Biotechnology
Methods of Transfection

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TRANSGENIC ANIMAL

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- Is an animal whose genome contains a foreign gene or genes introduced by the technique of tranfection
- TRANS GENE : Gene introduced by transfection
- **Transfection**: Specifies the introduction of a DNA segment either naked or integrated in a vector into an animal cell to produce a transgenic animal.
  - Transient : Introduced gene gradually lost from transfected daughter cells (not heritable)
  - Stable: Permanent integration of a transgene into the organism's germ cell for heritability

OBJECTIVES

- To obtain the protein encoded by the gene by allowing their expression either

  i) in cultured cells OR

  ii) as animal products (urine, blood etc.)

  Such animals known as ***BIOREACTERS*** and the process is known as ***Molecular/Gene farming***

- Improve the genetic make up of the animal

  i) increase quality of milk, meat, wool etc.

  ii) Increase efficiency of food utilization therefore can be grown into a marketable size sooner

  iii) Increase resistance power to certain diseases

- Gene therapy : Introduction of the normal functional copies of a defective gene into a patient to reduce a genetic disease
- For the creation of special animal strains to fulfill experimental / biomedical needs eg: Knock out mice

TRANFECTION

- Manipulate only specialised cells (Eg: Mammary cells for milk production) or embryo to create a new whole organism
- However charge of DNA, multitude of enzyme, membrane barriers make the taking up of exogenous DNA very difficult

METHODS OF TRANSFECTION

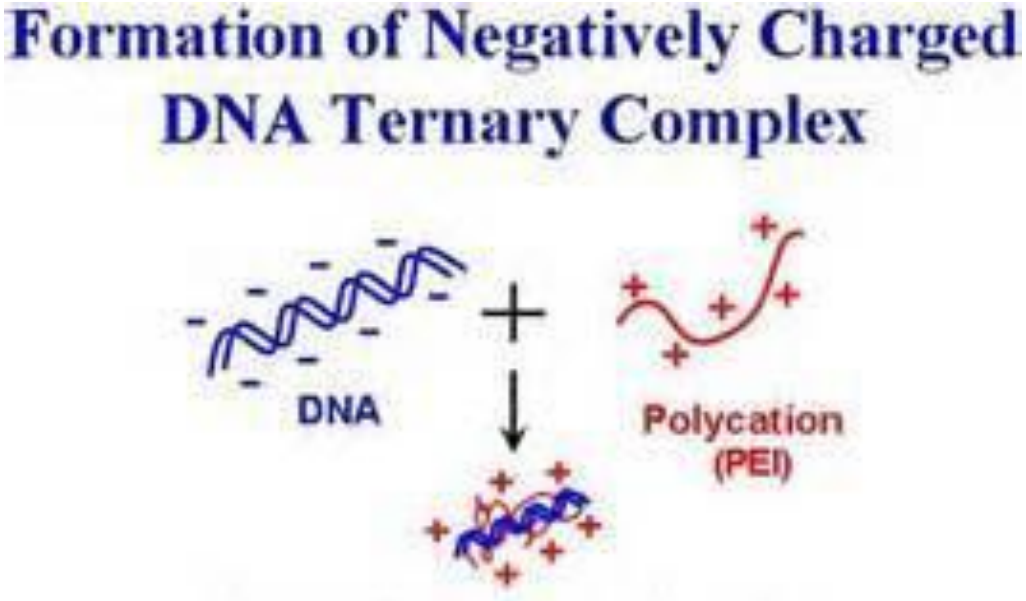
1. $CaPO_4$ PRECIPITATION

- DNA preparation to be used is dissolved in PO_4 buffer and added $CaCl_2$. This leads to the formation of insoluble Ca PO_4 which precipitate with DNA
- The precipitate is added to the cells which is transfected
- The precipitate particles are taken by the cells by phagocytosis. 1-2% cells may get transfected by this method

2. DEAE –Dextran mediated method

- Is a water soluble polycation (a chemical substance or molecule with multiple +ve charges) Added to DNA which is –vely charged

