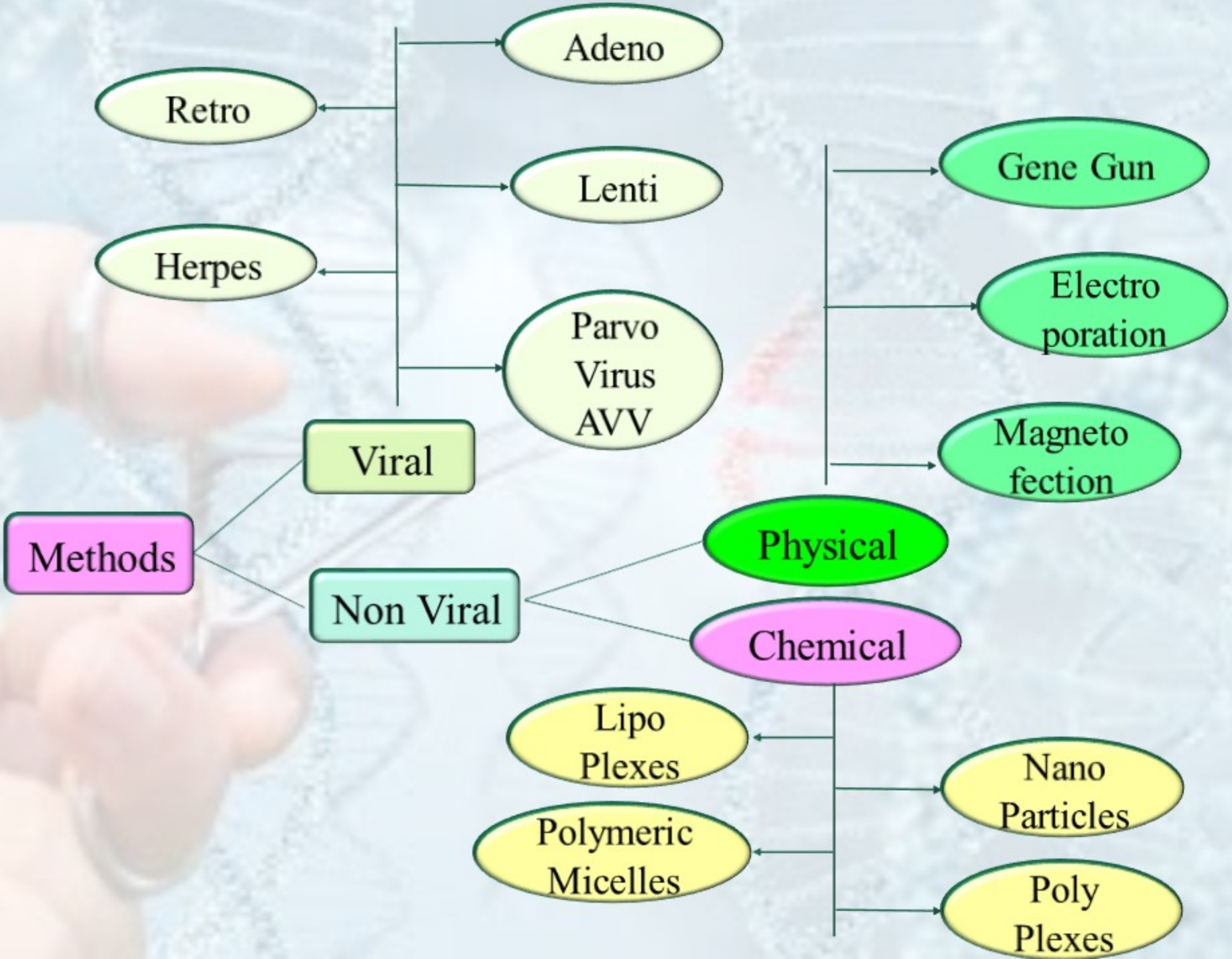
- Must target the correct cell
- Must integrate the genes into cells
- Activate the gene
- Avoid harmful side effects

