Cloning and Gene therapy

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Cloning

وارد کردن ژن مورد نظردر حامل ← جداسازي ژن مورد نظر ← انتخاب ژن مورد نظر

↓

تکثیر در میزبان مناسب

کلون کردن (Cloning)

انتقال حامل حاوي ژن به سلول هدف ← تکثیر سلول هدف ← محصول یا صفت مورد نظر

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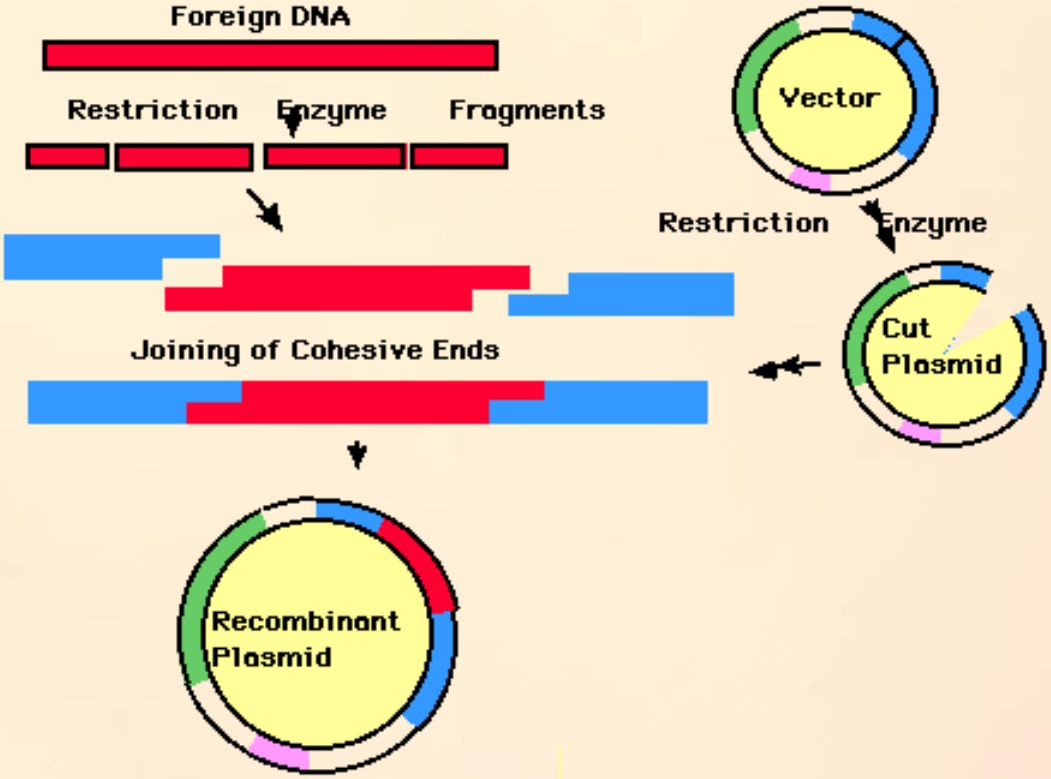
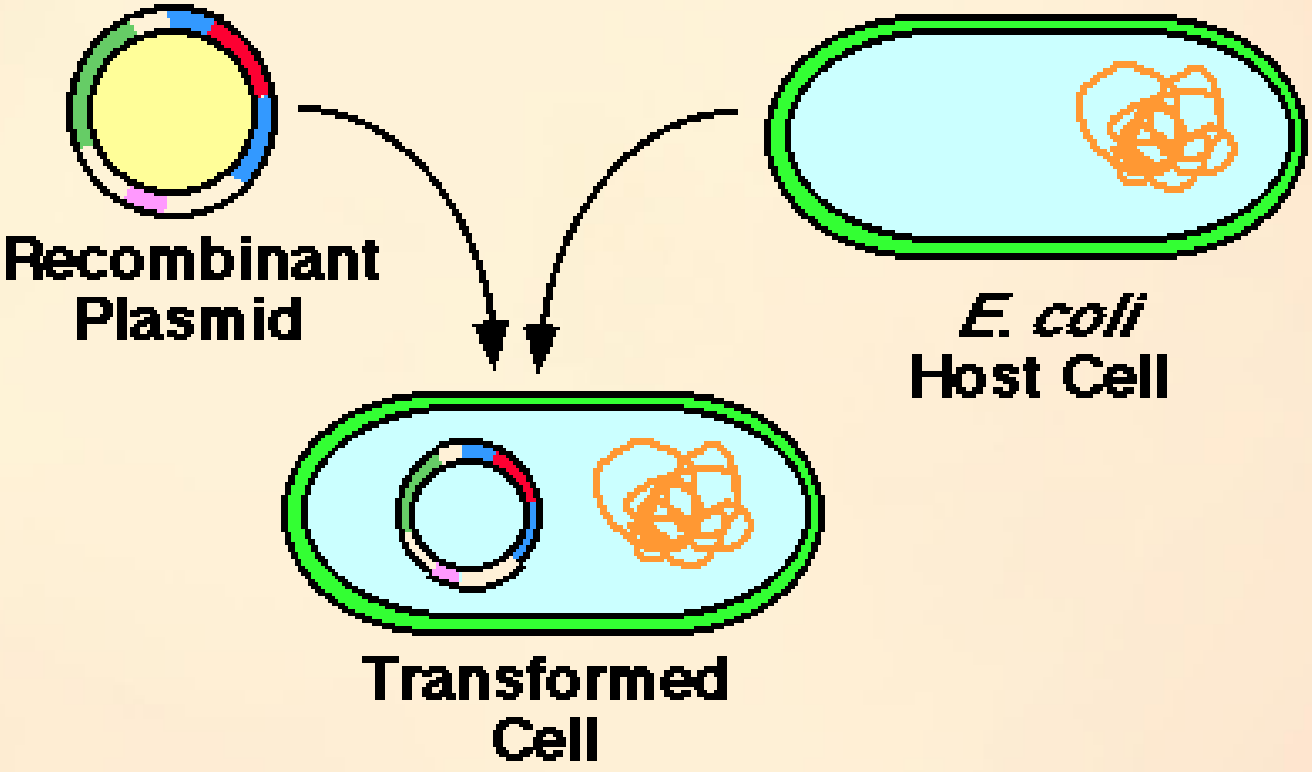
انتقال دادن (Transformation)