- In some unknown way DEAE dextran brings about DNA uptake through endocytosis

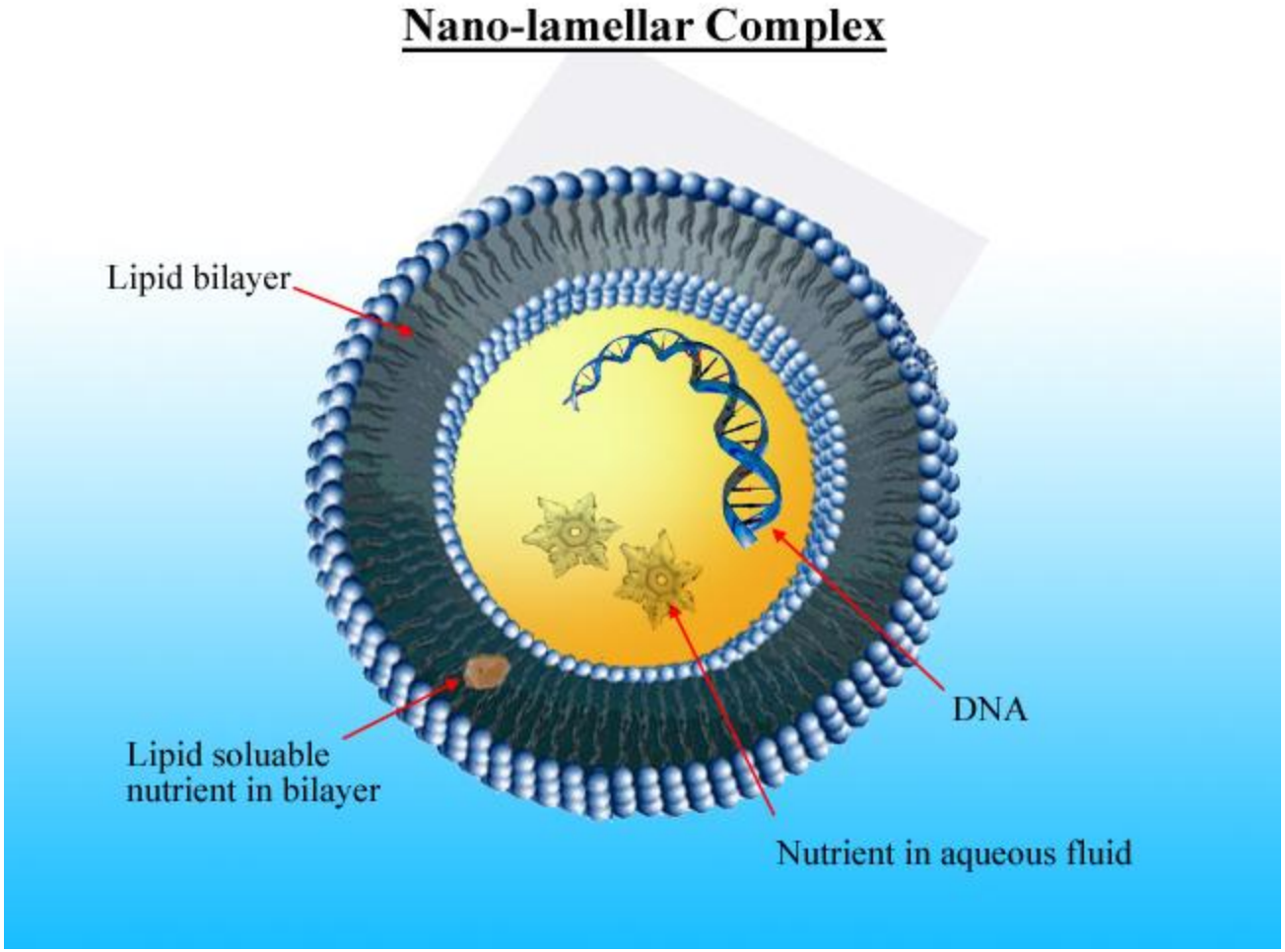


3. LIPOFECTION

- With the help of liposomes

Liposomes – are very small vesicles prepared from a suitable lipid

- The required DNA is enclosed within the Liposome
- The Liposomes being permeable through plasmamembrane easily enters into the cell and facilitate the delivery of functional DNA into the cell



- Used in both tissue culture cells and live animals by intravenous injections.