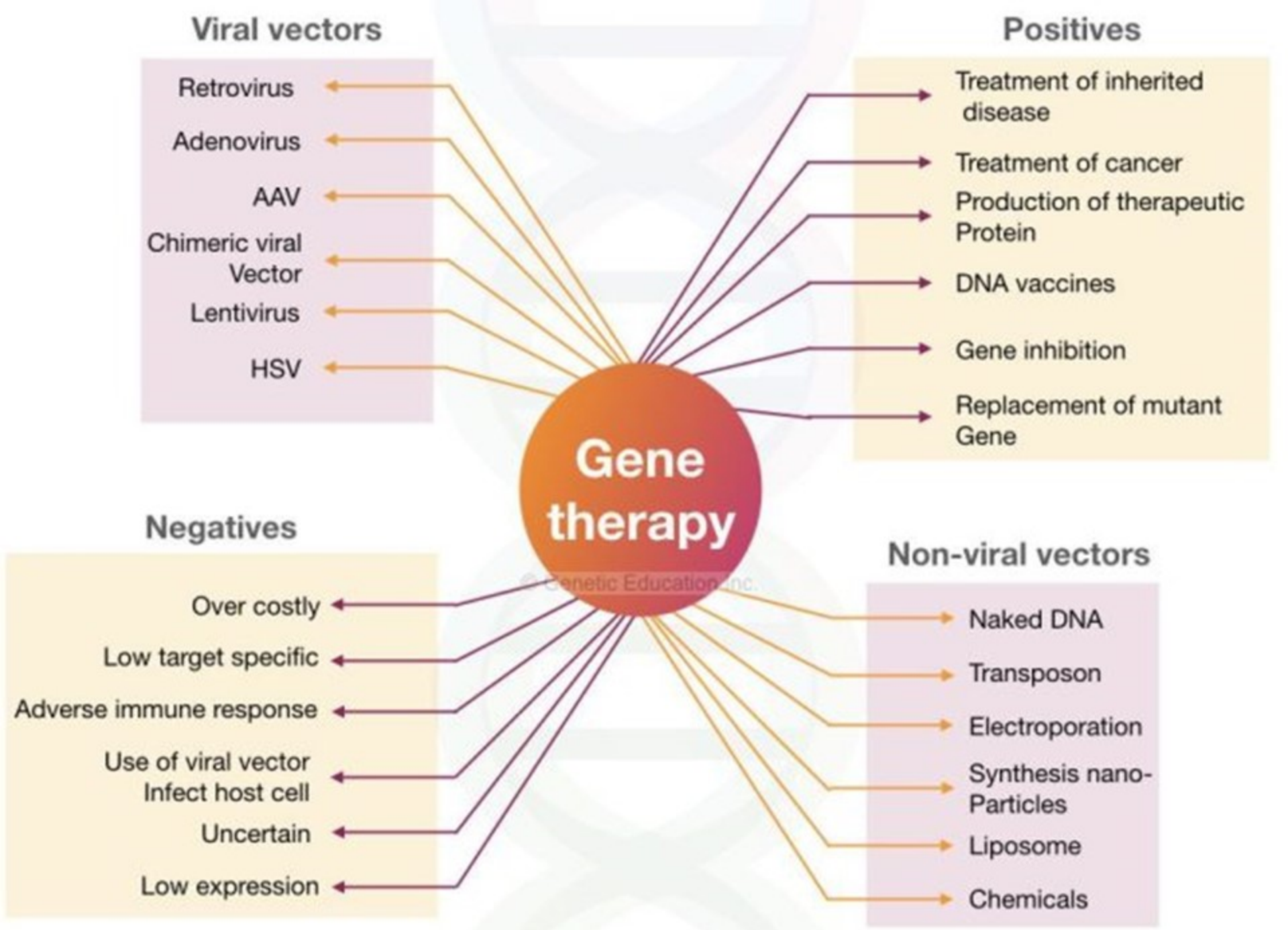
Types

- There are two types of vectors
  - Viral vectors
    - Retro virus
    - Adeno virus
    - Adeno-associated virus
    - Herpes simplex virus
  - Non viral vectors

Types

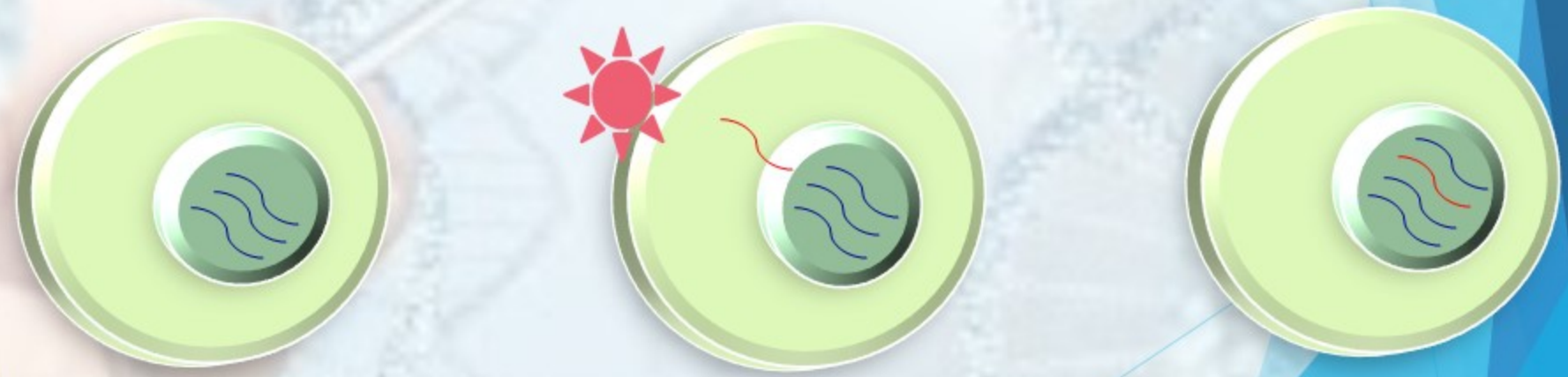
- Non viral vectors
  - Pure DNA construct
  - DNA molecular conjugates
  - Lipoplexes
  - Human artificial chromosome
  - polymers


Adeno
Retro
Lenti
Herpes
Parvo
Virus
AVV
Viral
Gene Gun
Electro
poration
Magneto
fection
Methods
Physical
Non Viral
Chemical
Lipo
Plexes
Nano
Particles
Polymeric
Micelles
Poly
Plexes


Viral vectors
Retrovirus
Adenovirus
AAV
Chimeric viral Vector
Lentivirus
HSV
Positives
Treatment of inherited disease
Treatment of cancer
Production of therapeutic Protein
DNA vaccines
Gene inhibition
Replacement of mutant Gene
Gene therapy
Negatives
Over costly
Low target specific
Adverse immune response
Use of viral vector Infect host cell
Uncertain
Low expression
Non-viral vectors
Naked DNA
Transposon
Electroporation
Synthesis nano-Particles
Liposome
Chemicals