همسانه سازي ژن (Gene Cloning) چيست ؟

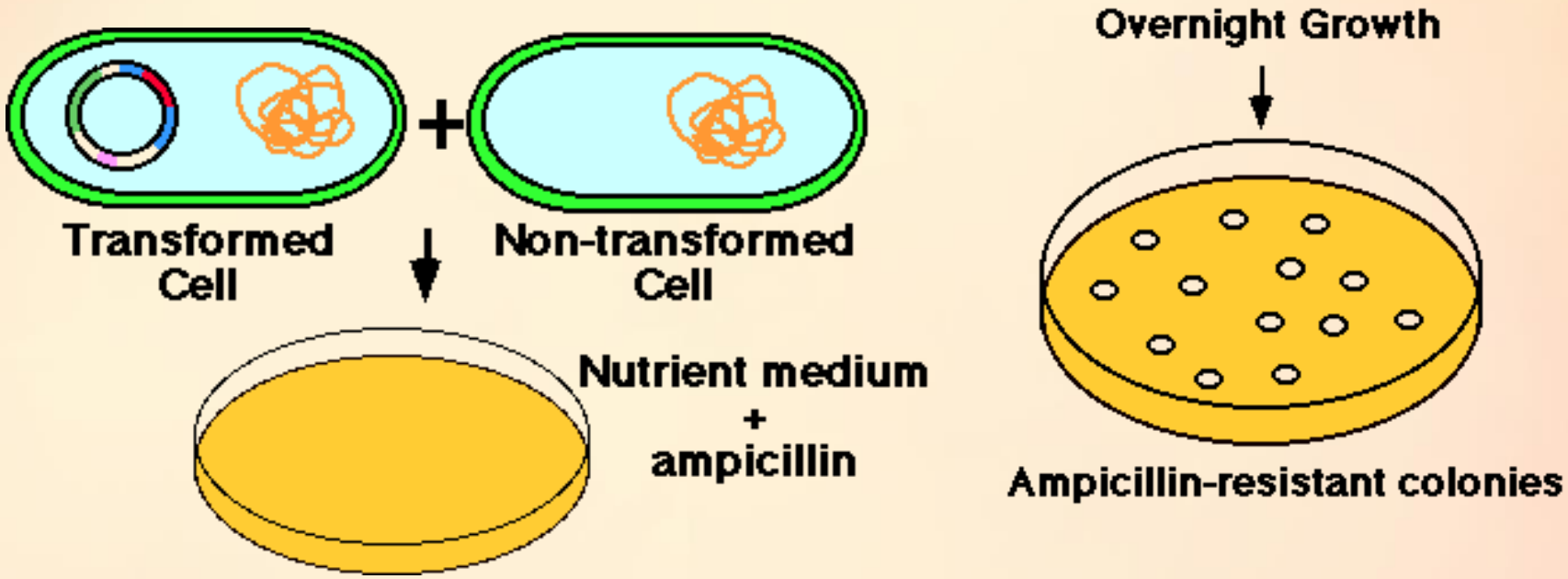
- **Creating Recombinant DNA**



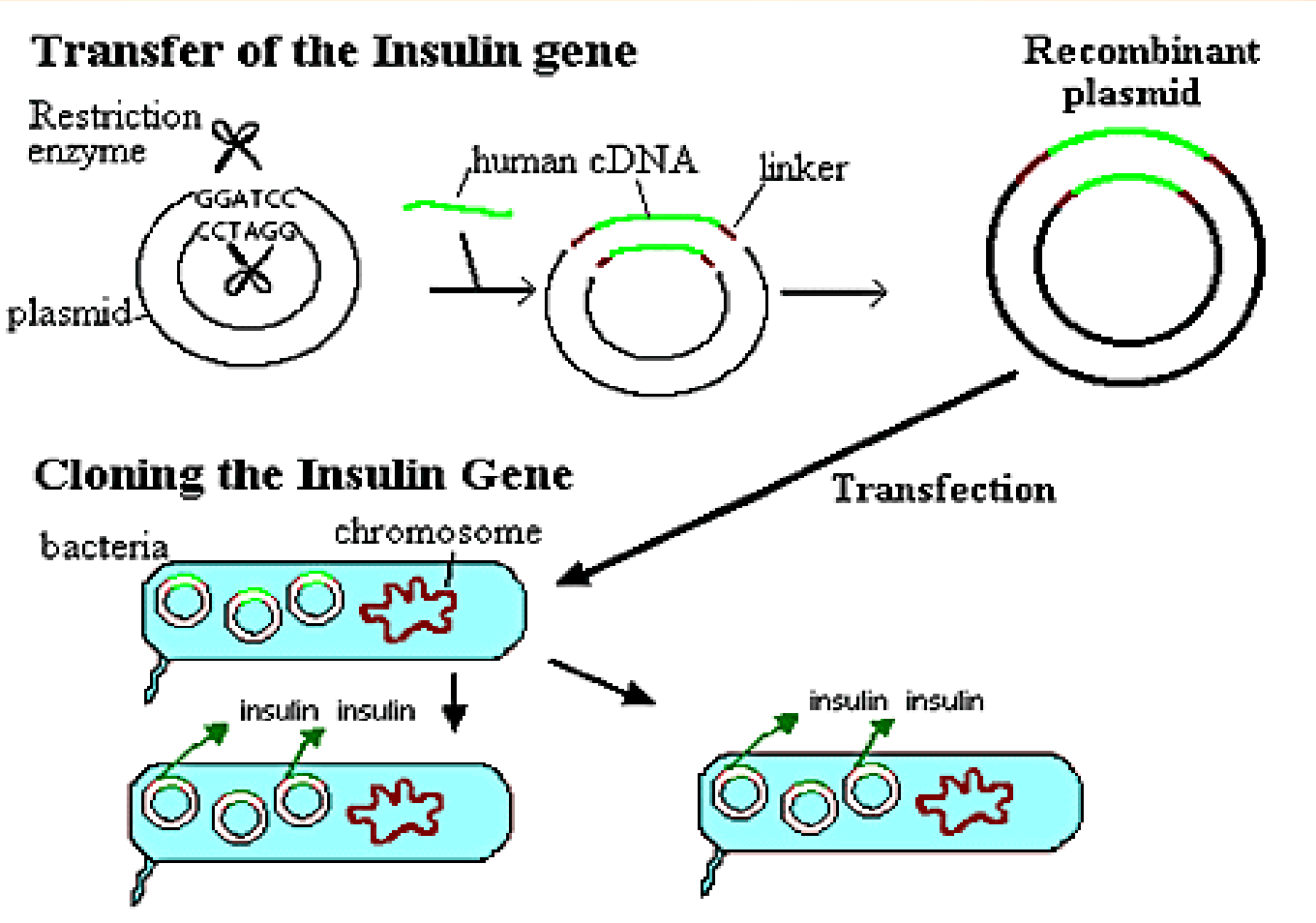
Inserting vectors into host cells

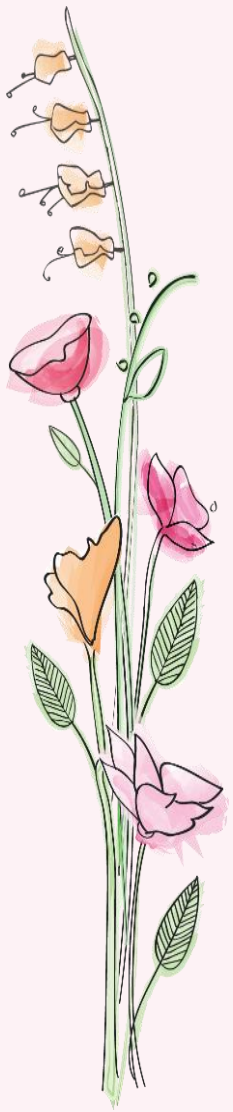


Selecting transgenic cells



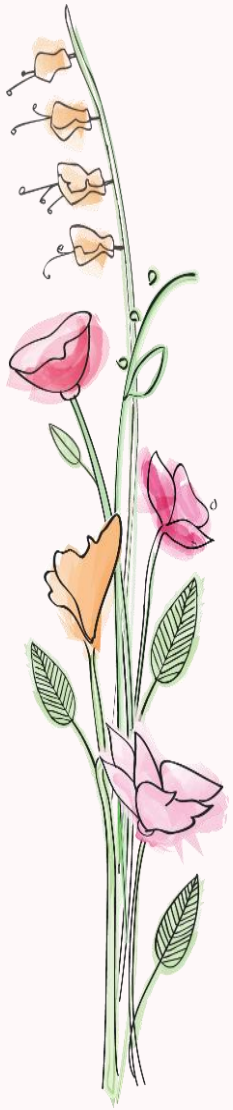
Transfer and Cloning of the Insulin Gene





History

- Early 1970s : scientists proposed "gene surgery"
- 1983 : Lesch-Nyhan disease treatment
- Late 1980s : scientists' increasing ability to identify the specific genetic malfunctions that caused inherited diseases. . In fact, some scientists theorize that all diseases may have a genetic component.
- On September 14, 1990 : first person to undergo gene therapy
- 1991 : , the U.S. government provided $58 million for gene therapy research



- Currently, there are a host of new gene therapy agents in clinical trials. nucleic acid based (*in vivo*) treatments and cell-based (*ex vivo*) treatments .