4. Electroporation

- Cells are mixed with DNA construct and briefly exposed to pulses of high electrical voltage for few milli seconds. This induce transient pores through which DNA seems to enter into the cells

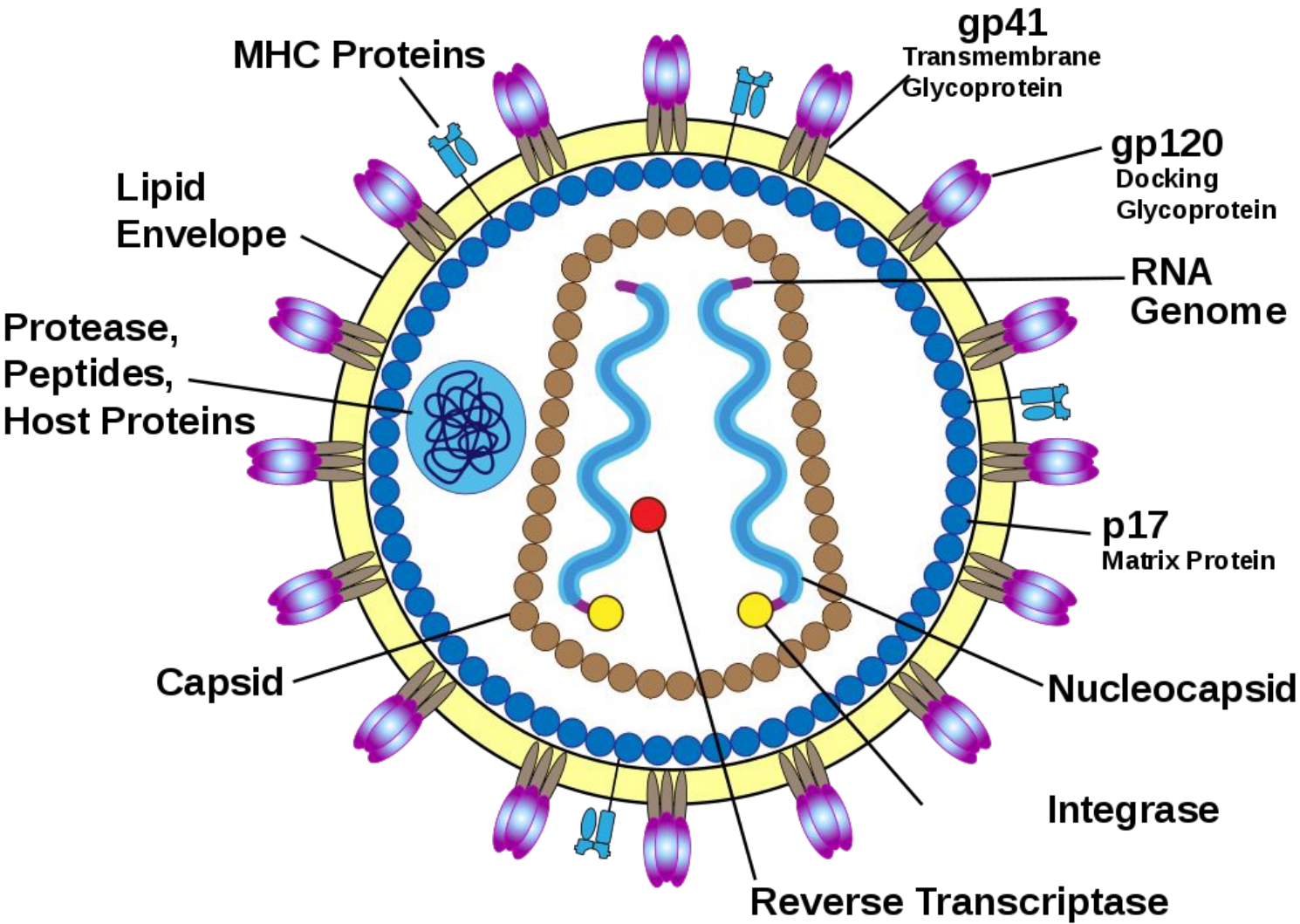
- Treatment of cells with colcemide* before electroporation increases the frequency of tranfection