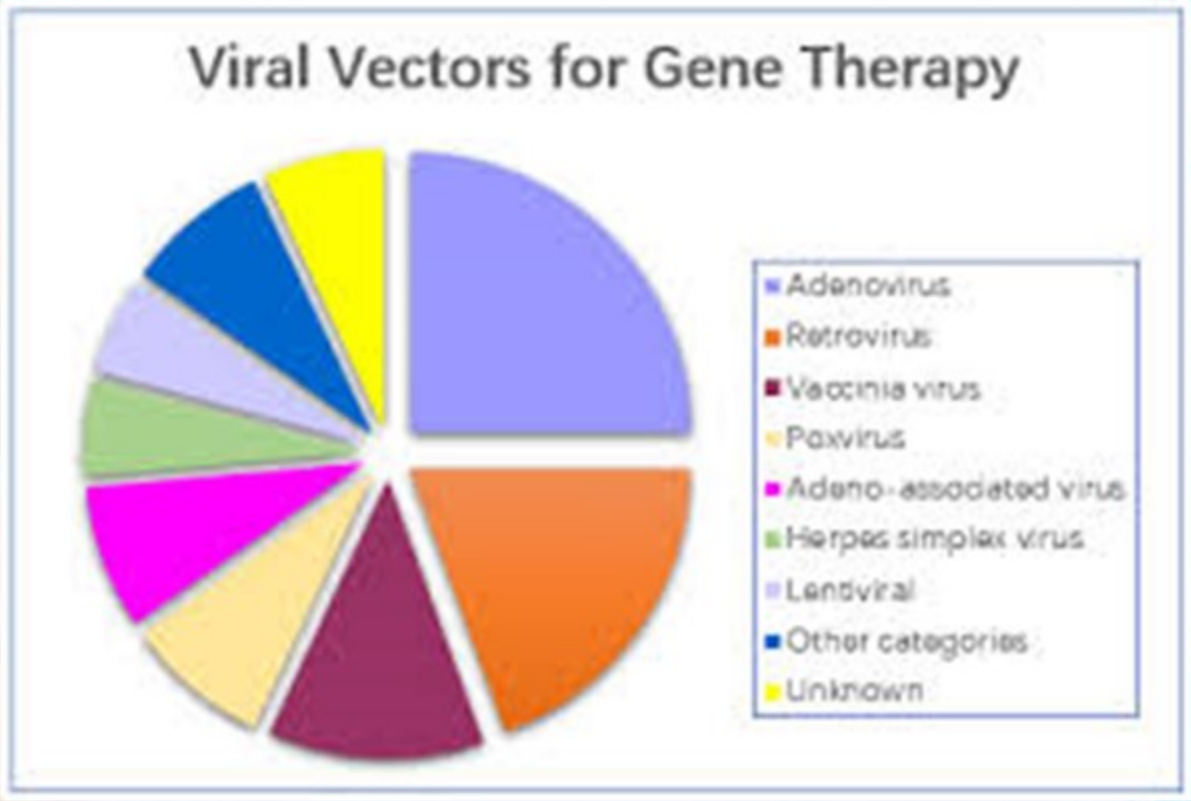
Viral Vectors

- The viruses inject their genetic material into host cell – During their replication.
- Removal of viral DNA nullifies the harmful effects.
- The Viral DNA removed virus can be used as carrier.



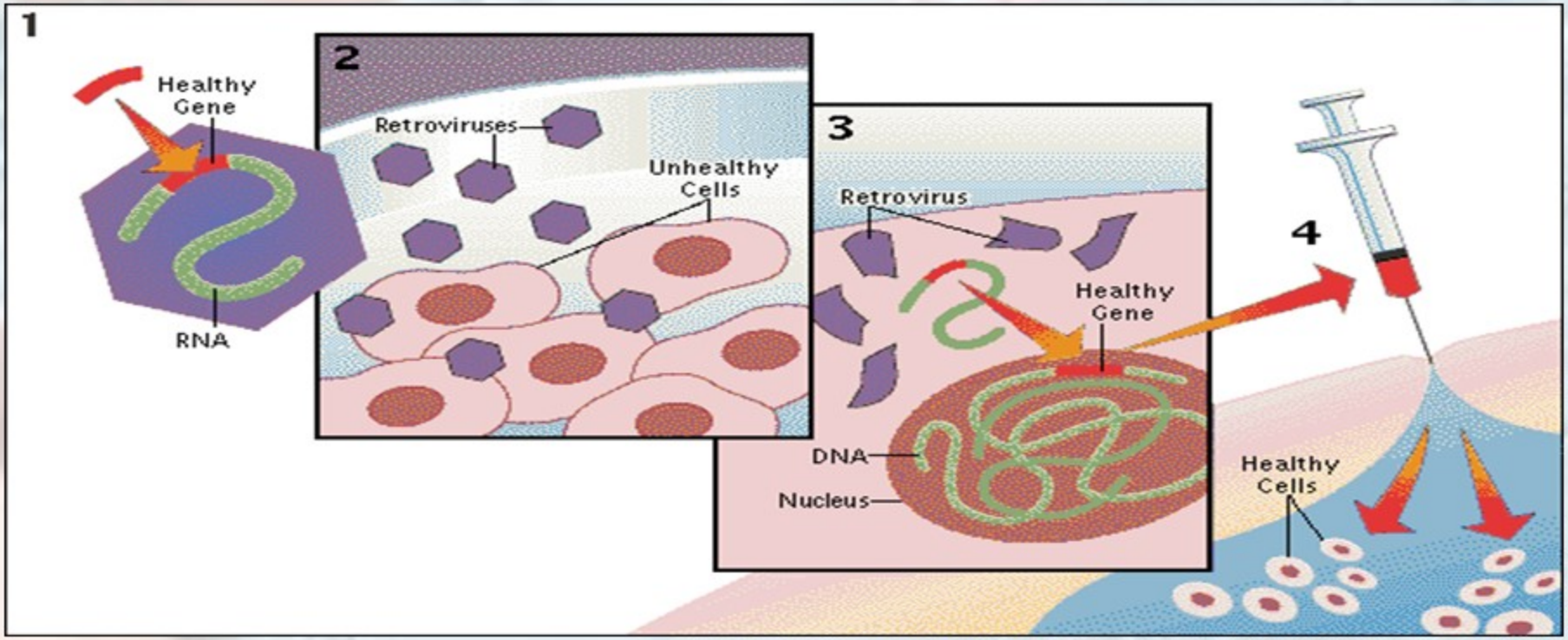
Actual Cell Vector inserting New gene New gene introduced

