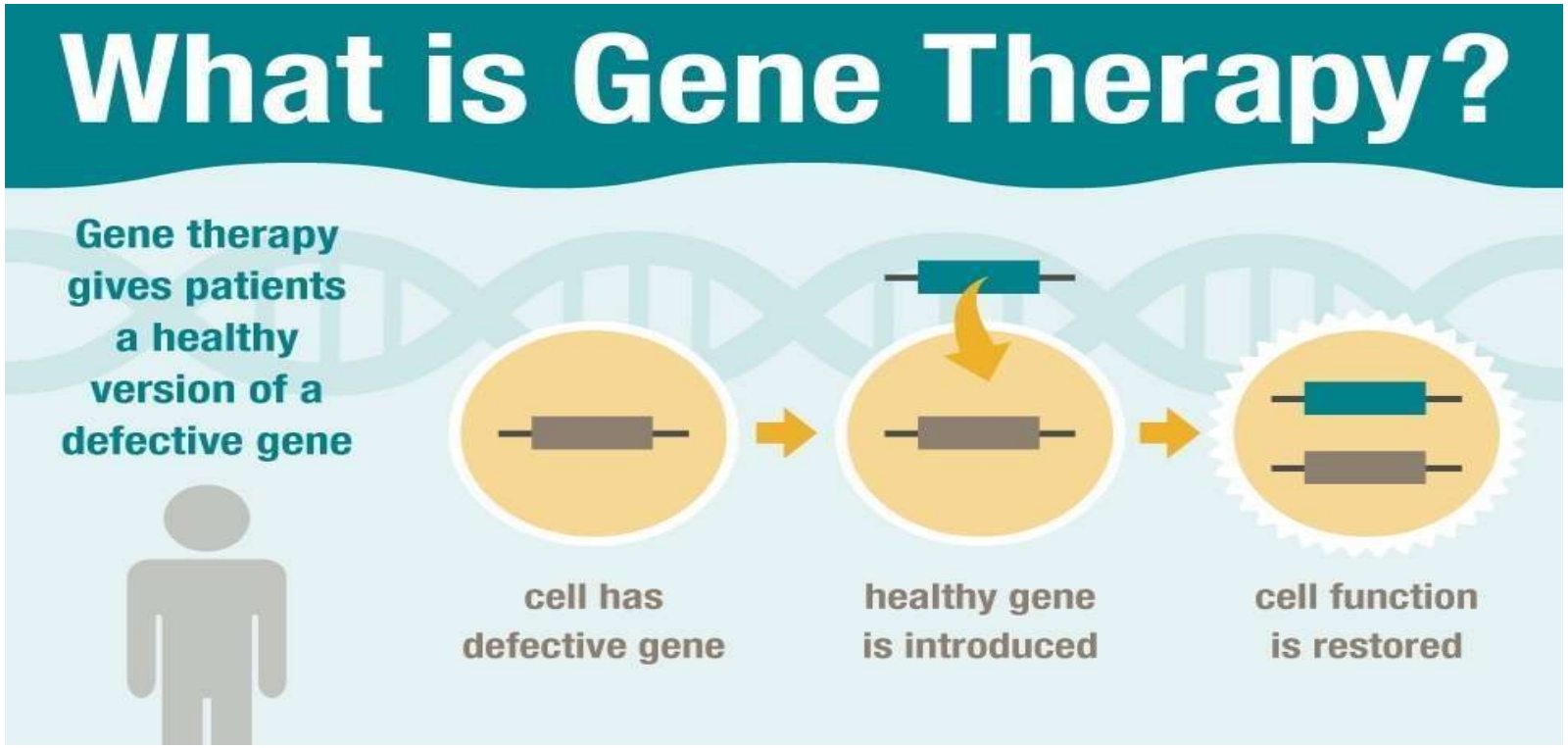
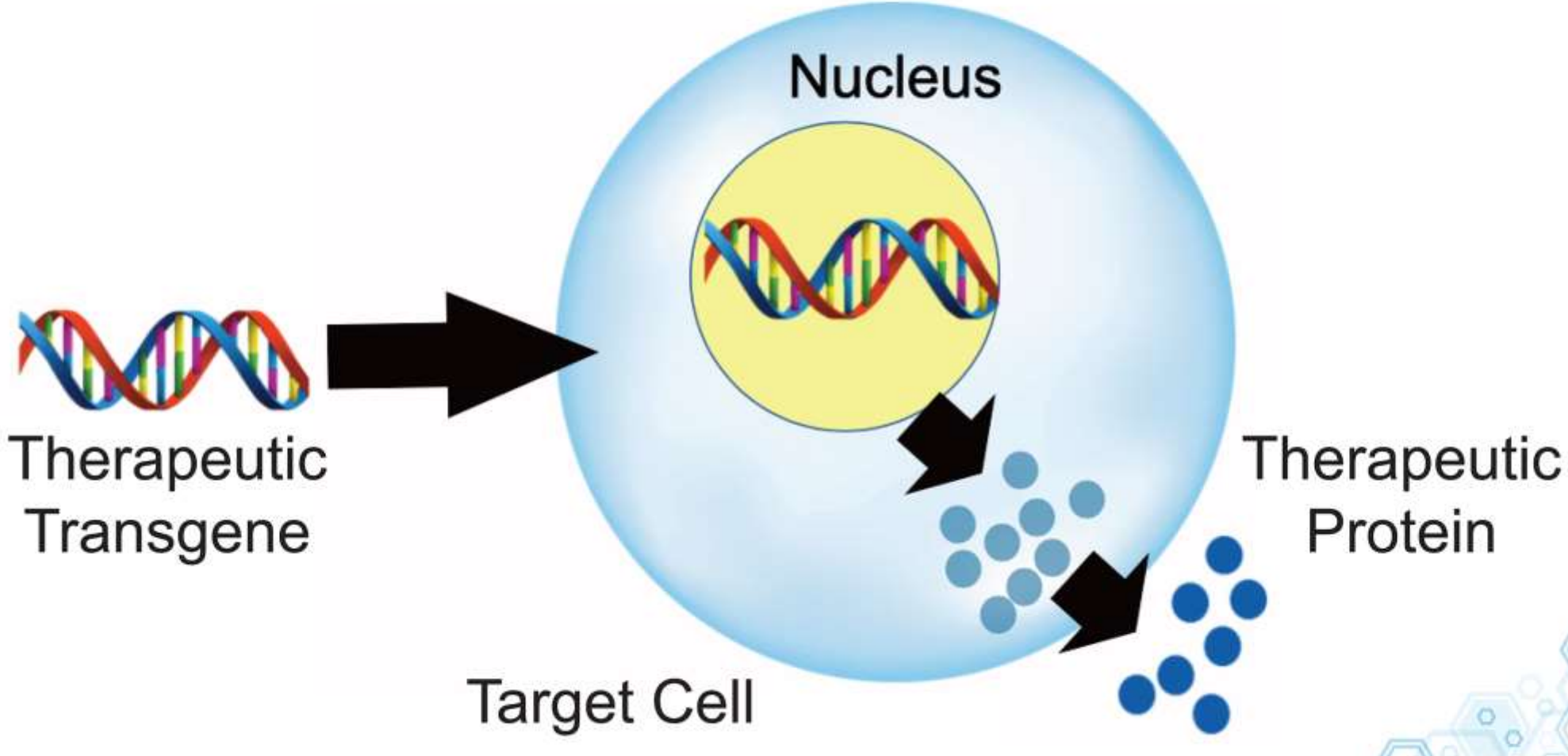
Cell Culture Systems
Course 9

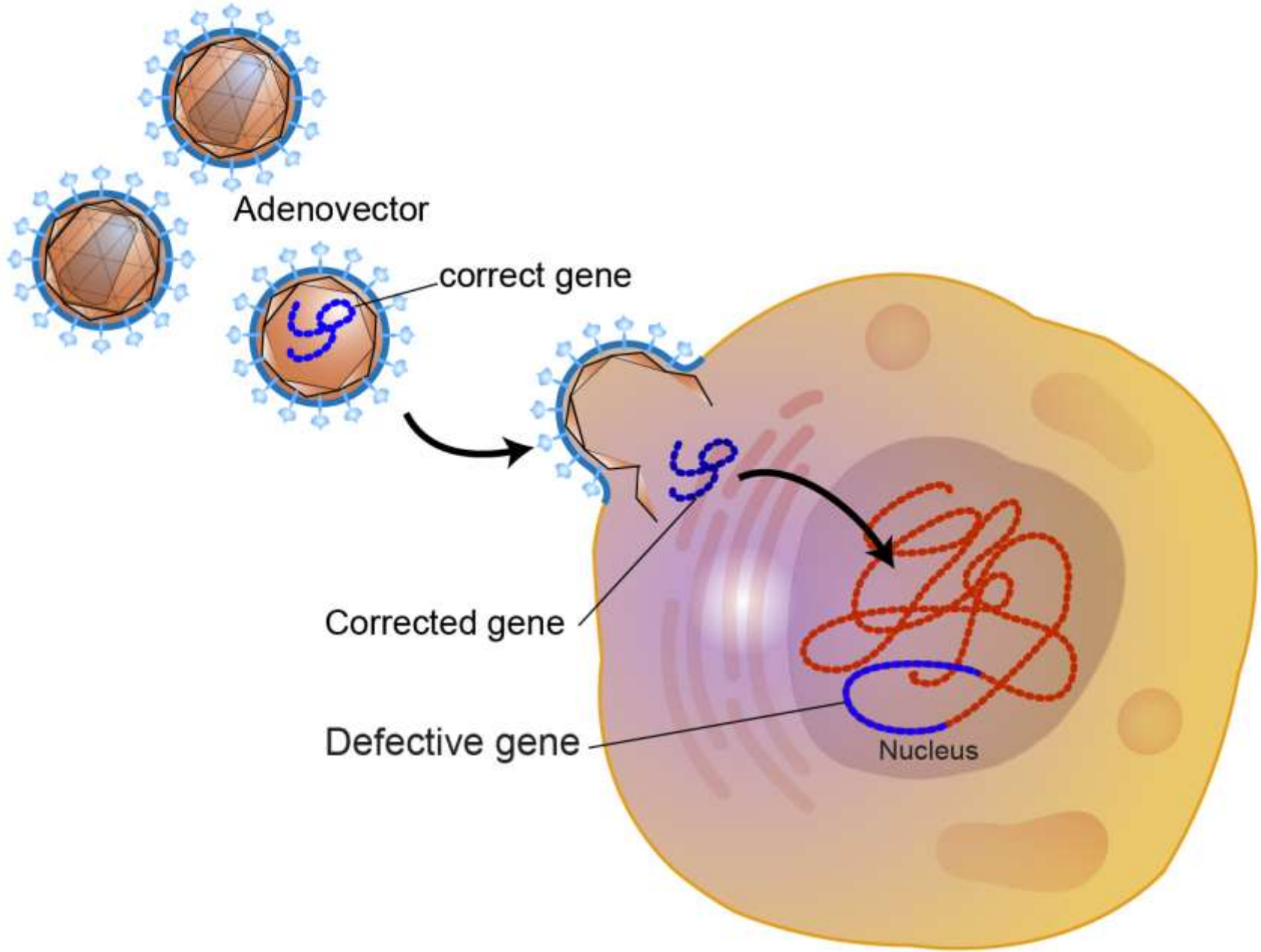
Gene Therapy



- Gene therapy is the process by which nucleic acids are transferred into cells to generate a therapeutic effect

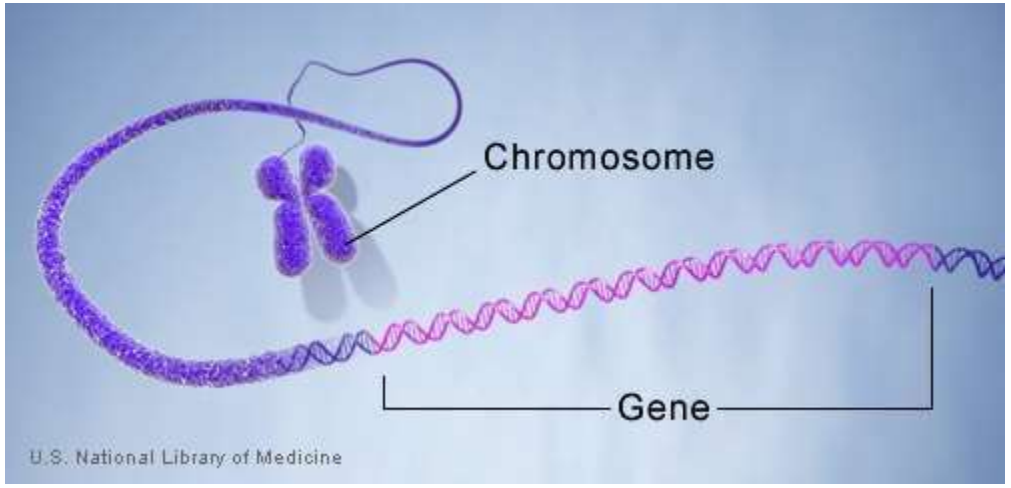
The Principle of Gene Therapy




Adenovector
correct gene
Corrected gene
Defective gene
Nucleus

What is a Gene ?