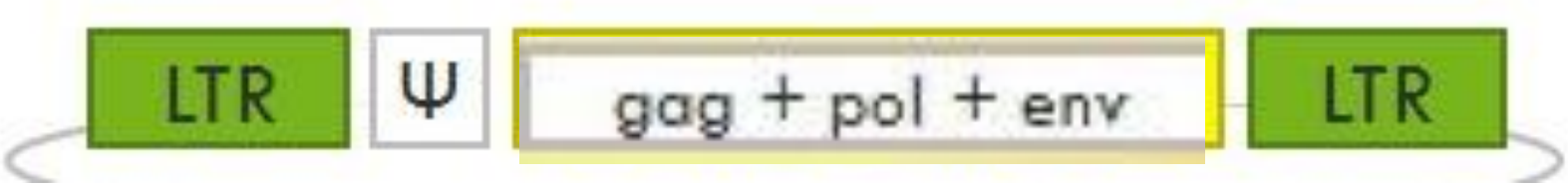
  *It arrest the cells at metaphase where nuclear membrane is absent

- Linear DNA is more efficient foe transfection than circular DNA

5. Retroviral infection

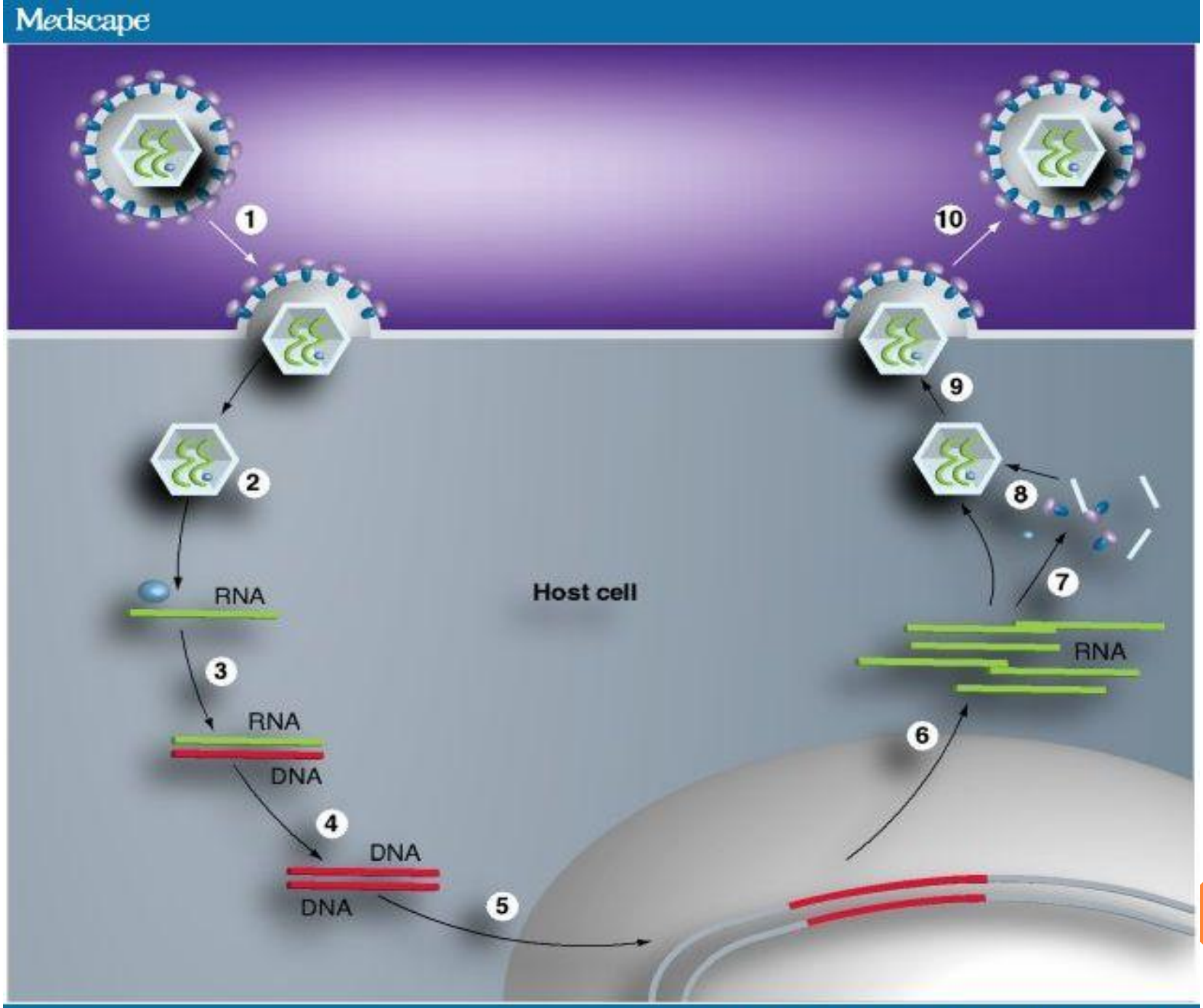
- A retrovirus contains a core of RNA as the genetic material with in a protein coat eg: HIV