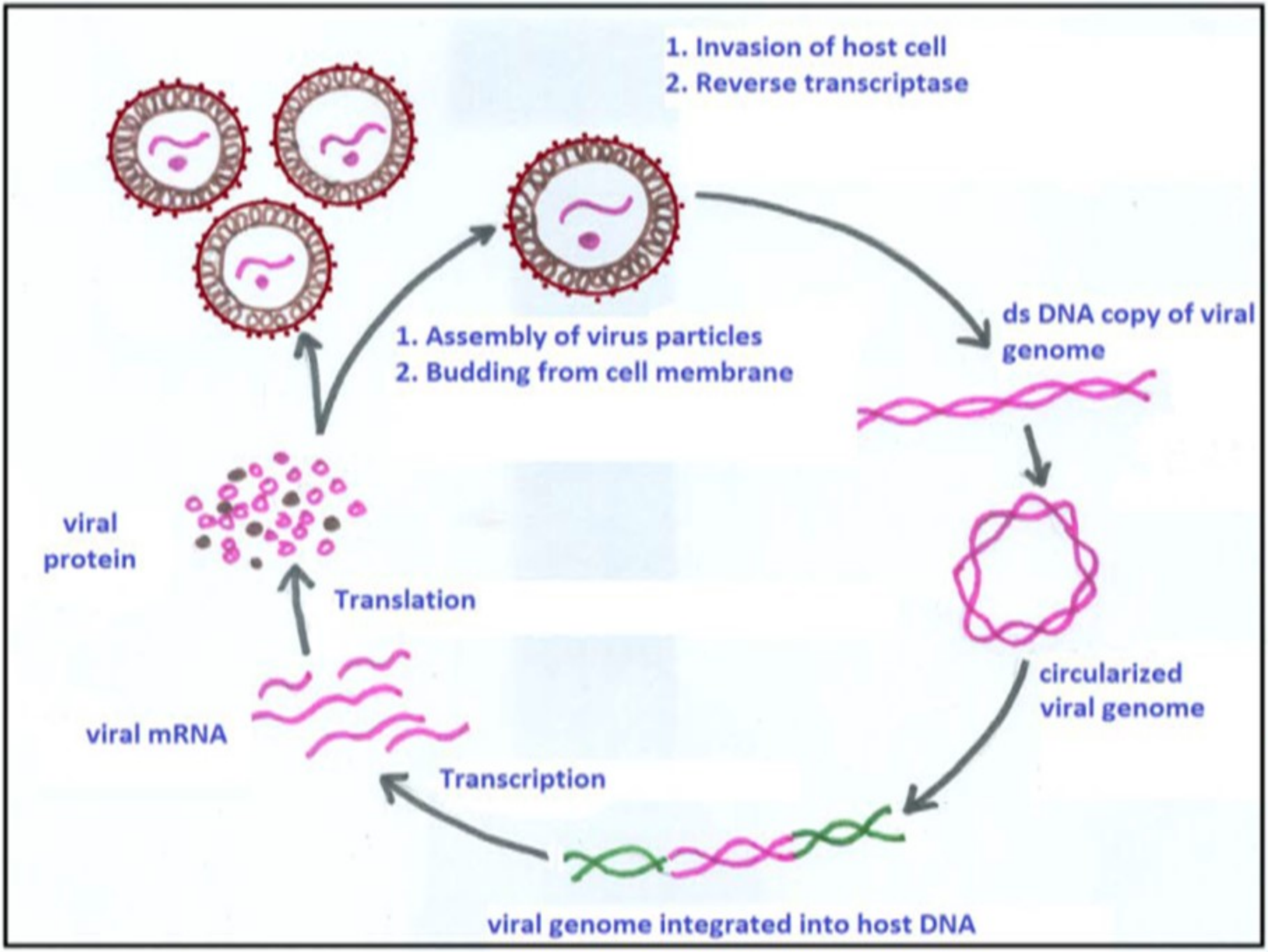
Proportionate use of different viral vectors



Retro Virus Vector

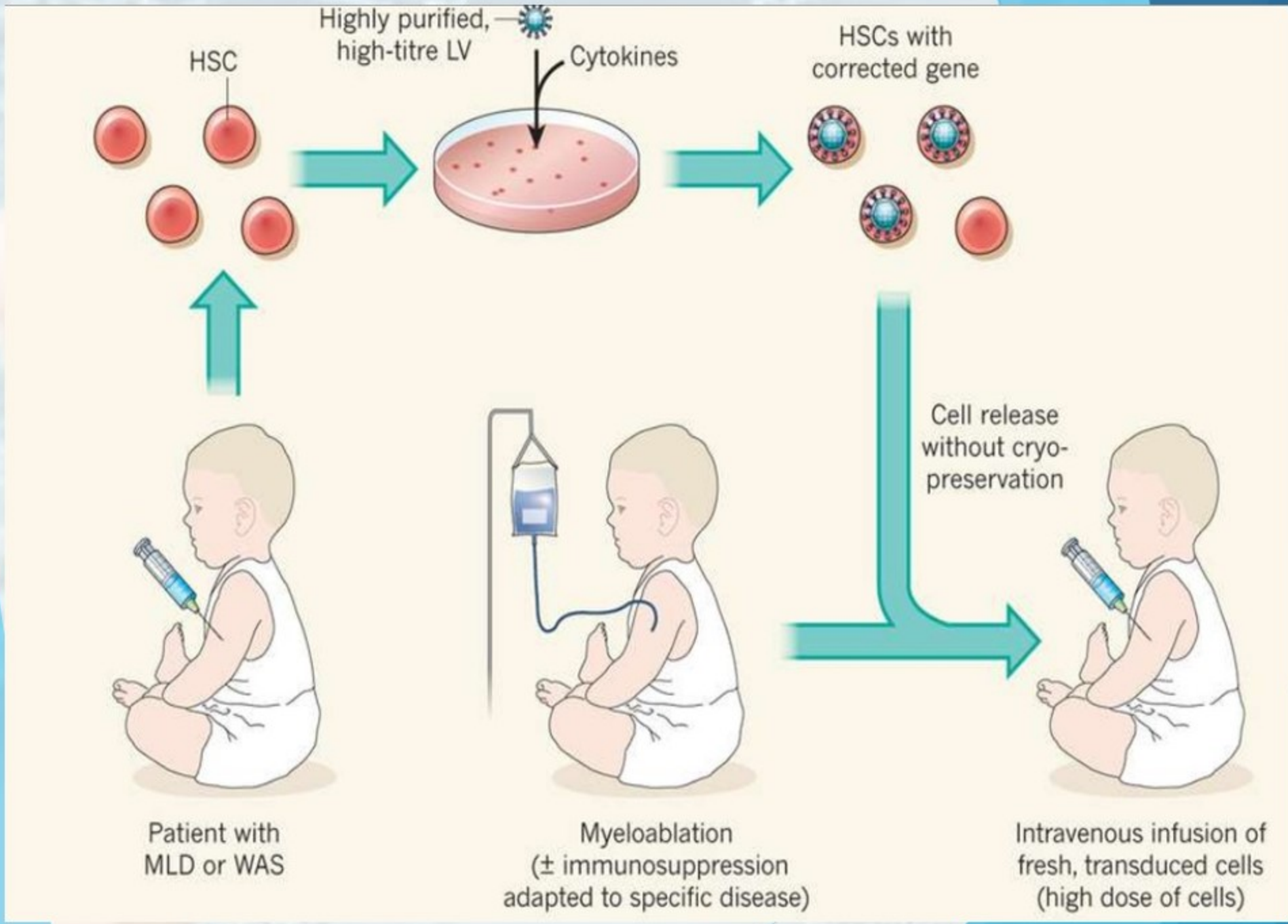
- Possess a reverse transcriptase activity, enabling them to synthesize a complementary DNA
- Carry DNA of size < 3.4 kb