- A gene is the unit of DNA that contains hereditary information that is passed down from generation to generation.
- All genes together are called the genome.



What is a Gene?

- Genes may contain information about visible traits, such as height or eye colour.
- Many genes contain the instructions for RNA or protein molecules that are not visible from the outside, but perform important functions in the body's cells.

Genetic Diseases

- Genetic diseases are caused by errors, or mutations, in genes that result in a loss or change of function of RNA or protein molecules.
- These mutations can be passed down from parents to children or can happen spontaneously.

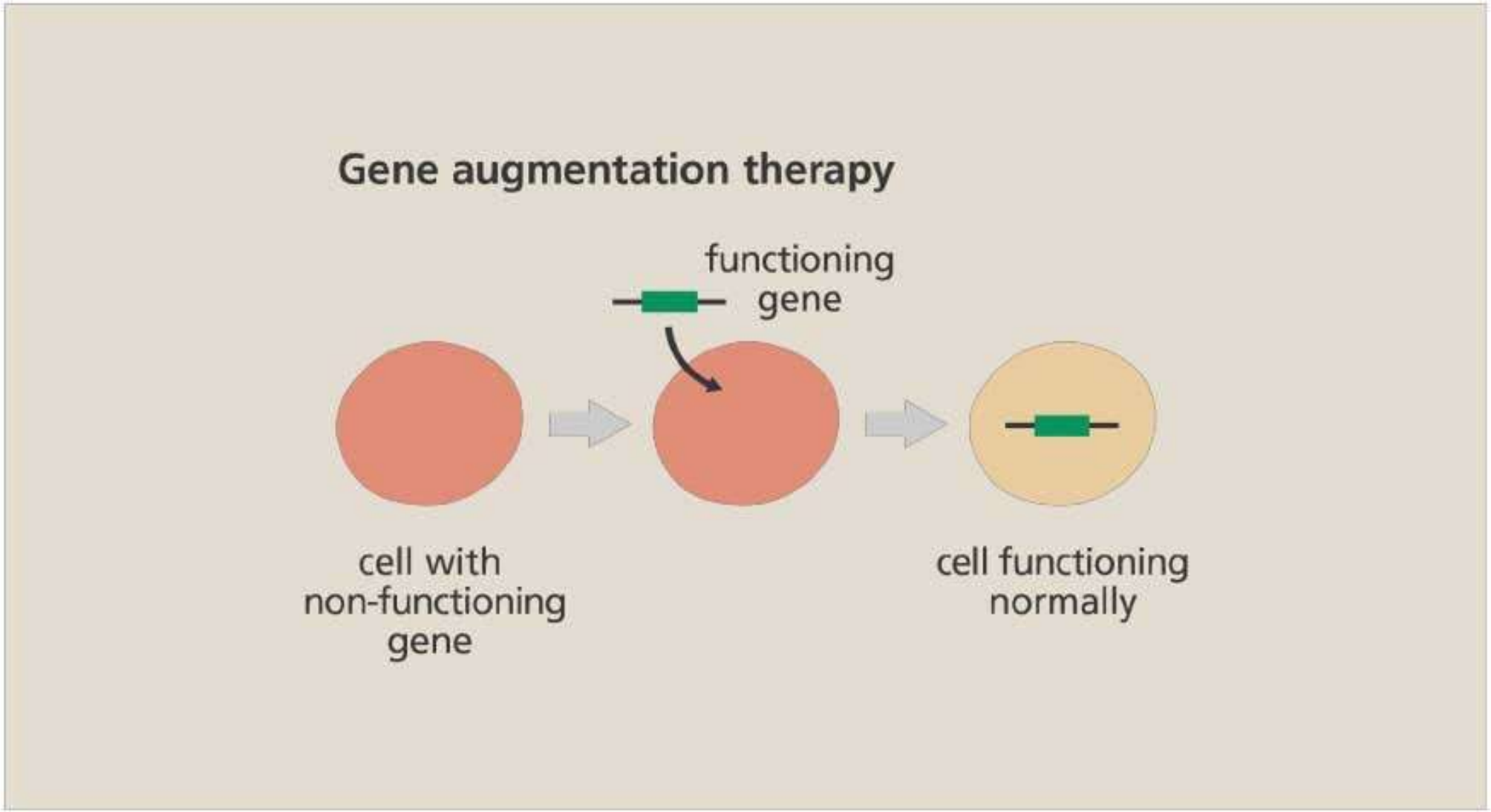
Gene Therapy

Genetic diseases can be healed by 4 different ways:

- Gene addition (augmentation)
- Gene replacement
- Gene inhibition
- Suicide genes

Gene Augmentation

- In gene insertion, **it is aimed to give a functional copy of the defective gene.** The mutant gene remains in the cell but functional gene is added to it. It is useful in the correction of genetic diseases that cause loss of function. For example, cystic fibrosis is a suitable disease for gene addition.

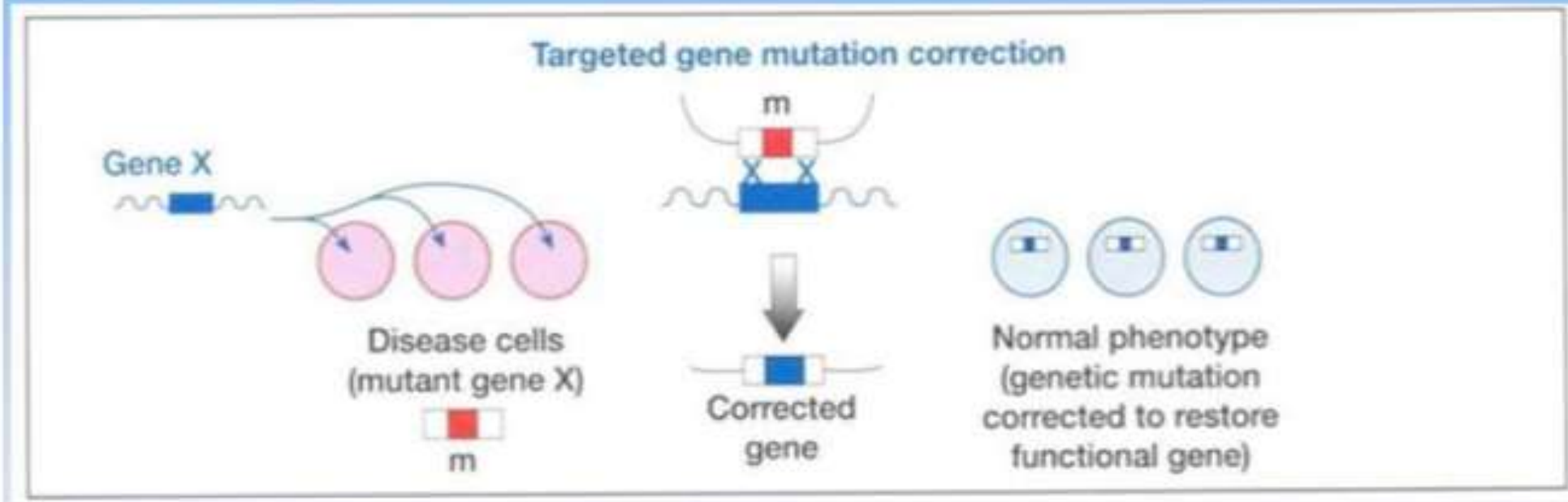

Gene augmentation therapy
functioning gene
cell with non-functioning gene
cell functioning normally

Gene Replacement

- Gene replacement is much more difficult and complex. **The goal is to replace the mutant gene with its functional copy or to correct the mutation in situ.** It is a method that will be preferred in genetic diseases that can recover as a result of the recovery of the function that the mutant gene product damages the cell.

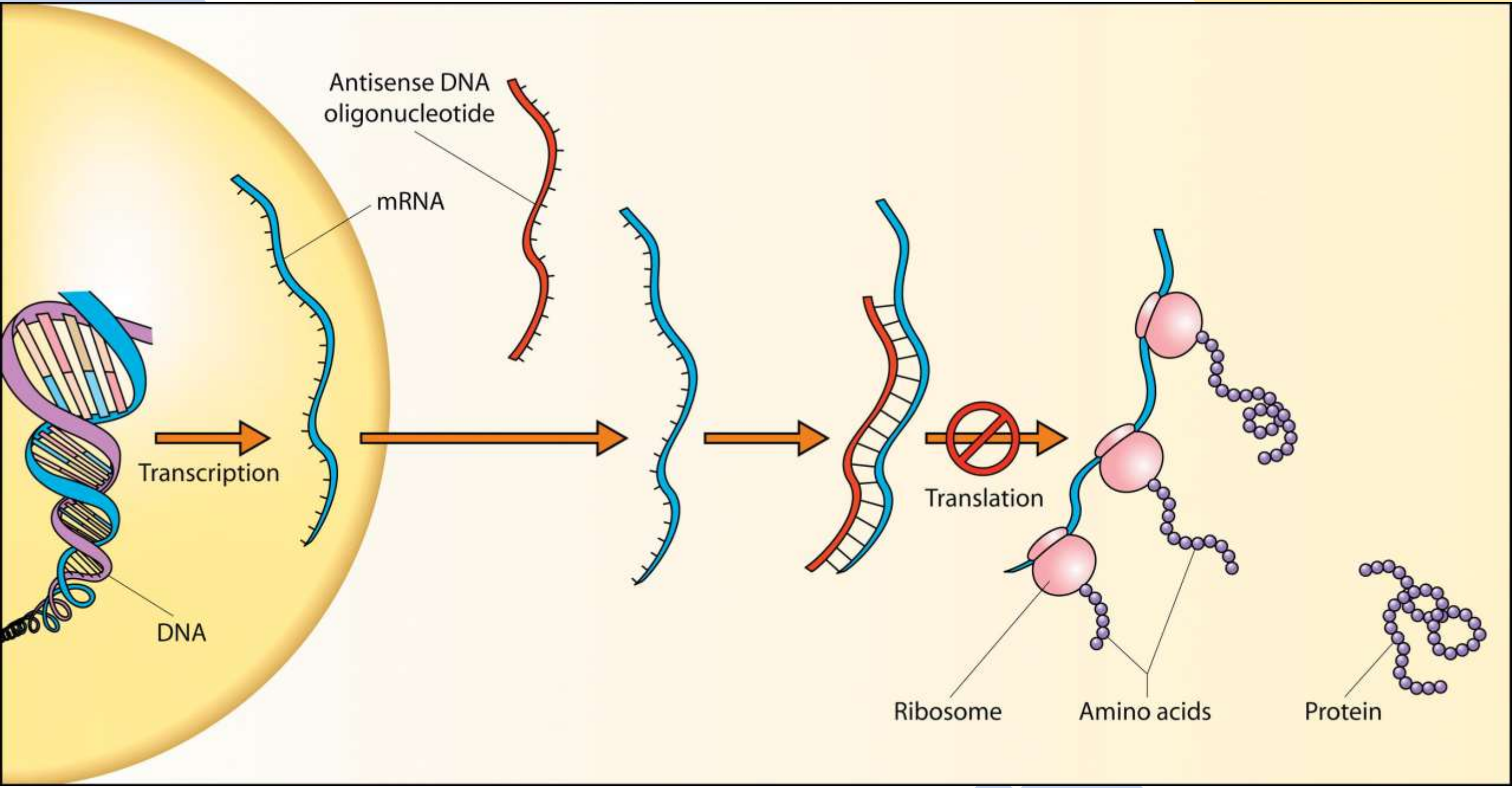
Gene replacement

Delivery of a gene whose function is absent due to loss-of-function mutations in the affected gene. This can be used in autosomal recessive diseases (RP or LCA) or in those that are autosomal dominant due to haploinsufficiency or dominant-negative mutations (RP).