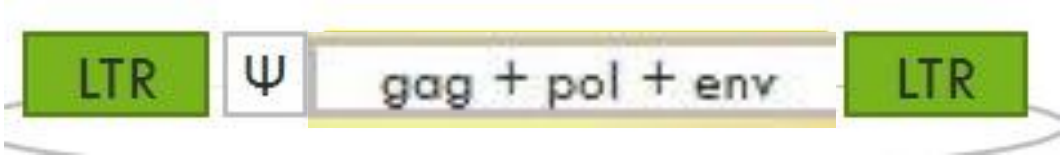


- LTR: Carry promoter and termination site
- *gag*: codes for viral coat protein
- *pol*: codes for enzyme reverse transcriptase
- *env*: Codes for the outer envelop
- *psi*: provides the packaging signal for directing assembly of RNA in forming virus particle

1. During infection the RNA genome of virus is injected into the host cell
2. Converted to DNA by reverse transcriptase
3. Newly formed viral DNA is then integrated to into host chromosomal DNA as ***PRO VIRUS***
4. Integrated viral DNA transcribed with other cellular genes and synthesis necessary viral proteins
5. The viral RNA and proteins are assembled during process into new retroviruses


Medscape
1
2
RNA
3
RNA
DNA
4
DNA
DNA
5
Host cell
6
RNA
7
8
9
10
Source: Future Microbiol © 2010 Future Medicine Ltd

- Retroviruses are infectious & therefore must be modified for using as transfective agents

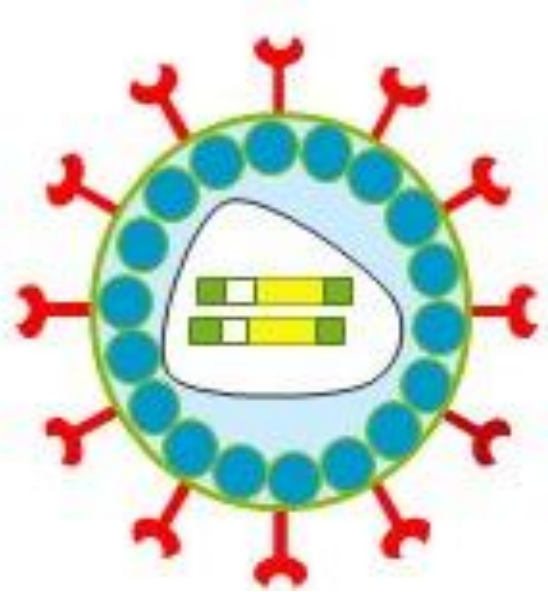


- A recombinant provirus DNA is constructed by deleting the viral genes in provirus replacing them with therapeutic genes