1. Invasion of host cell
2. Reverse transcriptase
ds DNA copy of viral genome
circularized viral genome
viral genome integrated into host DNA
Transcription
viral mRNA
Translation
viral protein
1. Assembly of virus particles
2. Budding from cell membrane

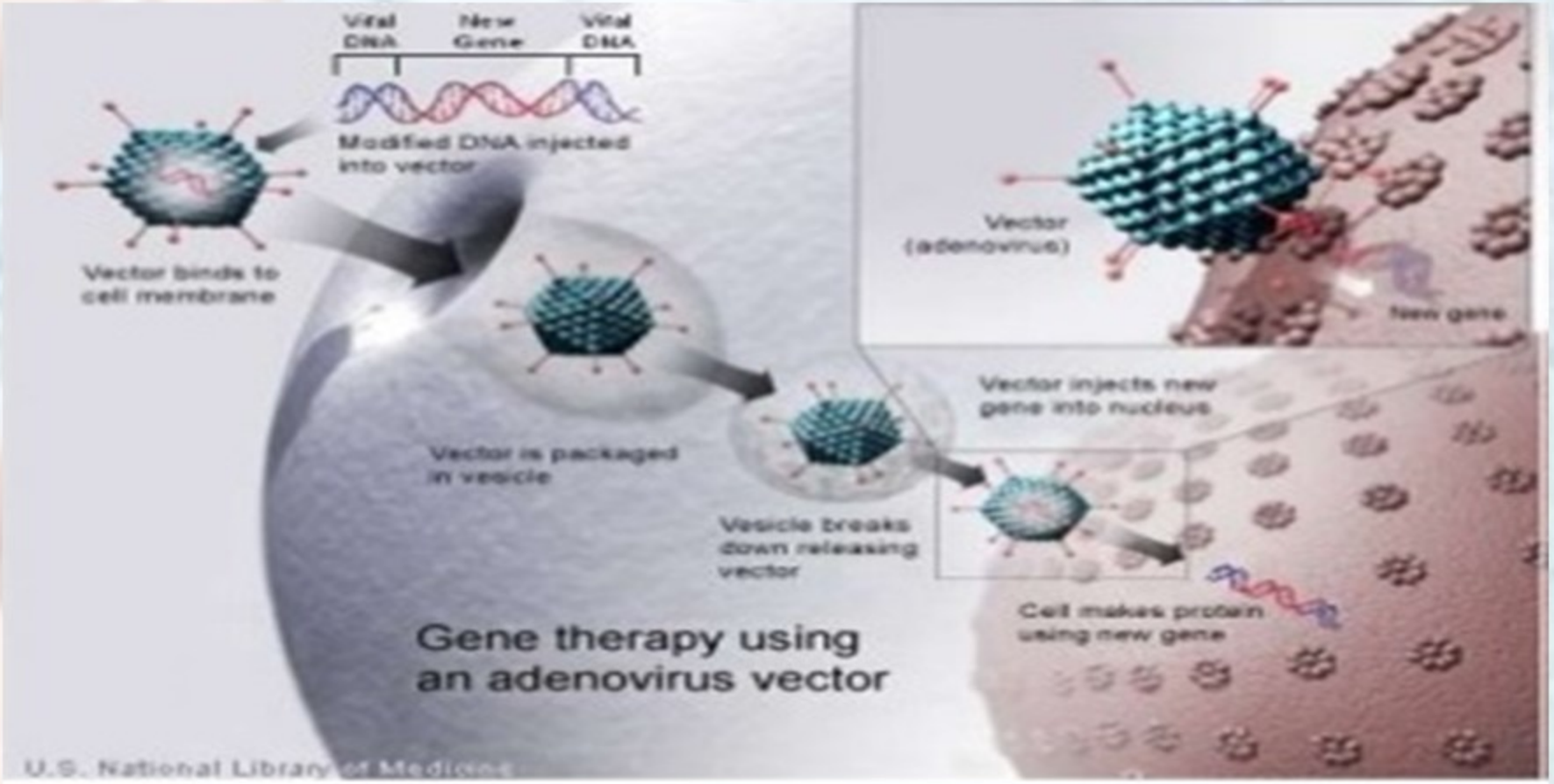
Lenti Virus

- Sub class of retro virus
- The viral genome reverse transcribed when virus enters the cell to produce DNA.
- This is inserted randomly into the genome by integrase enzyme
- Can target both dividing and non-dividing cells


HSC
Highly purified, high-titre LV
Cytokines
HSCs with corrected gene
Cell release without cryo-preservation
Patient with MLD or WAS
Myeloablation (± immunosuppression adapted to specific disease)
Intravenous infusion of fresh, transduced cells (high dose of cells)

Adeno Virus Vector

Adenoviruses are large linear double-stranded DNA viruses that are commonly used for preparing gene transfer vectors



Adeno Viral Vector - Types

- **First generation adenoviral** Vectors
- Their large size enables them to accept large inserts (gene of 3-4kb)

Advantages

- They are human viruses produced at very high titers in culture.
- They can infect a wide range of human cell types including non-dividing cells.
- They enter into cells by receptor mediated endocytosis with a very high transduction efficiency reaching upto 100% *in vitro*

Disadvantages

• Expression of foreign gene is for short period of time as they do not integrate into the chromosome.