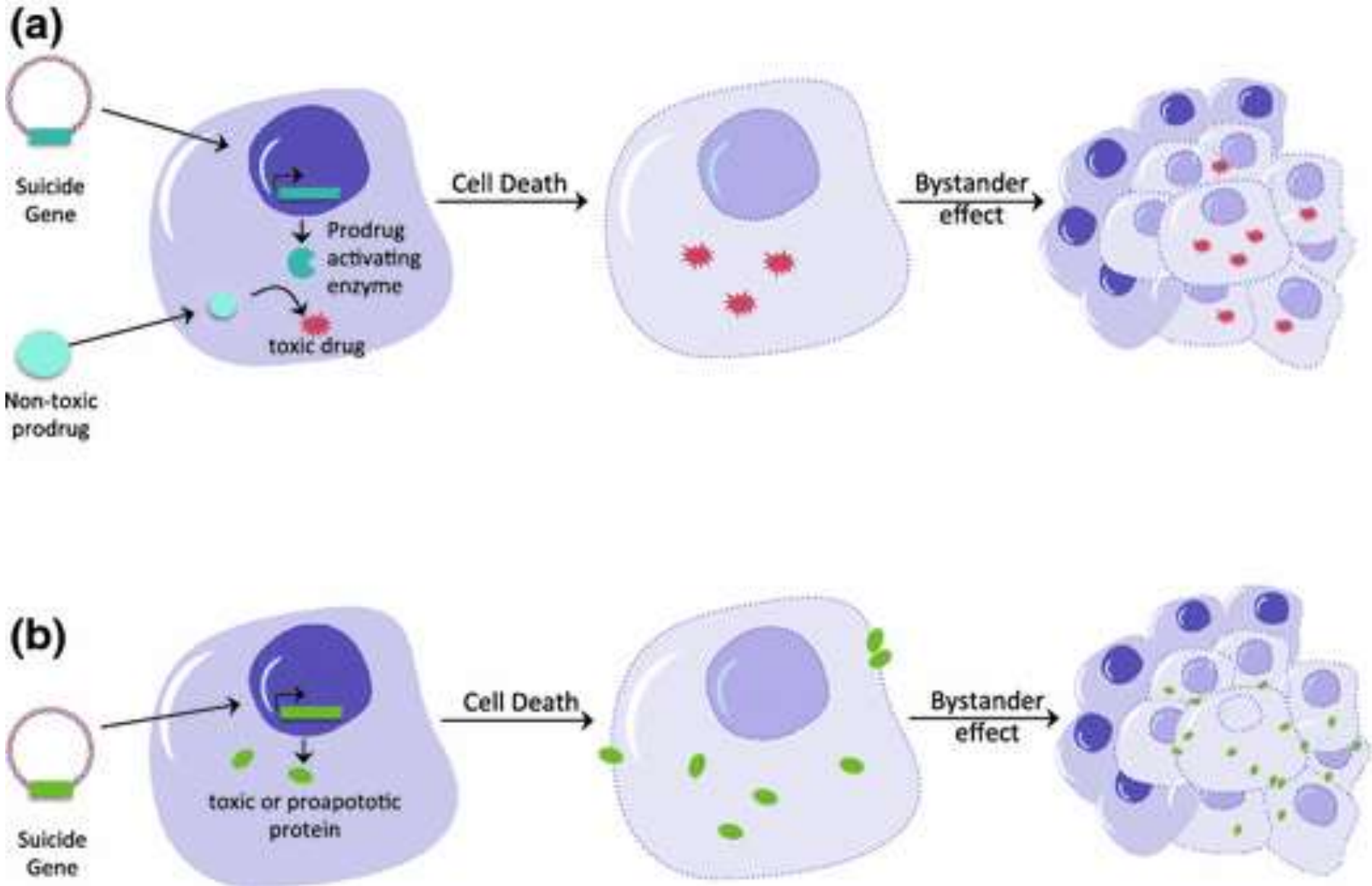
Inhibition of Gene Expression

- Suppression of gene expression is an approach especially used in the treatment of infectious diseases. **In this method, it is aimed to suppress the function of the target pathogen. In addition, this approach can be used in silencing cancer-activated oncogenes,** autoimmune diseases and perhaps genetic diseases due to function gain.

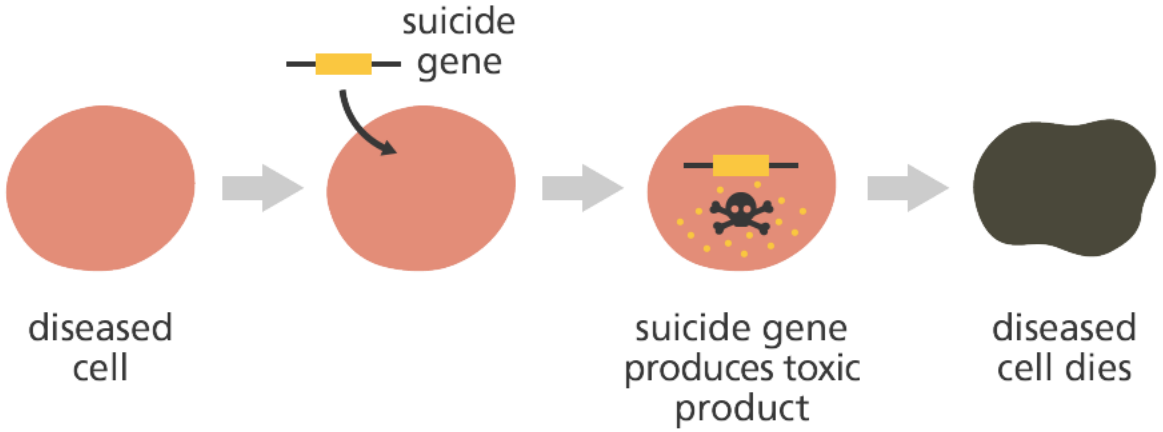

Antisense DNA
oligonucleotide
mRNA
Transcription
DNA
Translation
Ribosome
Amino acids
Protein

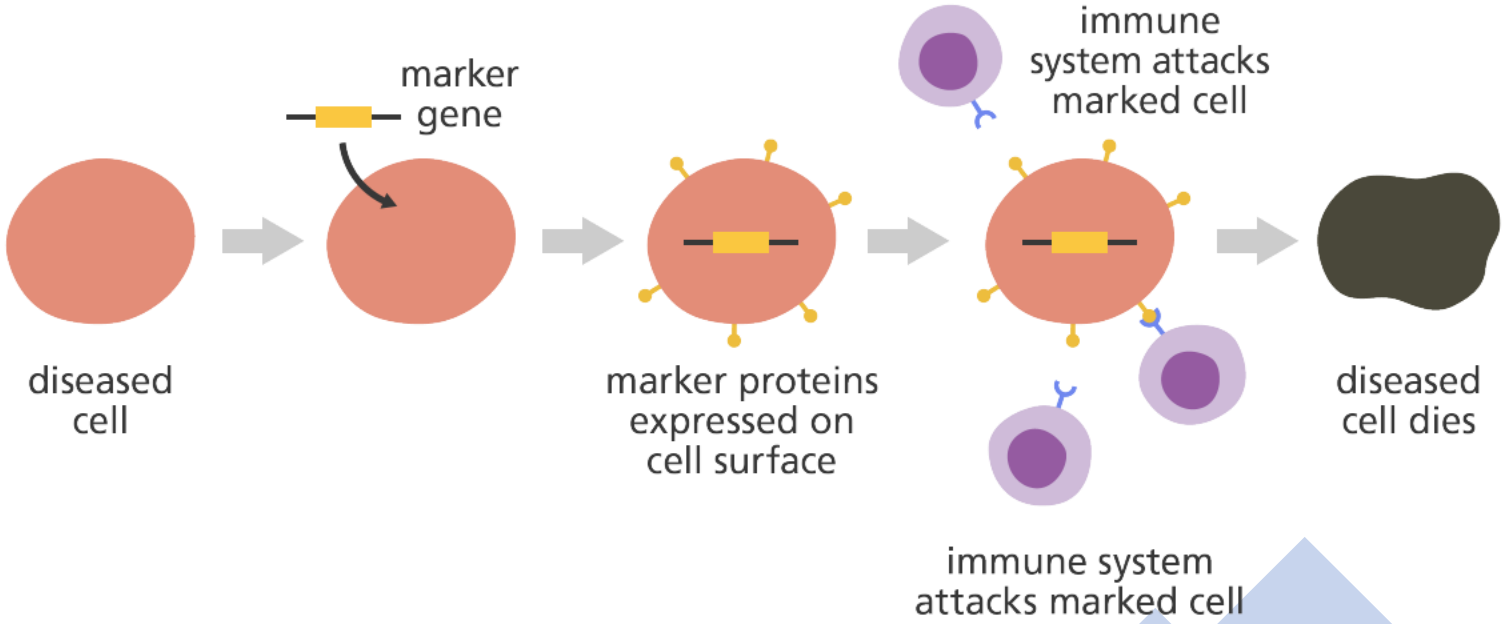
Killing of Specific Cells

- The method of killing specific cells is especially used in cancer treatment. **For this purpose, the toxic chemotherapeutic agent is administered to the body in the form of a non-toxic prodrug, while the gene encoding the enzyme that activates this prodrug is transferred to cancer cells.** This approach is called suicide gene therapy.
- In practice, the active prodrug also spreads to untargeted neighboring cells, creating a strong (bystander) effect.


(a)
Suicide Gene
Prodrug activating enzyme
toxic drug
Non-toxic prodrug
Cell Death
Bystander effect
(b)
toxic or proapototic protein
Suicide Gene
Cell Death
Bystander effect

Killing of specific cells





Types of Gene Therapy

Target cell →
- Germ line gene therapy
- *Somatic* gene therapy

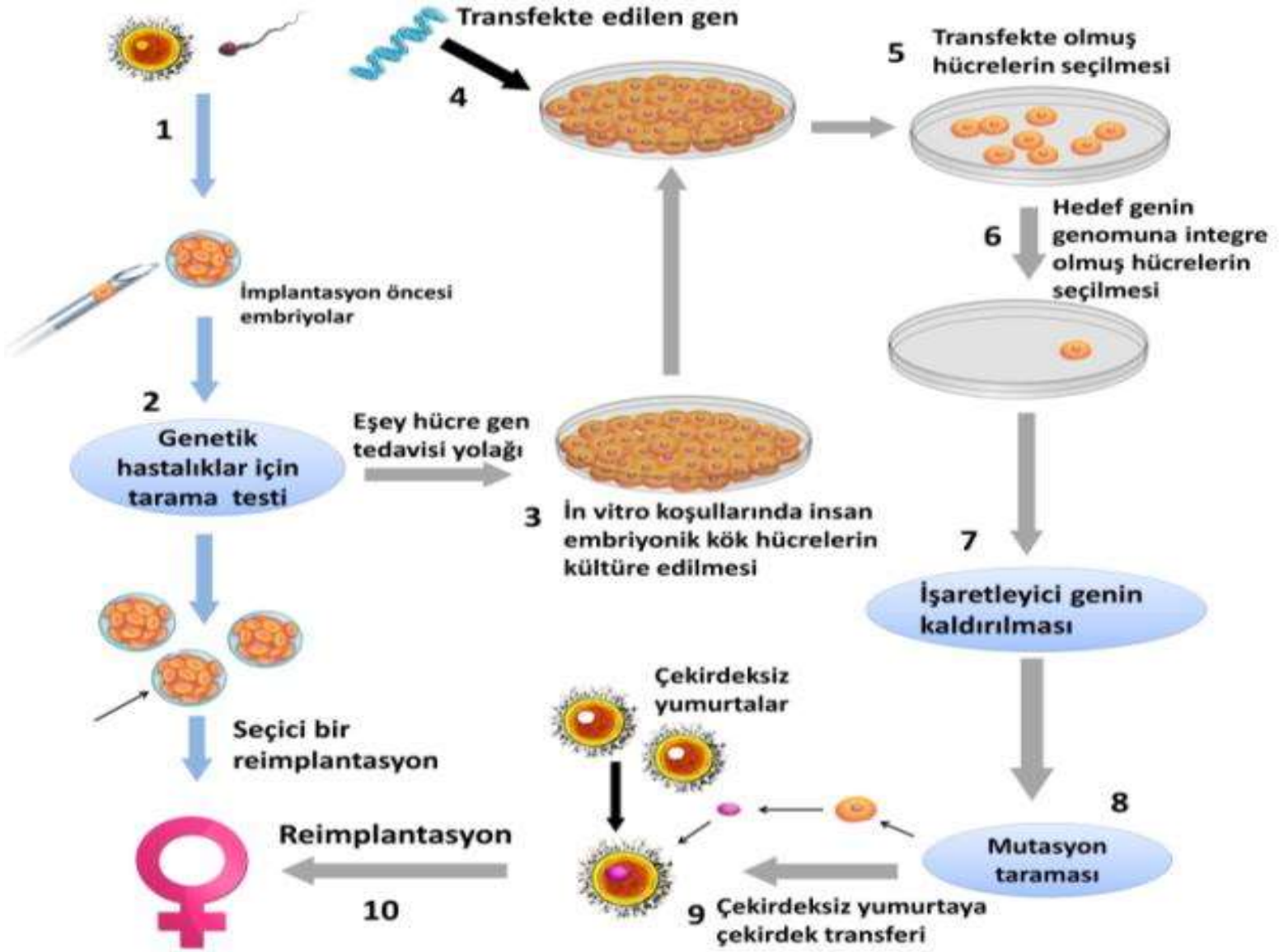
Delivery method →
- *ex vivo* gene therapy
- *in vivo* gene therapy
  - *in situ*
  - *systematical*

Germline Gene Therapy

- Germline gene therapy; **It is a gene therapy performed on gametes (sperm and eggs) and zygote, where the change is transferred to the next generations.** Although the use of Germline gene therapy has been banned in Europe for ethical reasons; In the USA, the work done in this area by the FDA is permitted.