- Presently, gene therapies for the following diseases are being developed: **cystic fibrosis** (using adenoviral vector), **HIV infection** (cell-based), **malignant melanoma** (cell-based), **Duchenne muscular dystrophy** (cell-based), **hemophilia B** (cell-based), **kidney cancer** (cell-based), **Gaucher's Disease** (retroviral vector), **breast cancer** (retroviral vector), and **lung cancer** (retroviral vector).

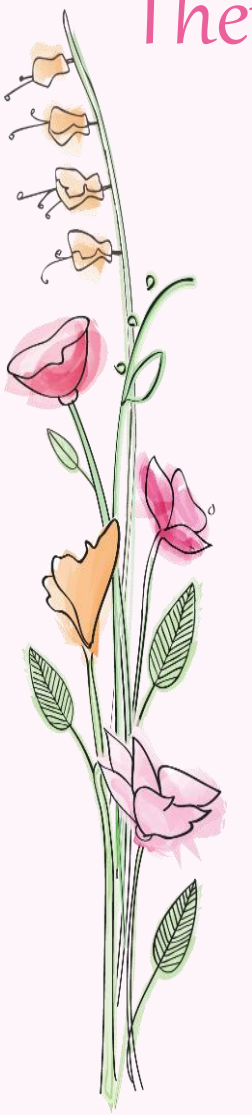
What is gene therapy?

- Gene therapy is the insertion of genes into an individual's cells and tissues to treat a disease, and hereditary diseases in which a defective mutant allele is replaced with a functional one.

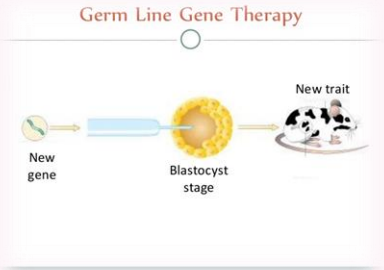
The biological basis of gene therapy

Scientists have known how to manipulate a gene's structure in the laboratory since the early 1970s through a process called gene splicing. The process involves removing a fragment of DNA containing the specific genetic sequence desired, then inserting it into the DNA of another gene. The resultant product is called recombinant DNA and the process is genetic engineering.