• These vectors may generate immune response causing chronic inflammation.

Second generation adenoviral Vectors

These vectors have been developed to overcome these difficulties of First Generation Adenoviral Vectors.

Advantages

- It has improved safety and increased transgene expression.

Disadvantages

- These viral vectors are associated with immunological problems.
- Construction of these vectors is difficult.

Third generation adenoviral Vectors

- Otherwise called as **gutless adenovirus**.
- Also known as **helper dependent adenovirus** as they lack all the coding sequences & Require helper virus which carries all the coding sequences.
- The size of insert DNA can be 36kb – high capacity adenoviruses.

Advantages

- These are non-integrative and high-capacity vectors.
- It can be produced in high titer and the construction of these vector is easy.
- It shows longer stability and reduced immune response.

Disadvantages

- Helper virus contamination

contamination can cause diseases like conjunctivitis, pharyngitis, cold and respiratory disease.

Adeno- Associated Virus (AAV)

Adeno-associated viruses (AAVs) are a group of small, single-stranded DNA viruses which cannot usually undergo productive infection without co-infection by a helper virus, such as an adenovirus.

- The insert DNA size is 4.5 kb.
- It can integrate into chromosome 19.
- It is single stranded
- After entering into host cell it becomes double stranded and gets integrated into chromosome.

Herpes Virus

- Herpes simplex virus-1 (HSV-1) is a 150 kb double- stranded DNA virus with a broad host range that can infect both dividing and non- dividing cells.
- The insert size is comparatively larger (>20kb) but have a disadvantage of short-term expression due to its inability to integrate into the host chromosome.

Advantages & Disadvantages of Viral Vectors

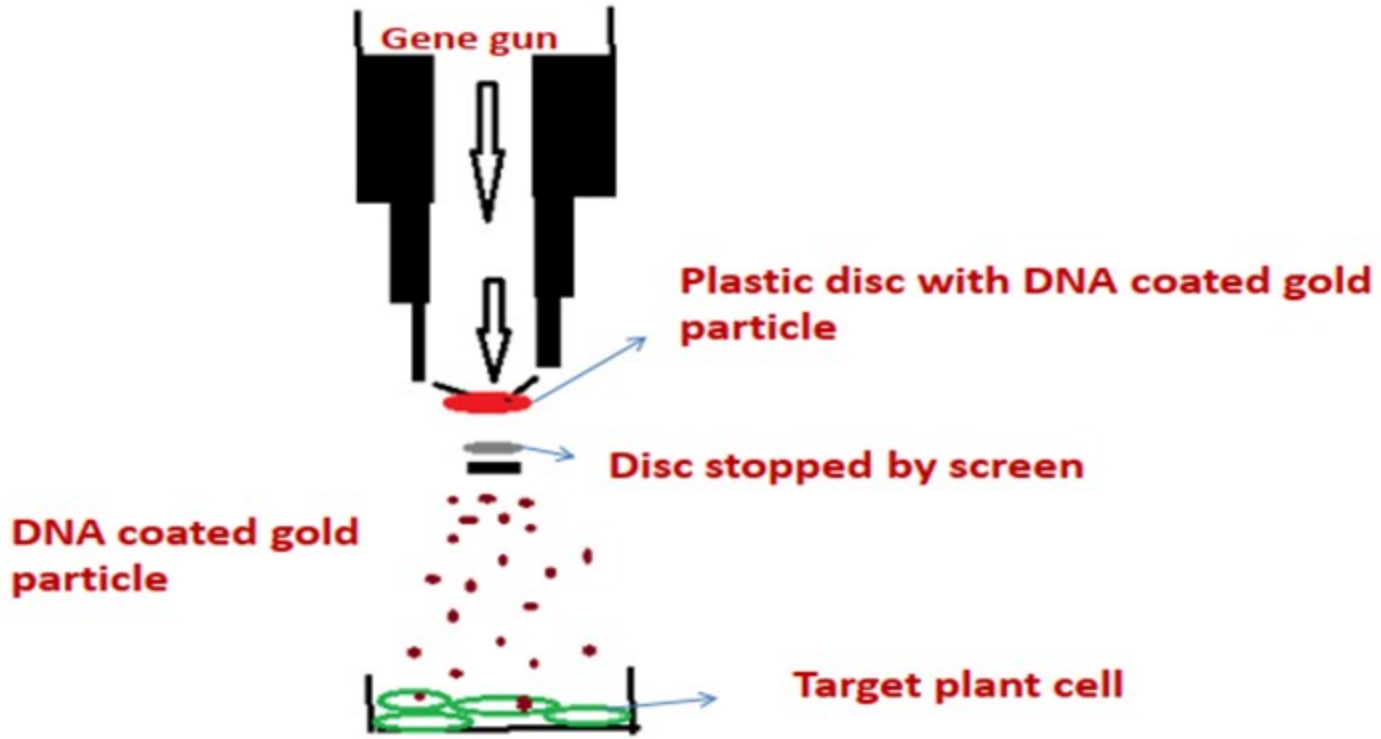
Advantages

- Targets specific cells
- Good at invasion

Gene Gun

- Particle bombardment technique: 'gene gun'
- DNA is coated on to metal micro particles and fired from a ballistic gun into cells/tissues.
- This technique is used to transfer the foreign DNA and its transient expression in mammalian cells in vitro and in vivo as well.
- It can cross the physical barriers like skin, muscle layer for which it is used for vaccination.

- Particle bombardment is used to deliver drugs, fluorescent dyes, antigenic proteins etc.



GENE GUN MEDIATED GENE TRANSFER

Advantage

- Simple and comparatively safe.