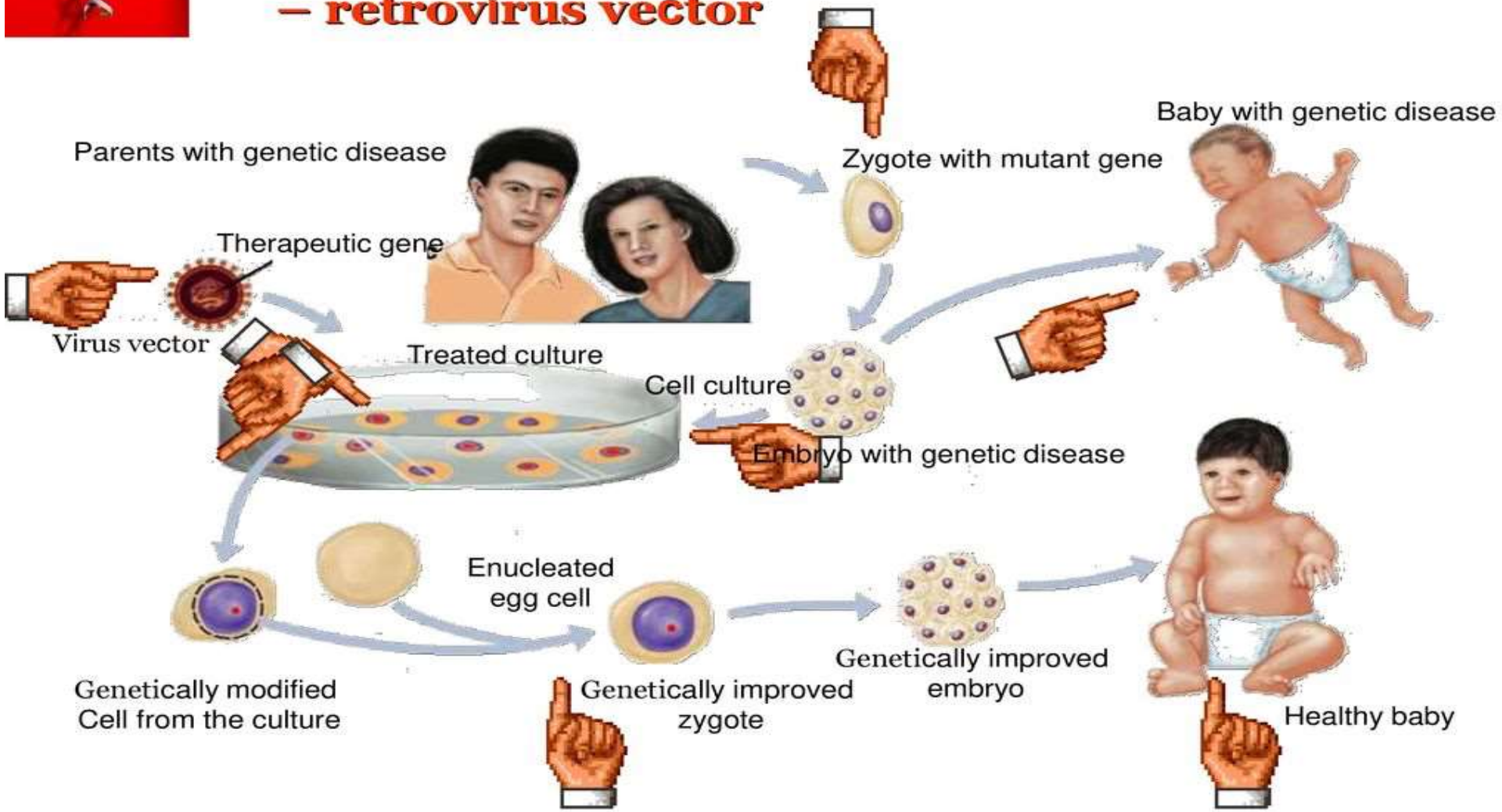
Germline Gene Therapy

- The purpose of the germ cell gene therapy is to transfer the therapeutic gene to both the body and germ cells. As a result, both the disease of the person will be eliminated and healthy generations will be obtained by creating gametes with corrected genotype.


Transfekte edilen gen
1
4
5
Transfekte olmuş hücrelerin seçilmesi
6
Hedef genin genomuna integre olmuş hücrelerin seçilmesi
İmplantasyon öncesi embriyolar
2
Genetik hastalıklar için tarama testi
Eşey hücre gen tedavisi yolağı
3
İn vitro koşullarında insan embriyonik kök hücrelerin kültüre edilmesi
7
İşaretleyici genin kaldırılması
Çekirdeksiz yumurtalar
Seçici bir reimplantasyon
8
Mutasyon taraması
Reimplantasyon
10
9
Çekirdeksiz yumurtaya çekirdek transferi

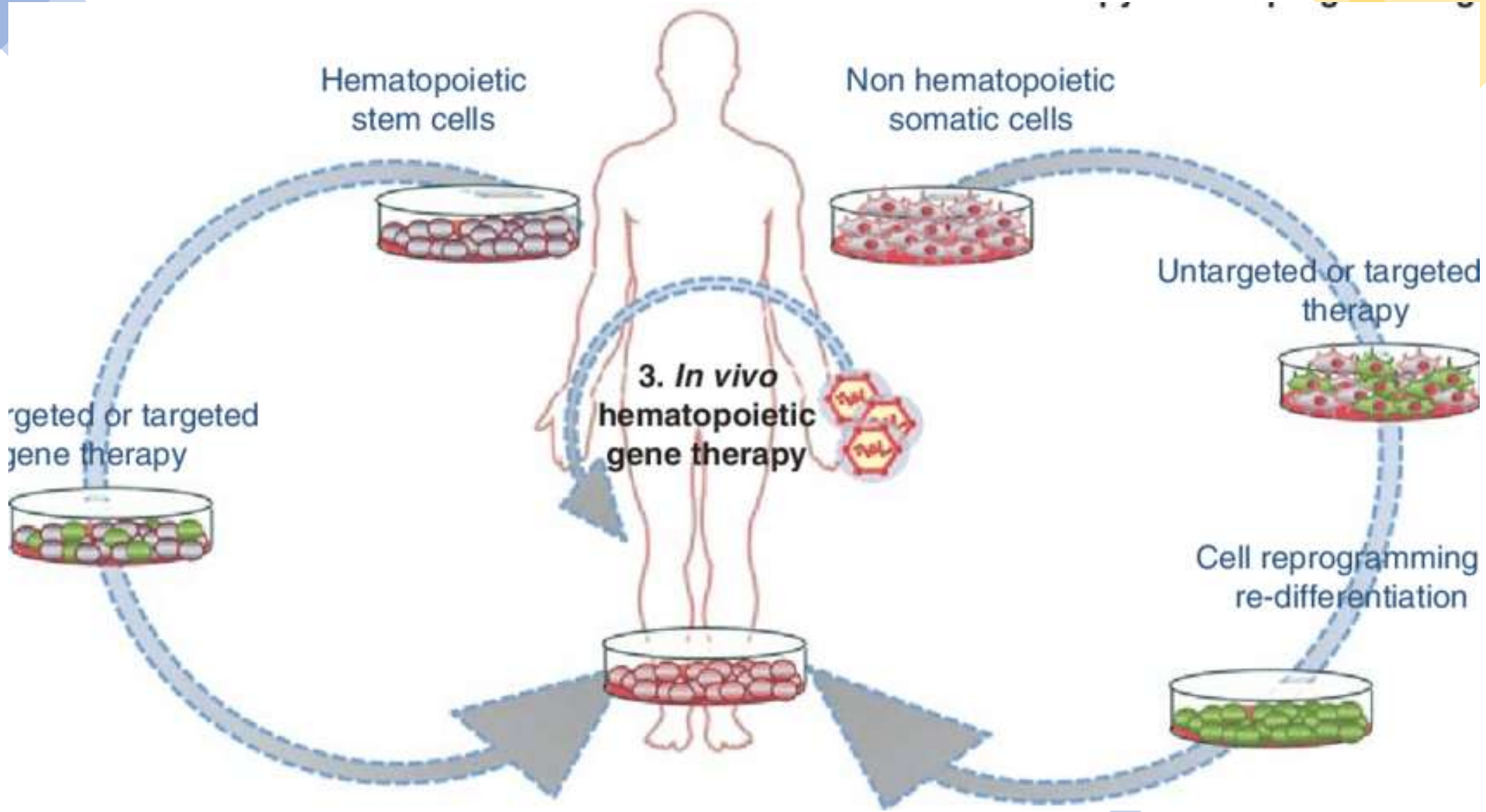
Germline gene therapy

– retrovirus vector



Somatic Cell Gene Therapy

- **In somatic cell gene therapy application, the therapeutic gene is transferred to somatic cells. Gene therapy is applied to cells isolated from bone marrow, blood and skin cells.**
- Ultimately, the changes made at the level of genes and the different effects of gene therapy remain only at the level of the body cells; not transferred to germ cells and subsequent generations

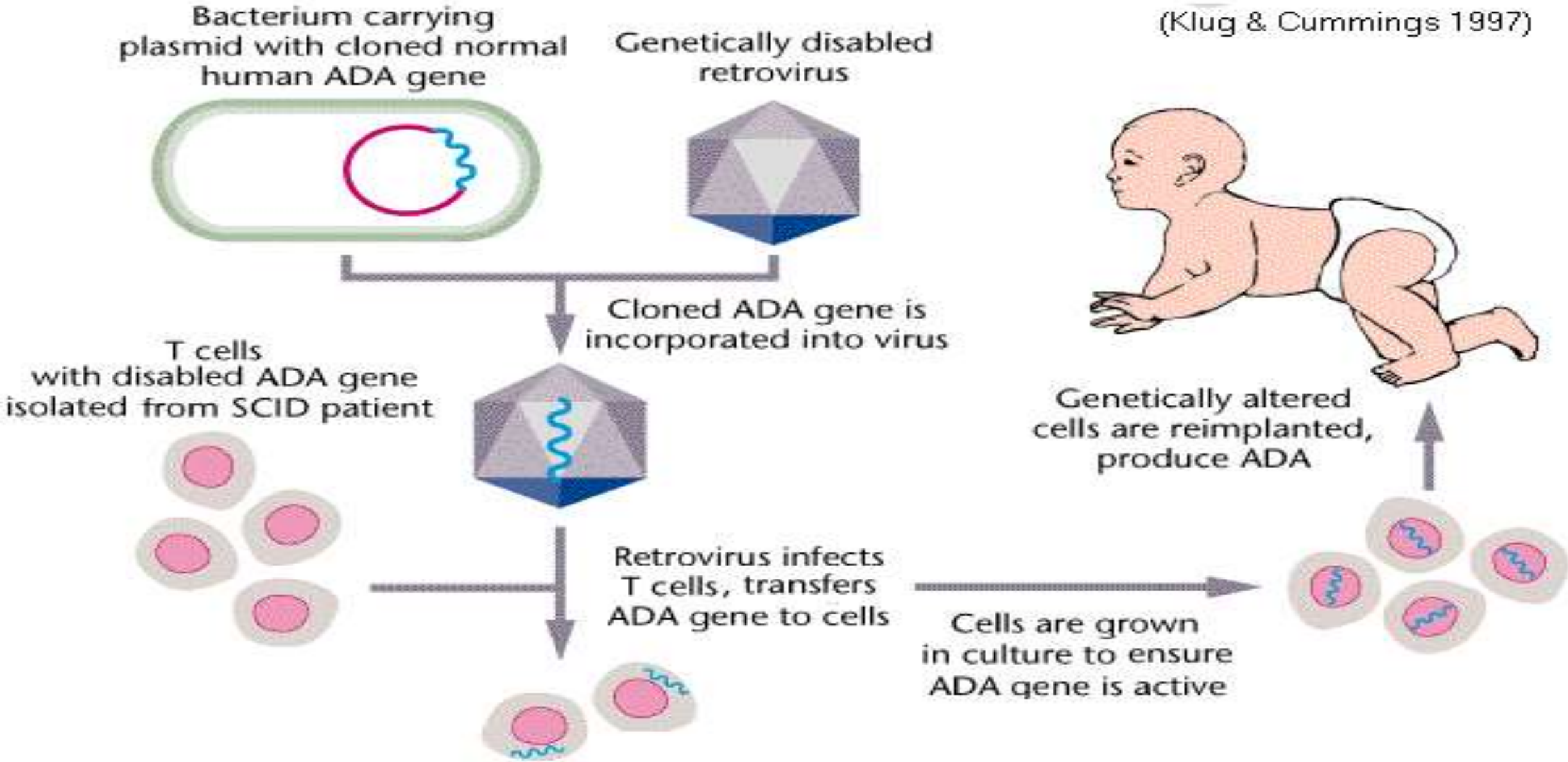

Hematopoietic
stem cells
Non hematopoietic
somatic cells
Untargeted or targeted
therapy
3. *In vivo*
hematopoietic
gene therapy
Cell reprogramming
re-differentiation