There are basically two types of gene therapy:

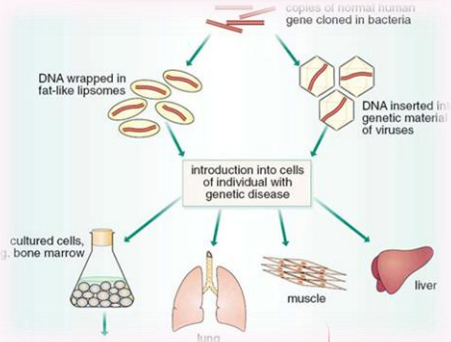


- Germ-line gene therapy
- somatic gene therapy



Or

- nucleic acid based (*in vivo*) treatments
- cell-based (*ex vivo*)




Gene therapy for Humans

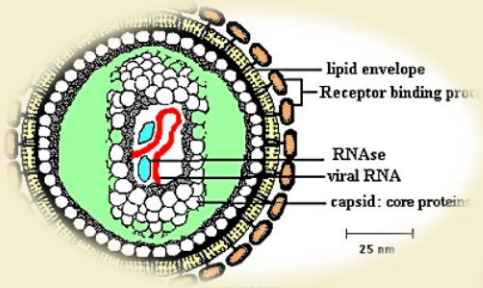
- Before the first human coding sequence had been determined, there was already speculation about the prospects for gene therapy. A prescient editorial published in Science in 1971 outlined many of the problems that would face clinical gene therapy, including construction of safe viral gene delivery vectors and efficient gene delivery to enough patient cells to correct the inherited gene defect.
- Some 40 years later, the same issues persist but substantial progress has been made!

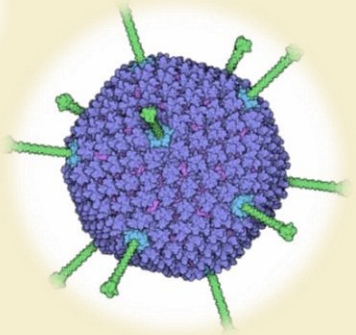
We can use it for which diseases?

- As a suggested remedy for body's immune reaction to tumors, new blood vessels in the heart to alleviate heart attacks and to stop HIV-replication, genetic diseases, such as hemophilia A and B, and cystic fibrosis, blood disorders, muscular dystrophy and diabetes.

Choices of vectors

❖ Viral vectors :