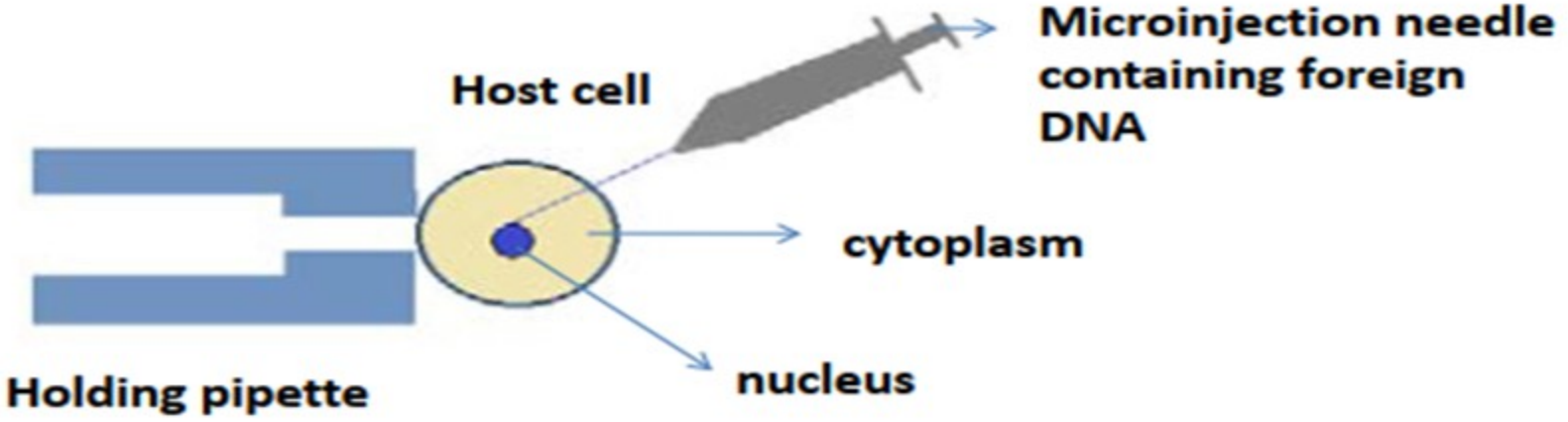
Disadvantage

- Poor efficiency of gene transfer.
- A low level of stable integration of the injected DNA.
- Repeated injection may cause damage in the proliferating cells.

Micro Injection

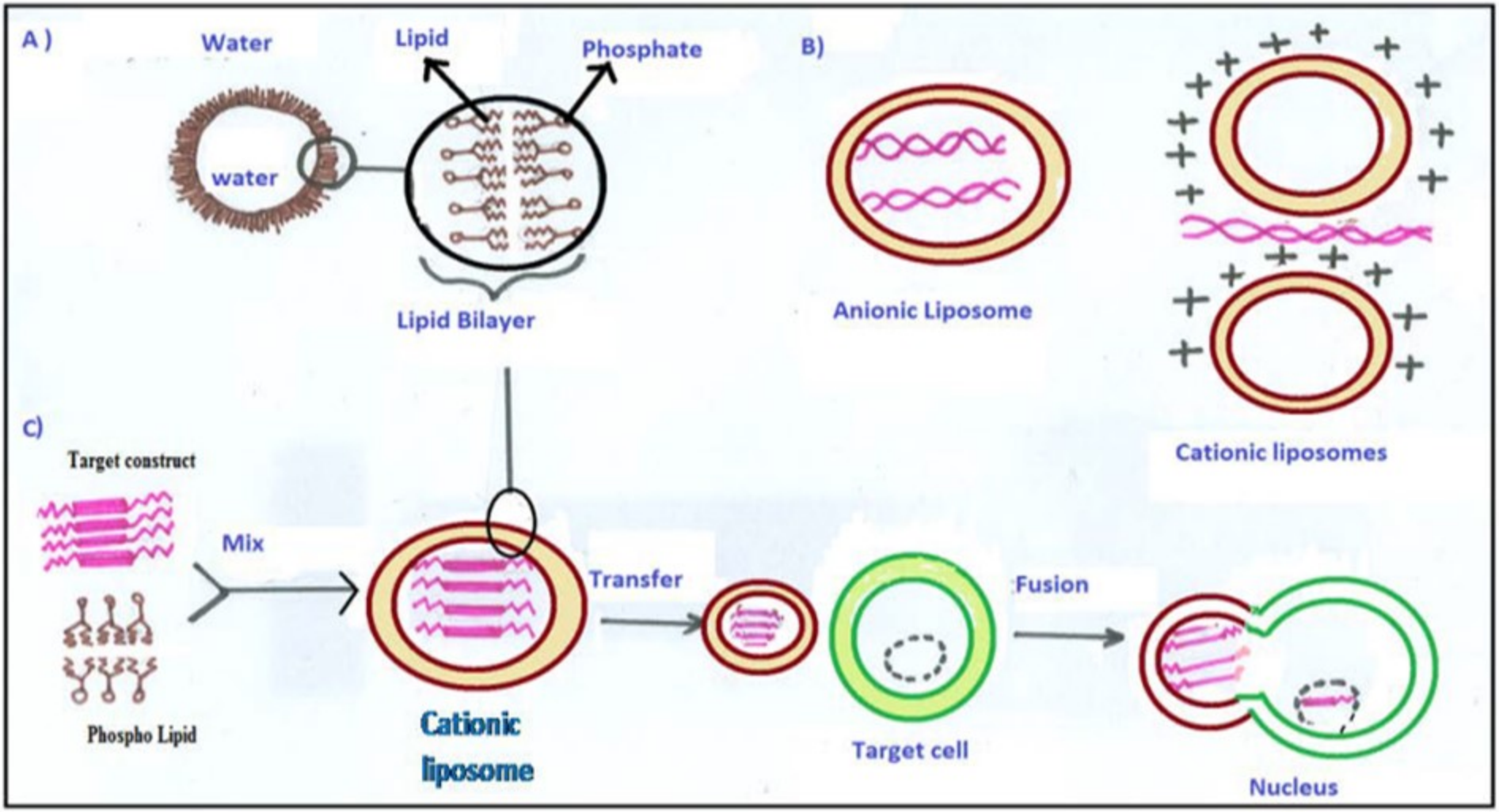
- Microinjection involves the delivery of foreign DNA, by the help of glass micropipette into a living cell.
- The cell is held against a solid support or holding pipette and micro needle containing the desired DNA is inserted into the cell.
- The tip of the pipette used is about 0.5 to 5 micro meter diameter which resembles an injection needle.