TYPES OF GENE THERAPY

SOMATIC CELL GENE THERAPY	GERM LINE GENE THERAPY
▪ Therapeutic genes transferred into the **somatic cells**. ▪ Eg. Introduction of genes into bone marrow cells, blood cells, skin cells etc. ▪ **Will not be inherited** later generations. ▪ At present all researches directed to correct genetic defects in somatic cells.	▪ Therapeutic genes transferred into the **germ cells**. ▪ Eg. Genes introduced into eggs and sperms. ▪ **It is heritable** and passed on to later generations. ▪ For safety, ethical and technical reasons, it is not being attempted at present.

Ex Vivo Gene Therapy

- **Cells taken from the patient are produced in tissue cultures and transduced with a vector of recombinant genes and returned to the patient.** This transferred DNA sequence is stably linked to DNA in the chromosome of target cells.


Bacterium carrying plasmid with cloned normal human ADA gene
Genetically disabled retrovirus
(Klug & Cummings 1997)
Cloned ADA gene is incorporated into virus
T cells with disabled ADA gene isolated from SCID patient
Retrovirus infects T cells, transfers ADA gene to cells
Cells are grown in culture to ensure ADA gene is active
Genetically altered cells are reimplanted, produce ADA

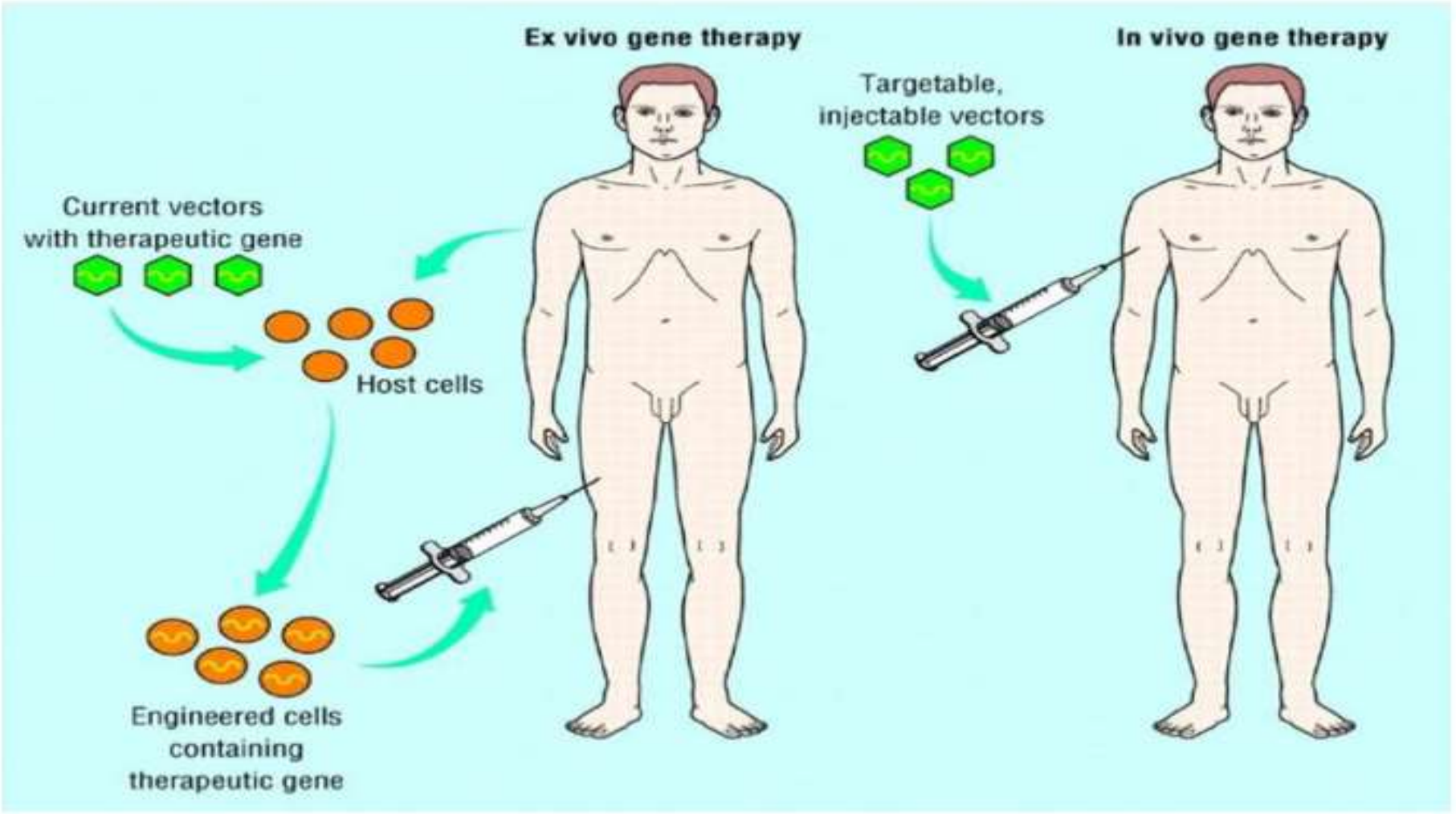
In Vivo Gene Therapy

- In vivo gene therapy **is the only option when recipient cells have insufficient in vitro culture (eg brain cells) or where cultured cells cannot be effectively re-implanted to the patient.**

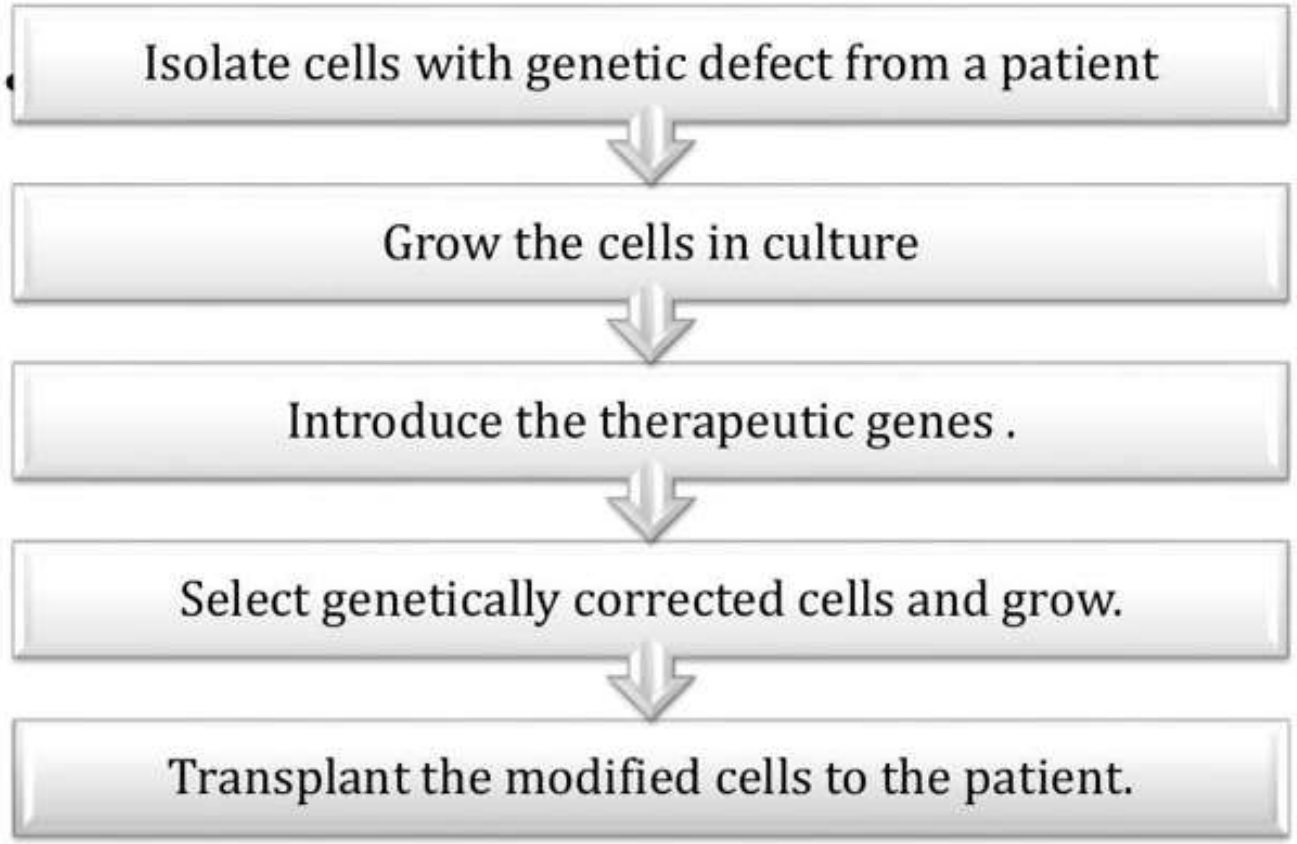
  Tissue targeting is a major challenge in this method.

In Vivo Gene Therapy

- **The transferred gene can be delivered directly to the target tissue or general circulation, but the vector used for transfer must be designed to be taken only by targeted cells or to be expressed only in targeted cells.**


Ex vivo gene therapy
In vivo gene therapy
Current vectors
with therapeutic gene
Host cells
Engineered cells
containing
therapeutic gene
Targetable,
injectable vectors

EX VIVO GENE THERAPY



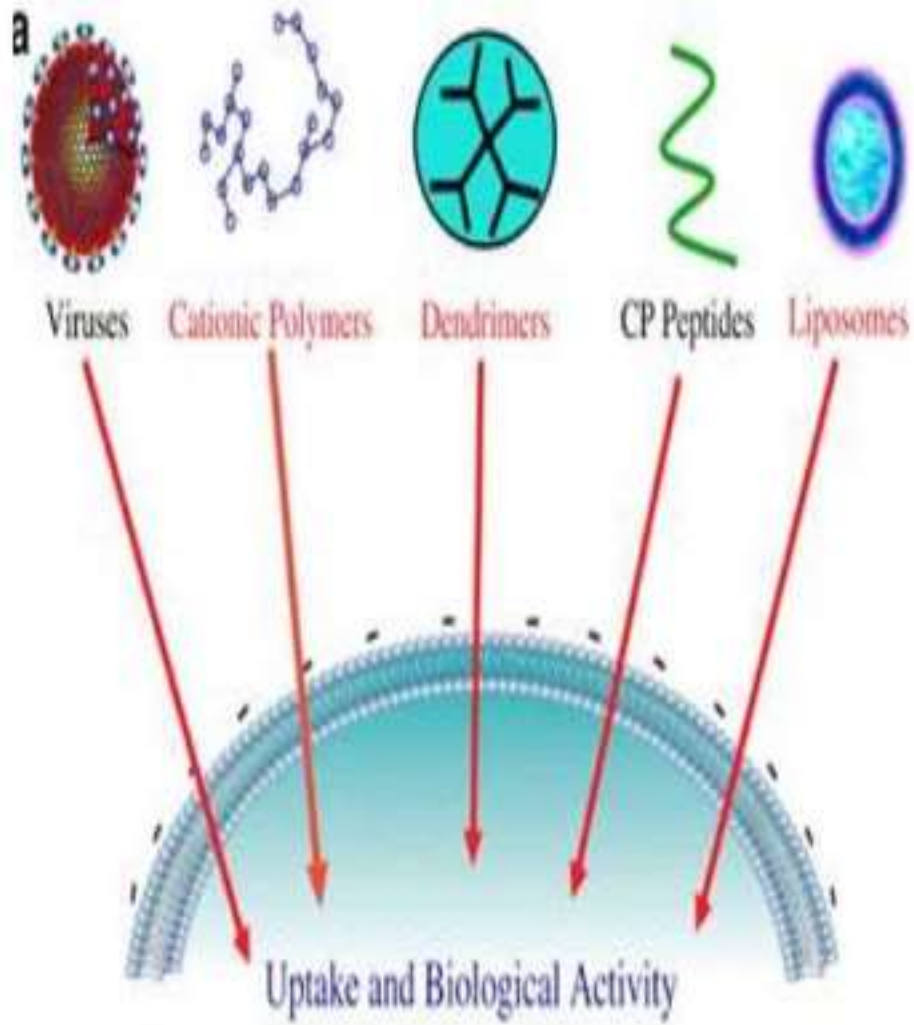
Vectors

- **Carriers used to deliver the therapeutic gene to target cells are called vectors.** The success of gene therapy largely depends on the vector that can transfer the gene fragment.
- **A suitable vector should be able to present the DNA fragment selectively and efficiently to the target cells with minimal toxicity.**

VECTORS IN GENE THERAPY

- To transfer the desired gene into a target cell, a carrier is required. Such vehicles of gene delivery are known as **vectors**.