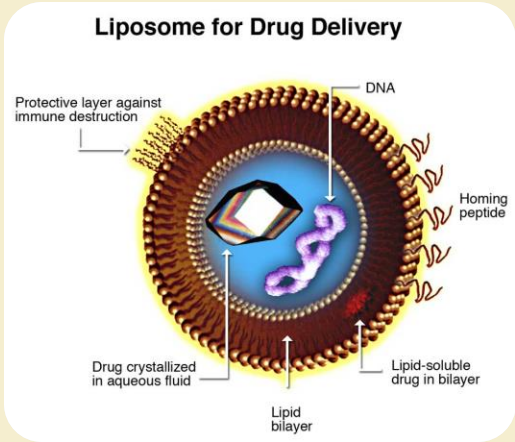


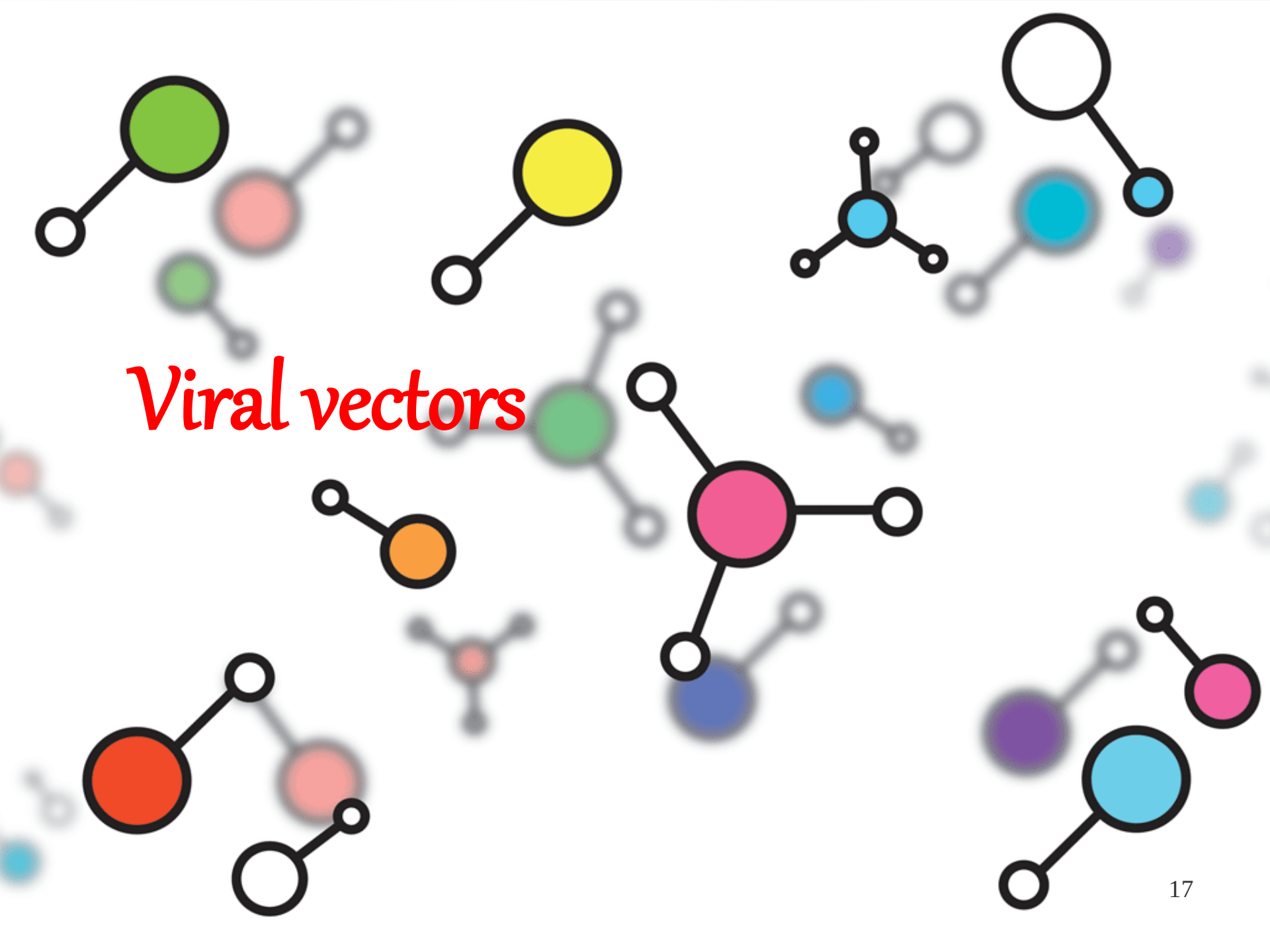


1. Retrovirus
2. Adenovirus
3. Adeno-associated virus
4. Herpes Simplex Virus

❖ Non-viral vectors :

1. Liposome
2. DNA–polymer conjugates
3. Naked DNA




Viral vectors

- They invade cells as part of the natural infection process.