- For this, glass micropipette is heated until the glass becomes somewhat liquefied and is quickly stretched to ressemble a injection needle.
- The delivery of foreign DNA is done under a powerful microscope (micro-manupulator).



Liposome mediated gene transfer

- Liposomes – Spherical vesicles which are made up of synthetic or natural phospholipid bilayers.
- DNA to be transferred is packaged into the liposome *in vitro* and transferred to the targeted tissue.
- The lipid coating helps the DNA to survives in vivo and enters into the cell by endocytosis.
- Cationic liposomes where the positive charge on liposomes is stabilized by binding of negatively charged DNA, are popular vehicles for gene transfer *in vivo*.



(A) formation of lipid bilayer in water

(B) Structure of anionic and cationic liposome

(C) Use of liposome to transfer genes into cells.

Liposome mediated gene transfer

Advantage

- The liposomes with the foreign DNA are easy to prepare.
- There is no restriction in the size of DNA that is to be transferred.

Disadvantage

- Efficiency of gene transfer is low and transient expression of the foreign gene is obtained as they are not designed to integrate into the chromosomal DNA.

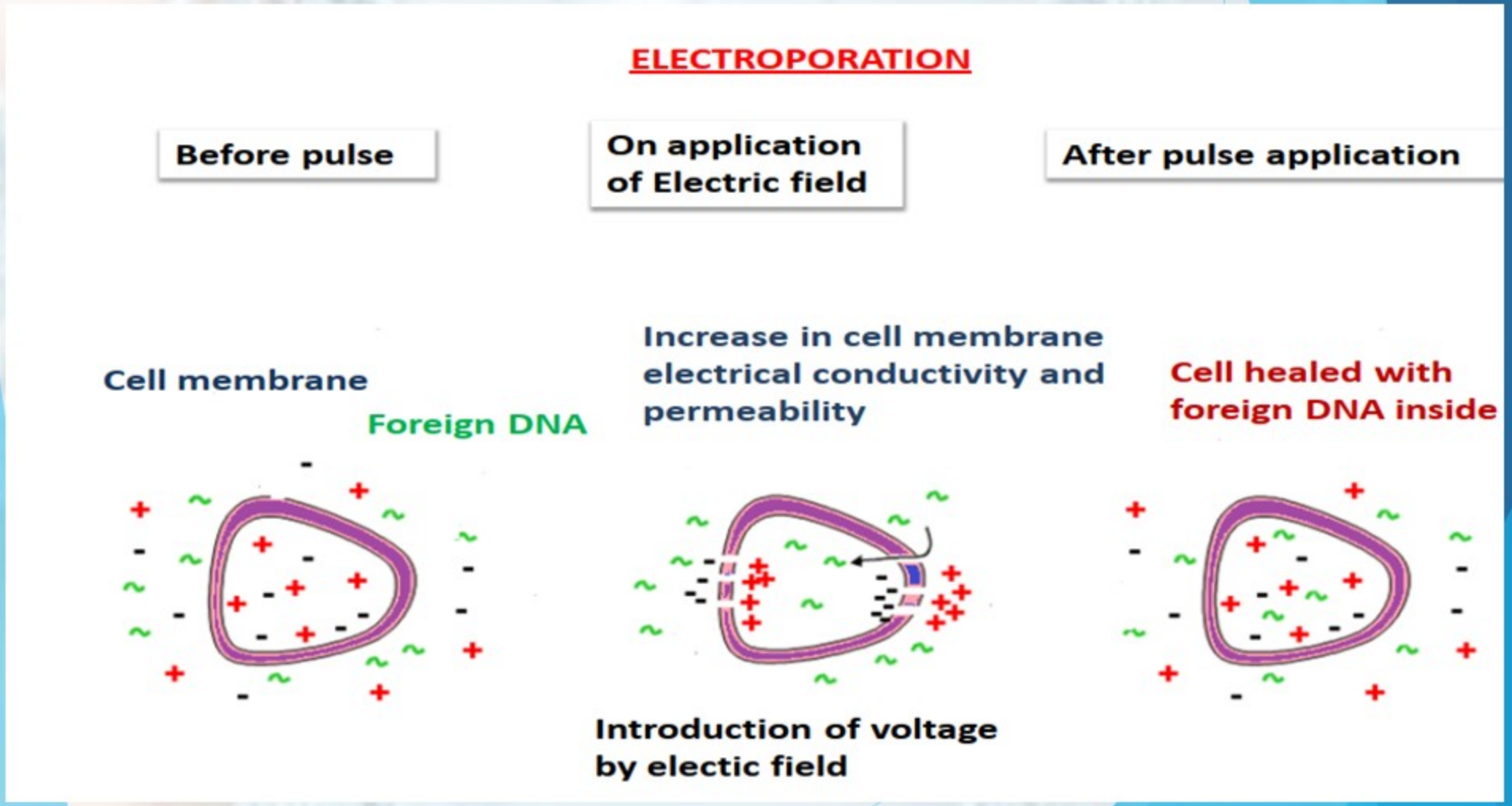
Lipoplexes

- Lipid DNA complexes – Lipoplexes
- DNA surrounded by artificial lipid layer (Liposomal based)
- Types - Cationic and anionic lipoplexes
- Mostly degraded by lysosomes

Electroporation

- The external electric field is applied to the protoplast, which changes the electrical conductivity and the permeability of cell membrane.
- The exogenous molecules found in the medium are taken up to either the cytoplasm (transient transfection) or into the nucleus (stable transfection).
- The efficiency of electroporation can be increased by giving the cell a heat shock prior to the application of electric field.

- Prior to the application of electric field, small quantity of PEG can be used to improve the efficiency.



DNA Molecular Conjugates

- Therapeutic DNA combine with conjugate – DNA Molecular Conjugates
- Common synthetic conjugate Poly L Lysine
- It avoids lysosomal break down of DNA