- 2 main classes
  - **Viral** vectors
  - **Non viral** vectors



Properties of Ideal Vector

- Sufficient capacity to carry DNA fragments
- Tissue-specificity
- Stability
- Small enough to penetrate through cell membrane
- Non-immunogenic
- Non-toxic
- Long-lasting effectivity
- Continuous gene expression

Viral Vectors

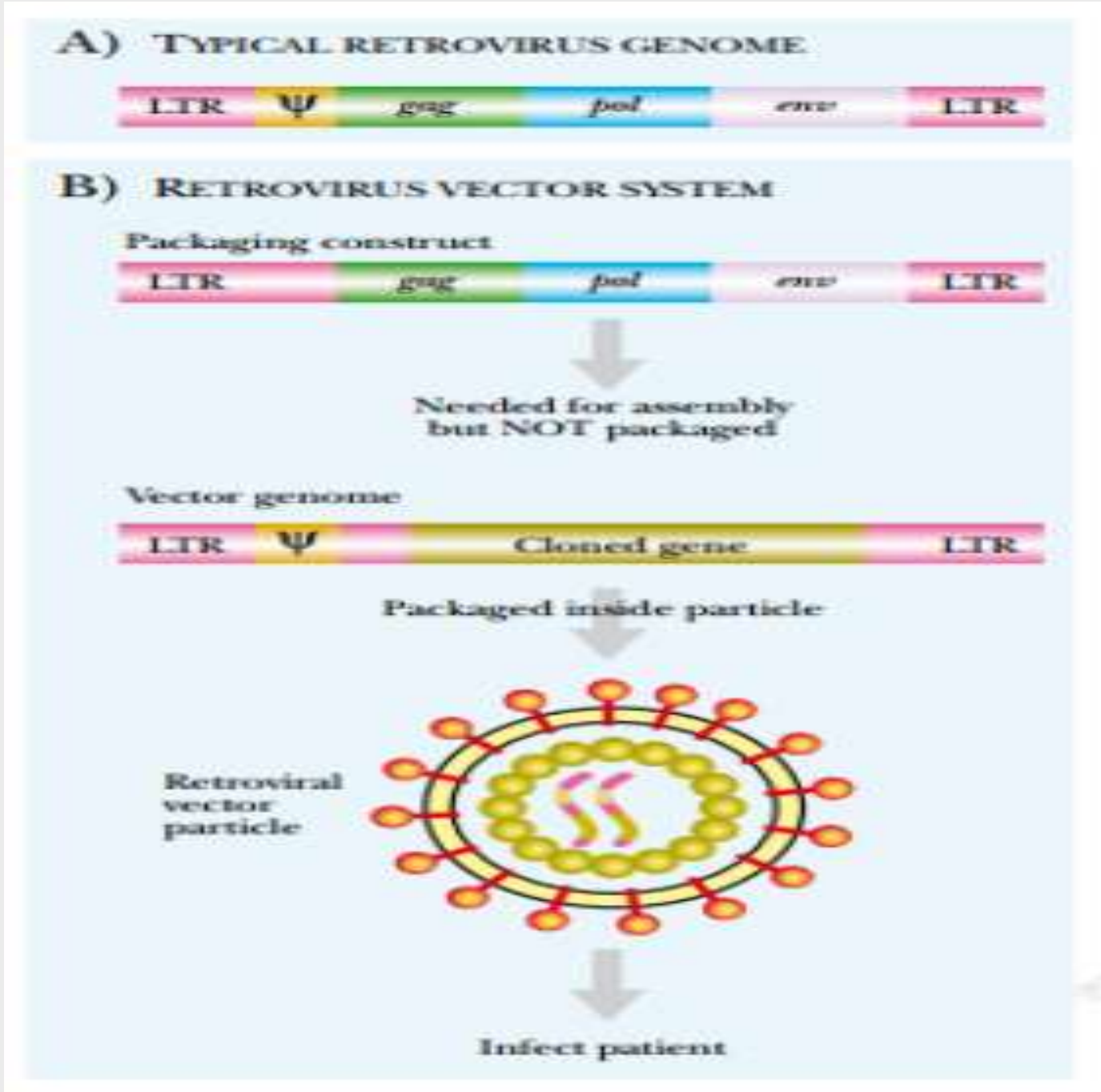
- **Non-pathogenic viruses that carry therapeutic genes are called viral vectors.** Disease-causing parts of the genes of viruses used as vectors are removed using genetic engineering practices and these genes are replaced by therapeutic genes.
- Most used viruses as carriers; adeno viruses, retro viruses, adeno-related viruses, herpes virus

Retrovirus Mediated Gene Therapy

Two forms of retrovirus are used for gene therapy

1. Therapeutic vector: contaning cloned healthy gene and packaging signal

2.Retrovirus : containing three functional genes but not including packaging signal


A) TYPICAL RETROVIRUS GENOME
LTR
Ψ
gag
pol
env
LTR
B) RETROVIRUS VECTOR SYSTEM
Packaging construct
LTR
gag
pol
env
LTR
Needed for assembly
but NOT packaged
Vector genome
LTR
Ψ
Cloned gene
LTR
Packaged inside particle
Retroviral
vector
particle
Infect patient

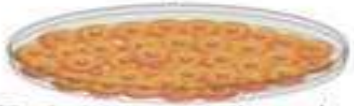
A) Retrovirüs genomunun yapısı

B) Retrovirüs vektörün üretilmesi için gereken DNA parçaları

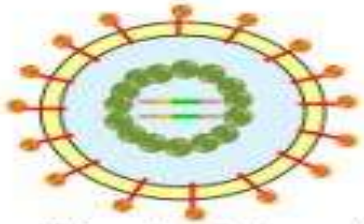
1- Yapısal vektör

2- Terapötik vektör

Yapısal ve terapötik vektör aynı anda bir memeli hücre hatına verilecektir



Paketleme hücre hattı

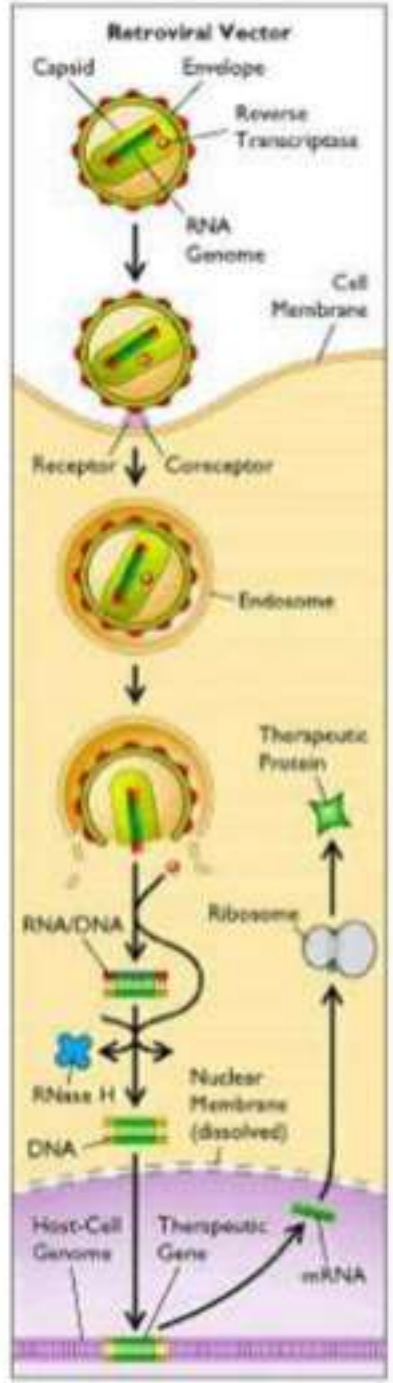


Terapötik retrovirüs partikülleri

Hastaya nakil edilmesi

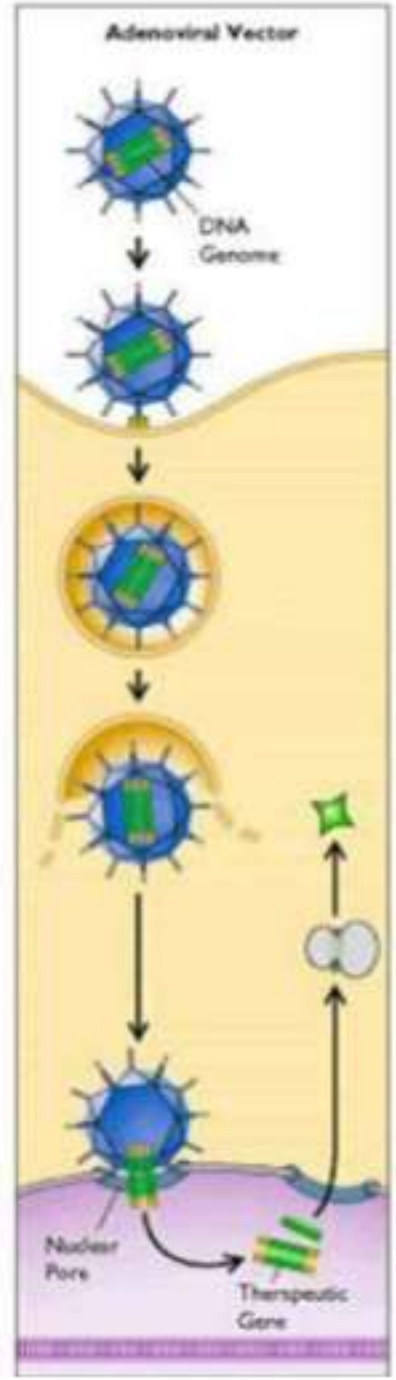
Vector	Packaging Ability (kb)	Genetic Material	Tropism	Vector Genome Form	Primary Advantages	Disadvantages
Adeno-associated virus	<5	ssDNA	Dividing/ nondividing cells	Nonintegrated (90%) Integrated (>10%)	Noninflammatory; nonpathogenic	Insert onto host genome; low production profile, low transduction efficiency; low packaging capacity
Adenovirus	7.5	dsDNA	Dividing/ nondividing cells	Nonintegrated	Excellent transduction efficiency and transduce most tissue/cells; long-term transgene expression	High antigenicity; elicit immune response; transient transgene expression; does not integrate host genome
Lentivirus	8	RNA	Dividing/ nondividing cells	Integrated	High transduction efficiency; long-term transgene expression	Pathogenic; random integration into host genome; less cost effective and elaborate procedures; major safety issue with potential to competent viruses
Retrovirus	8	RNA	Dividing cells	Integrated	Long-term transgene expression; strong tropism; large packaging capacity; high transduction efficiency	Low packaging capacity; cannot transduce non-dividing cells; random integration into host genome; target cell transformation.