- Have a specific relationship with the host in that they colonize certain cell types and tissues in specific organs
- Vectors are chosen according to their attraction to certain cells and areas of the body.

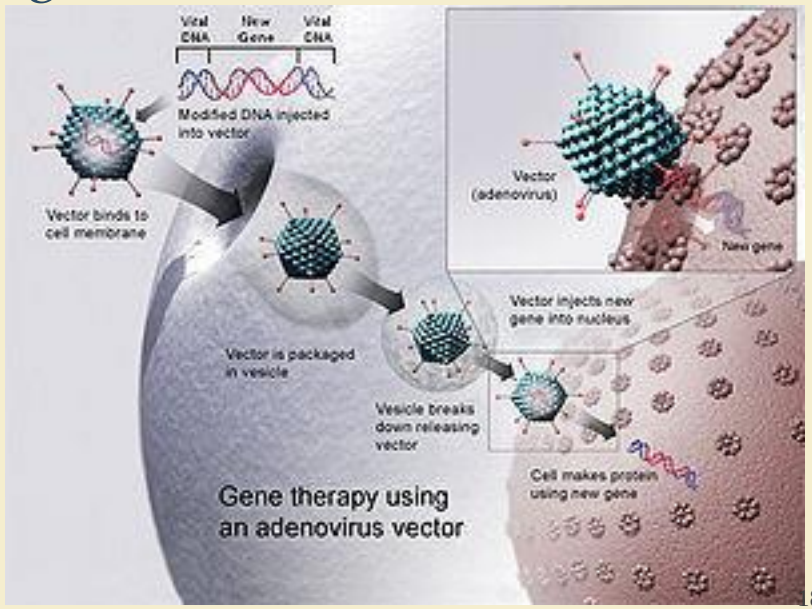


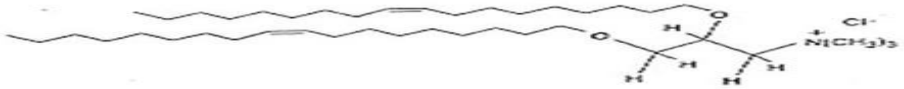
Table 1. Advantages and disadvantages of major viral vectors.