Retrovirus
RNA



Integration to the genome

Adenovirus
DNA



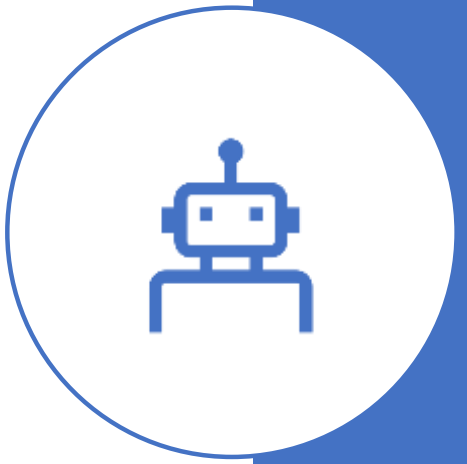
No integration to the genome

Disadvantages of Viral Vectors

- The retrovirus cannot infect non-dividing cells, the adenovirus shows an immunological response, the herpesvirus has a cytotoxic effect
- The common disadvantage of all viruses is the danger of the gene being placed in the wrong place.

Disadvantages of Viral Vectors

- In this way, it can cut other genes of the host cell, leading to cancer or other disorders. The ability to infect more than one cell variety and the possibility of penetration into germ cells are known as their other disadvantages.



Non-Viral Vectors

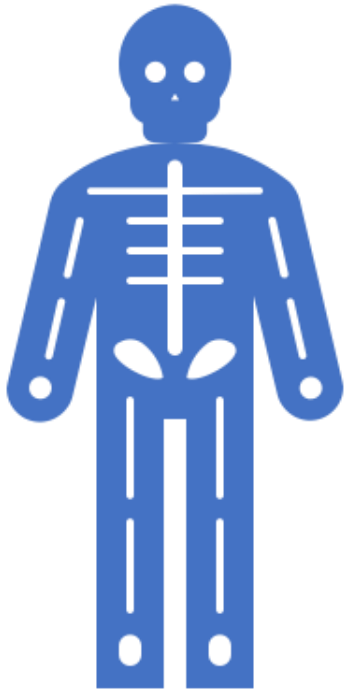
- Safer
- Low immunogenicity
- Easy to produce
- Low-cost
- Scale up ability

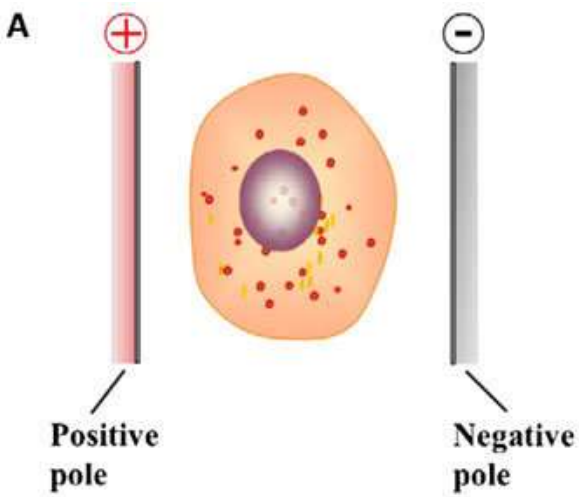
Non-Viral Vectors

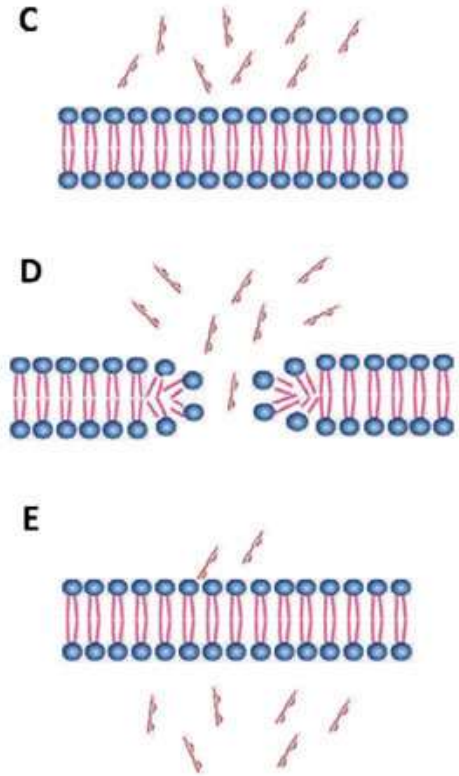
- **They can also carry different and large genes transport, and also they can store genetical materials for a long time due to their stability.**
- However, their low transfection efficiency limits their use in a wide range.

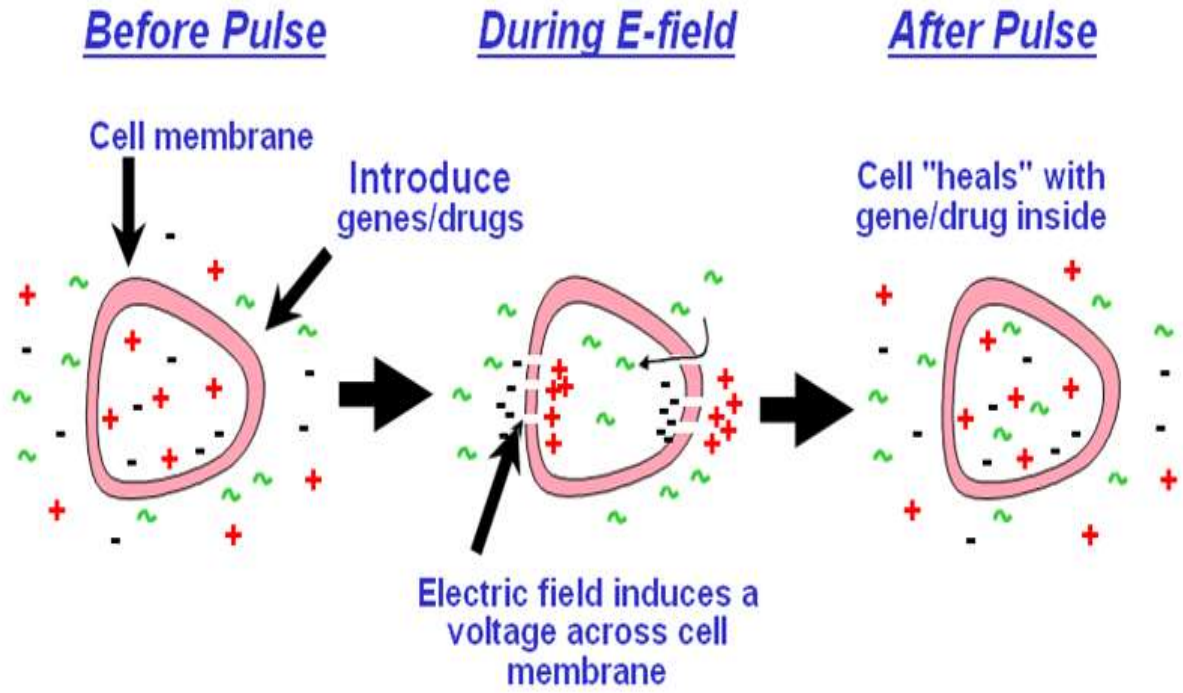
Electroporation

- **As a result of increasing the permeability of the cell membrane by applying a controlled electric field, the DNA is transferred into the cell.**
- It has been successfully used in various tissues (skin, muscle, liver and tumor, etc.).
- Although it is a safe, efficient and reproducible method, it has limitations in in-vivo applications.

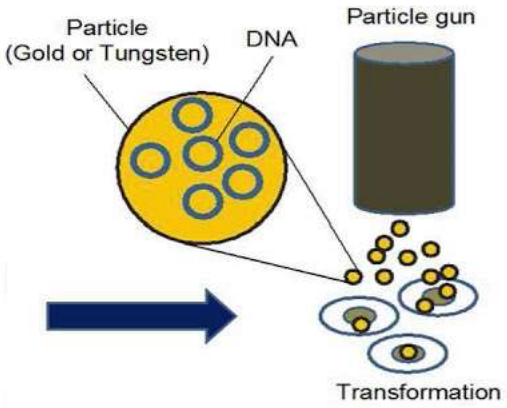



A
Positive pole
Negative pole
B
u
Threshold Voltage
Poration
Intact
Reseal
t


C
D
E

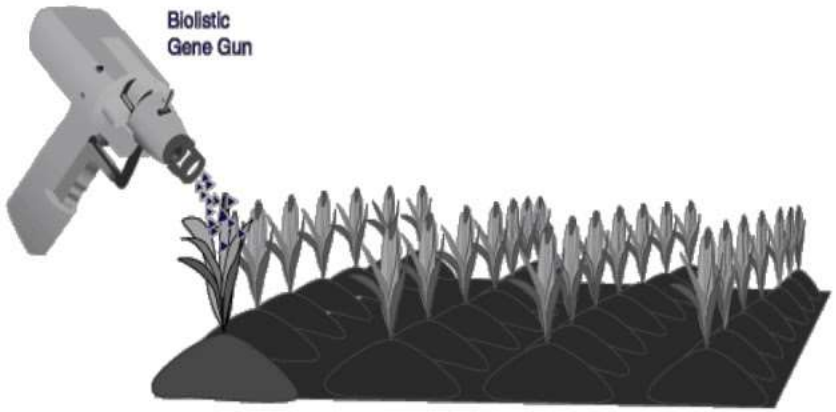

Before Pulse
During E-field
After Pulse
Cell membrane
Introduce genes/drugs
Cell "heals" with gene/drug inside
Electric field induces a voltage across cell membrane

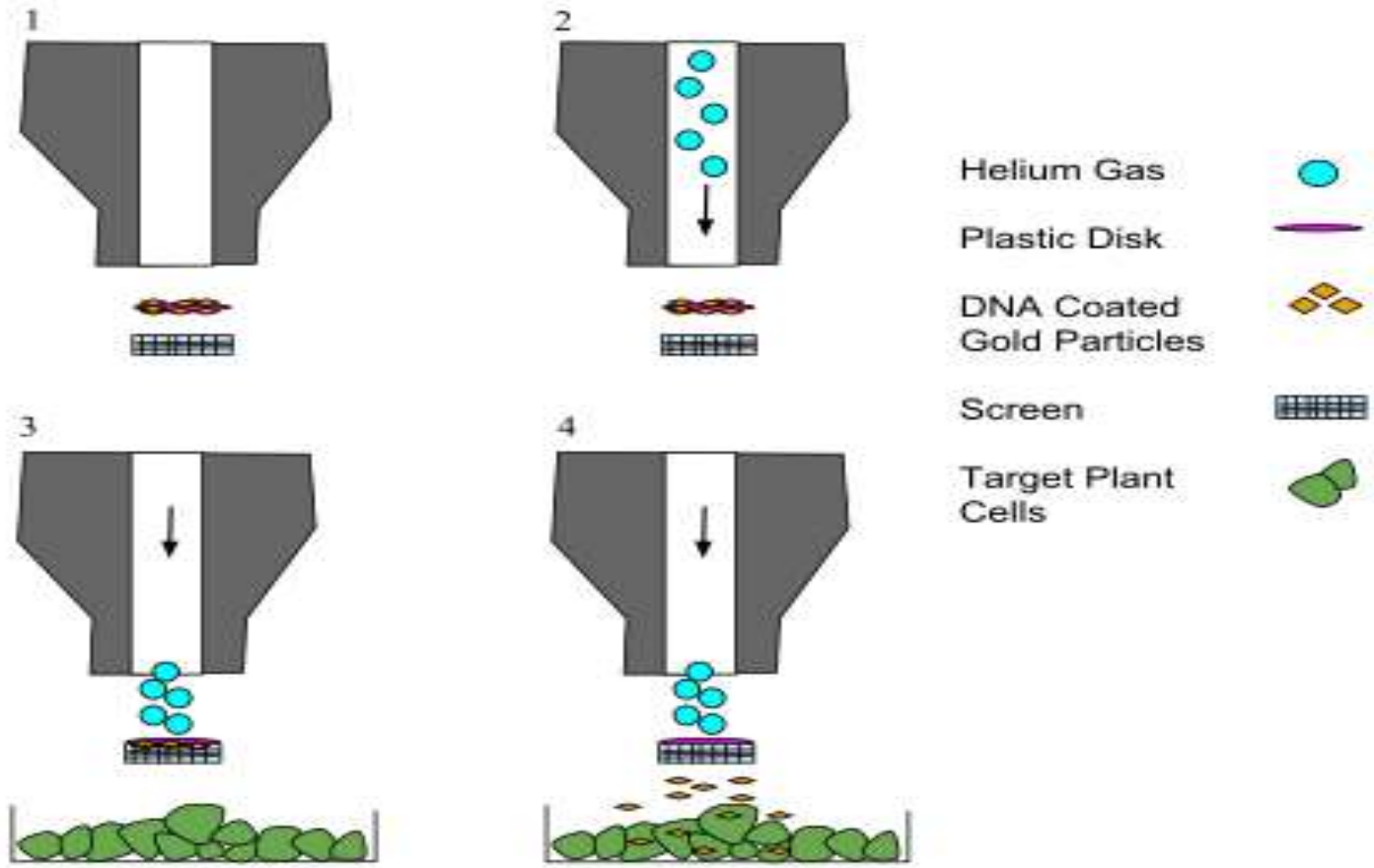
Gene Gun

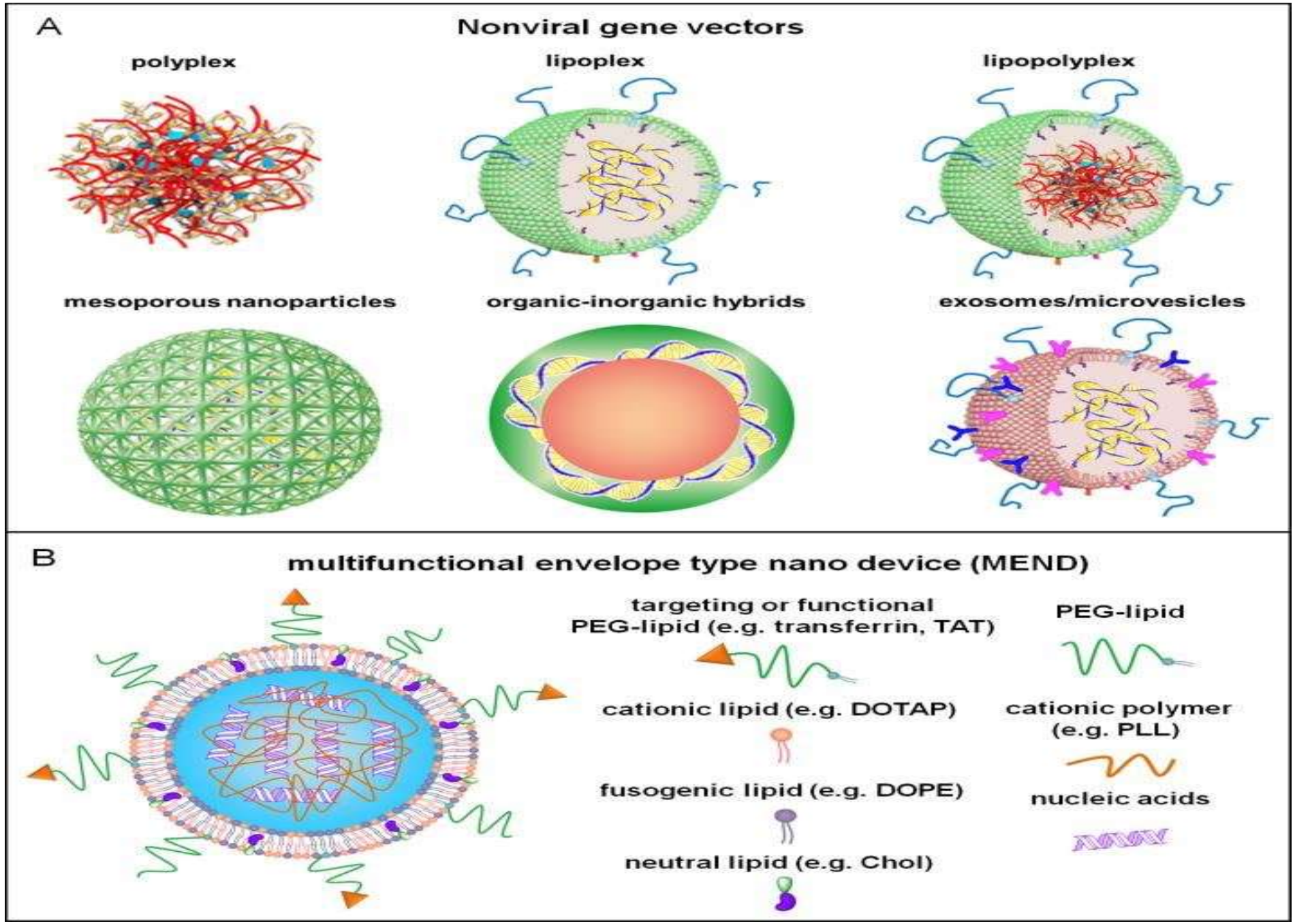


No vaccuum required. Portable

Microcarriers coated on the inside of a plastic tube and accelarated using pressurized He.

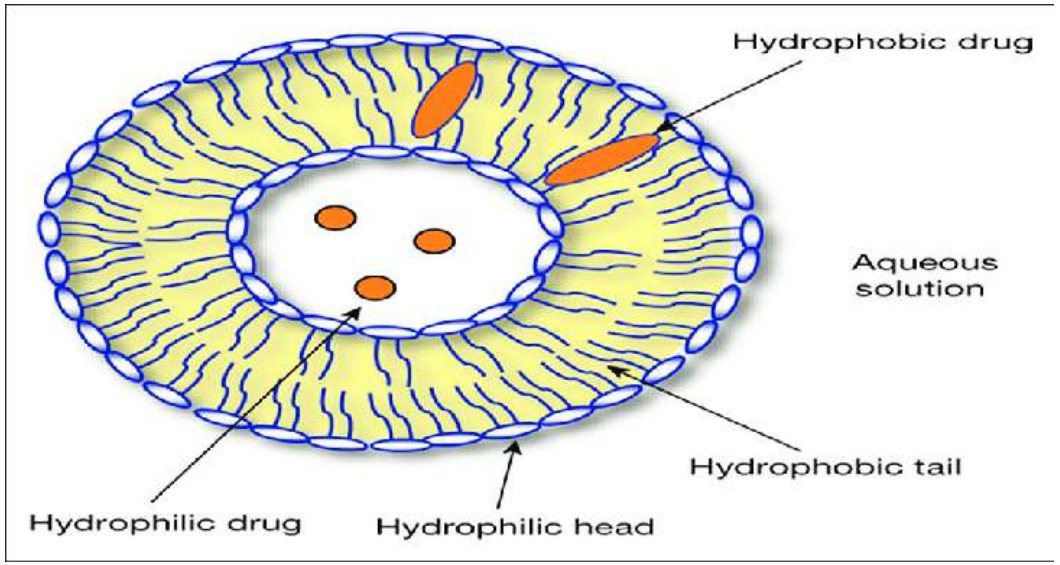


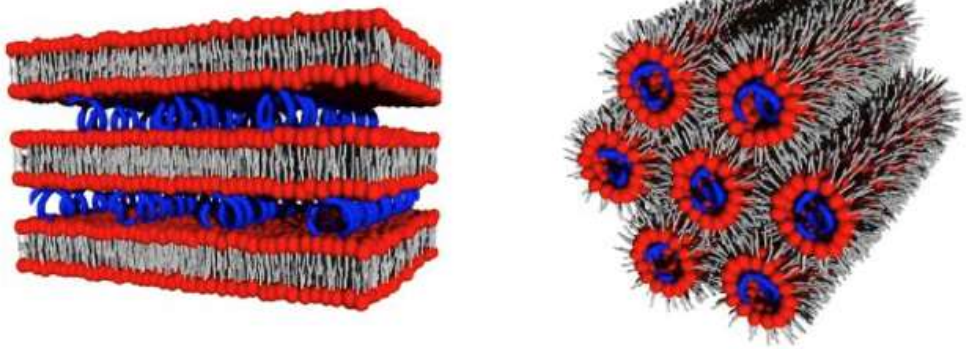

1
2
3
4
Helium Gas
Plastic Disk
DNA Coated
Gold Particles
Screen
Target Plant
Cells


A
Nonviral gene vectors
polyplex
lipoplex
lipopolyplex
mesoporous nanoparticles
organic-inorganic hybrids
exosomes/microvesicles
B
multifunctional envelope type nano device (MEND)
targeting or functional
PEG-lipid (e.g. transferrin, TAT)
PEG-lipid
cationic lipid (e.g. DOTAP)
cationic polymer
(e.g. PLL)
fusogenic lipid (e.g. DOPE)
nucleic acids
neutral lipid (e.g. Chol)

Liposomes

- Liposomes are synthetic tools created by mixing certain lipids in a liquid medium by modeling the membrane structure of mammalian cells

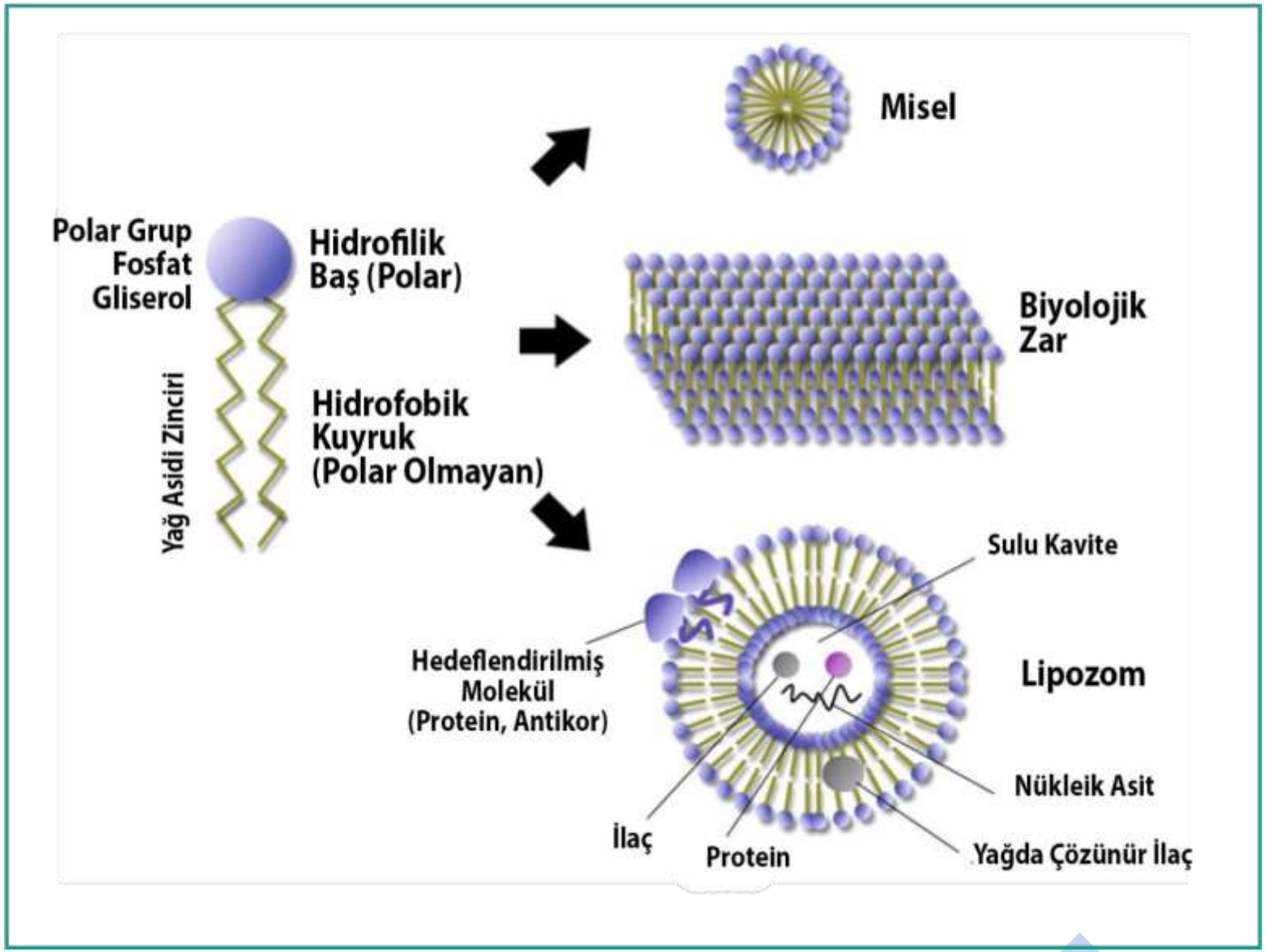




DNA

Lipoplex

Cationic Liposome


Misel
Polar Grup
Fosfat
Gliserol
Hidrofilik
Baş (Polar)
Yağ Asidi Zinciri
Hidrofobik
Kuyruk
(Polar Olmayan)
Biyolojik
Zar
Sulu Kavite
Hedeflendirilmiş
Molekül
(Protein, Antikor)
Lipozom
Nükleik Asit
İlaç
Protein
Yağda Çözünür İlaç