Virus	Advantages	Disadvantages	Major Clinical/Preclinical Studies
Retrovirus	Long-term gene expression	Generation of replication-competent virus Potential for tumorigenesis Infects dividing cells only	[6]
Lentivirus	Long-term gene expression Infects non-dividing and dividing cells High immunogenicity	Generation of replication-competent virus Potential for tumorigenesis	[7,8]
Vaccinia virus	Safety: used as a smallpox vaccine High titer production	Pre-existing immunity	[9]
Adenovirus	High immunogenicity Safety: used in many clinic trails High titer production	Pre-existing immunity	[10]
Adeno-associated virus	Long-term gene expression Non-pathogenic virus	Low titer production	[11]
Cytomegalovirus	Induces a unique CTL response Protects against SIV infection in an animal model	Pre-existing immunity Risk of pathogenesis in specific individuals	[12]
Sendai virus	High immunogenicity	Pre-existing immunity	[13]

Nonviral vectors

1. Liposome

2. Cationic polymers



3. Naked DNA

4. Peptide-mediated gene delivery

- These vectors rely on the natural biological process in which cells *uptake (or gather) macromolecules*
- Another possible vector under development is based on dendrimer molecules. A class of polymers (naturally occurring or artificial substances that have a high molecular weight and formed by smaller molecules of the same or similar substances, is "**constructed**" in the laboratory by *combining these smaller molecules*.

METHODS FOR GENE THERAPY OF CANCER

I. Viruses

II. Naked DNA (vector-free)

III. Liposomes

IV. Protein-DNA complexes

V. Gene gun

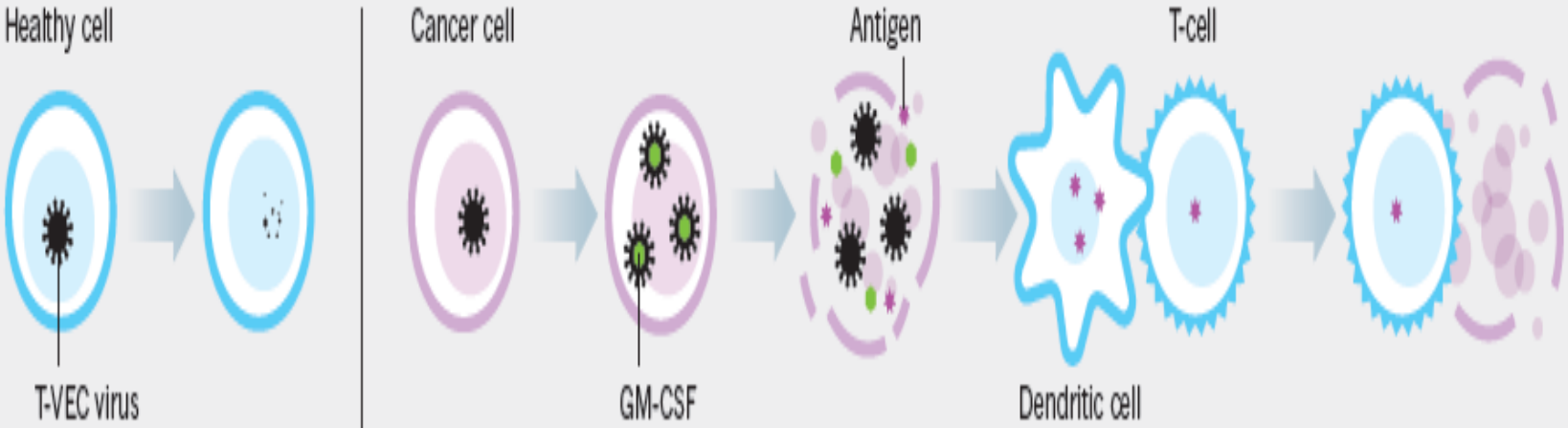
VI. Calcium phosphate precipitation

VII. Electroporation

VIII. Intracellular microinjection

GOING VIRAL AGAINST CANCER

The virus-based cancer therapy T-VEC infects tumour cells and destroys them by stimulating the immune system to direct an attack against malignant cells in the body.



T-VEC enters but cannot replicate in normal cells.

T-VEC destroys malignant cells directly, releasing the protein GM-CSF and antigens that enable the immune system to target cancerous cells nearby and throughout the body.

GM-CSF attracts dendritic cells, which present tumour antigens to the immune system's T-cells, programming them to destroy cancer cells throughout the body.

Gene therapy for Plants

We consider two aspects:

I. Using plant viruses and plants as a tool for treatments

II. Using gene therapy against plant diseases

- Plant virus expression vectors have been engineered to function as **rapid**, **inexpensive** and robust platforms for vaccine production.
- they are able to function in a much broader range of plants, and thus provide more choices of the production system to be used.
- The ability to express several proteins in tandem and at comparable levels from a single construct could provide added value over other virus vectors

DNA Virus Vectors for Vaccine Production in Plants: Spotlight on Geminiviruses

- Plants represent a safe, efficacious and inexpensive production platform by which to provide vaccines and other therapeutic proteins.
- Plant virus expression vector technology has rapidly become one of the most popular methods to express pharmaceutical proteins in plants.


Plant Form
Our Manufacturing Process
From seedling to high-value biopharmaceutical in eight weeks
Plant Nicotiana benthaminia seeds
Plants grow, generating biomass
6 weeks
Grow agrobacterium containing genetic sequence for target drug
3 days
Infiltrate plants with agrobacterium
Plants grow, producing drug in each cell
1 week
Extract drug
1 day
Purify drug
4 days
Fill vials
1 day
Package product
1 day

- Recent advances in plant virus molecular biology have yielded an alternative means of transiently expressing proteins through the use of virus expression vectors which are engineered to be delivery vehicles.
- These include greater expression levels over a *short period of time*, the ability to generate proteins which may impede plant growth, as well as reduced biocontainment issues and related public perception concerns related to genetically modified crops

- Plant virus expression vectors which have been engineered to generate vaccines and other pharmaceutical proteins have predominantly been the positive-sense RNA viruses such as **Tobacco mosaic virus**, **Potato virus X**, **Cucumber mosaic virus** and **Cowpea mosaic virus**. Geminiviruses were among the first viruses to be considered as potential gene vectors but their use was limited because of the **limitations on the size of insert tolerated**

Plant treatment

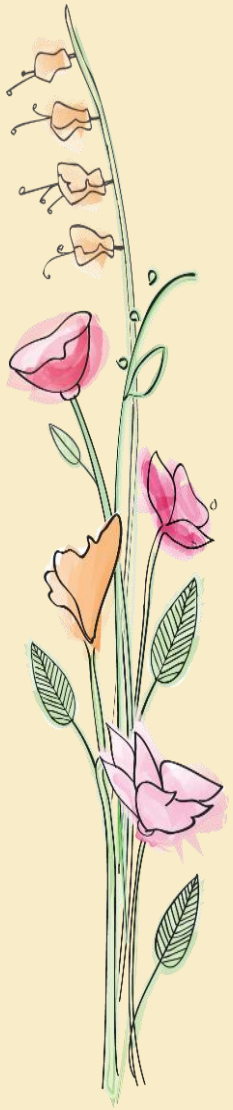
Gene Therapy for Plants: Using Carbon Nanofibres

- The researchers discovered that the **microRNA mechanism** that controls whether a particular cell destroys or simply represses the mRNA molecules in plants relies on *'switcher' genes*
- Because the basic microRNA system is present in both plants and animals, similar switchers are likely to exist in humans.

- Regulating the switcher mechanism should allow them to boost the capacity for environmental adaptation without interfering with development. This has clear applications for plants affected by **climate change**.
- The researchers worked collaboratively for three years analysing the plant model Arabidopsis to reveal the underlying mechanism.

- The new developments in gene therapy in plants have to be compared with similar approaches in animal systems.
- Delivery of COs, their alignment to chromosomal sequences, mismatch recognition, and mismatch elimination should be considered.
- Packaging COs in various coats may **secure express delivery** and **nuclease resistance**; and providing an extra supply of mismatch repair proteins for the target cells at the time of delivery may increase efficiency and precision of repair.

The future of gene therapy



- There are many obstacles and some distinct questions concerning the viability of gene therapy
- Viral vectors must be carefully controlled lest they infect the patient with a viral disease
- One of the most pressing issues, however, is **gene regulation**
- Learning how to make the gene go into action **only when needed**

- In the area of gene therapy, it is clear that many exciting innovations are emerging. While many of these new gene-therapy and biotech products might yet have **unknown risks**, they also have the potential for tremendous patient benefit

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Good Luck