  However, the LTR region and psi region are retained in the provirus

- Missing genes for viral protein are provided by a helper provirus

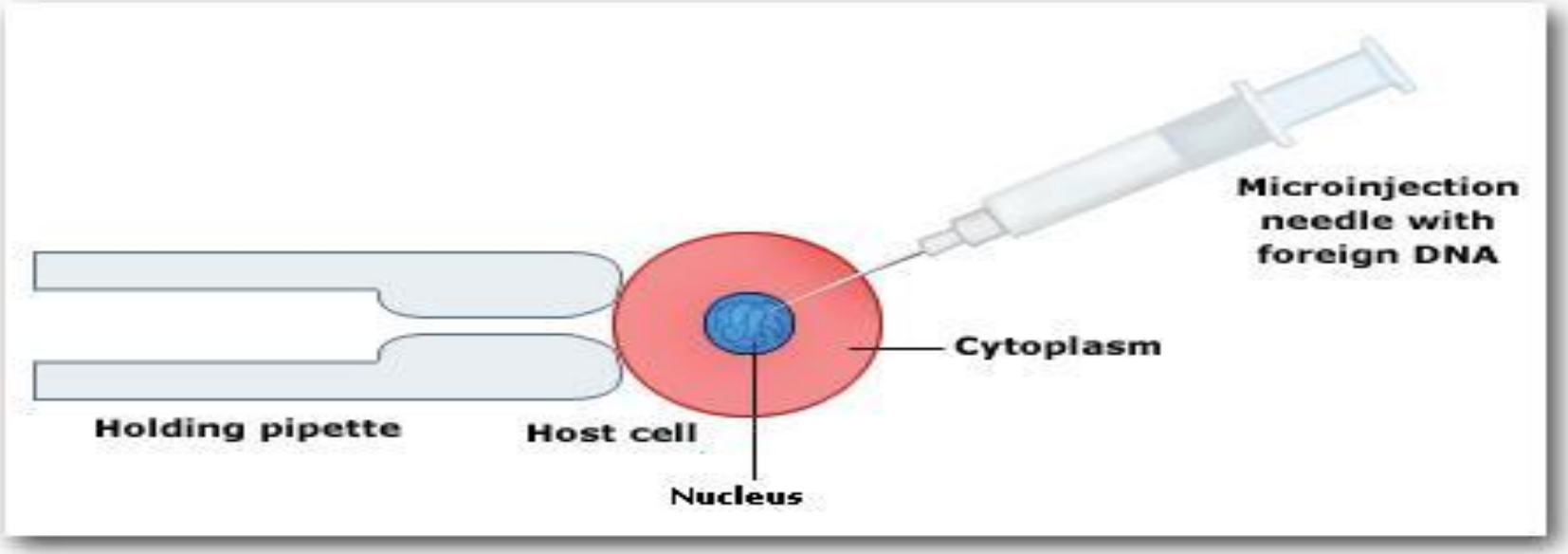
- Newly generated viruses are safe vectors that contain therapeutic RNA but no genes for viral proteins, they cannot regenerate new viruses



- The recombinant retroviral genome id copied by reverse transcriptase to yield a DNA copy

6. DNA MICROINJECTION

- Direct injection of DNA into the nucleus of a cell or into a male pronuleus (Since larger than female pronuclei)
- Through recombination the inserted gene get spread to the host genome and inherited in Mendelian manner



Require a

- ***Low power sterioscopic dissecting microscope &***
- ***Two micro manipulators –***

i) Micropipette to hold the ovum by partial suction

ii) Glass injection needle to introduce DNA into male pronucleus

- The female is treated with HCG for super ovulation and mated with fertile males to get large number of fertilised embyos
- The embryos are collected and transgene construct prepared in a buffer is injected to male pronucleus
- Most successful in fish and mice. The frquency is much lower in other animals
- In drosophila a recombinat P-element is generated through micro injaction

7. Shotgun method or

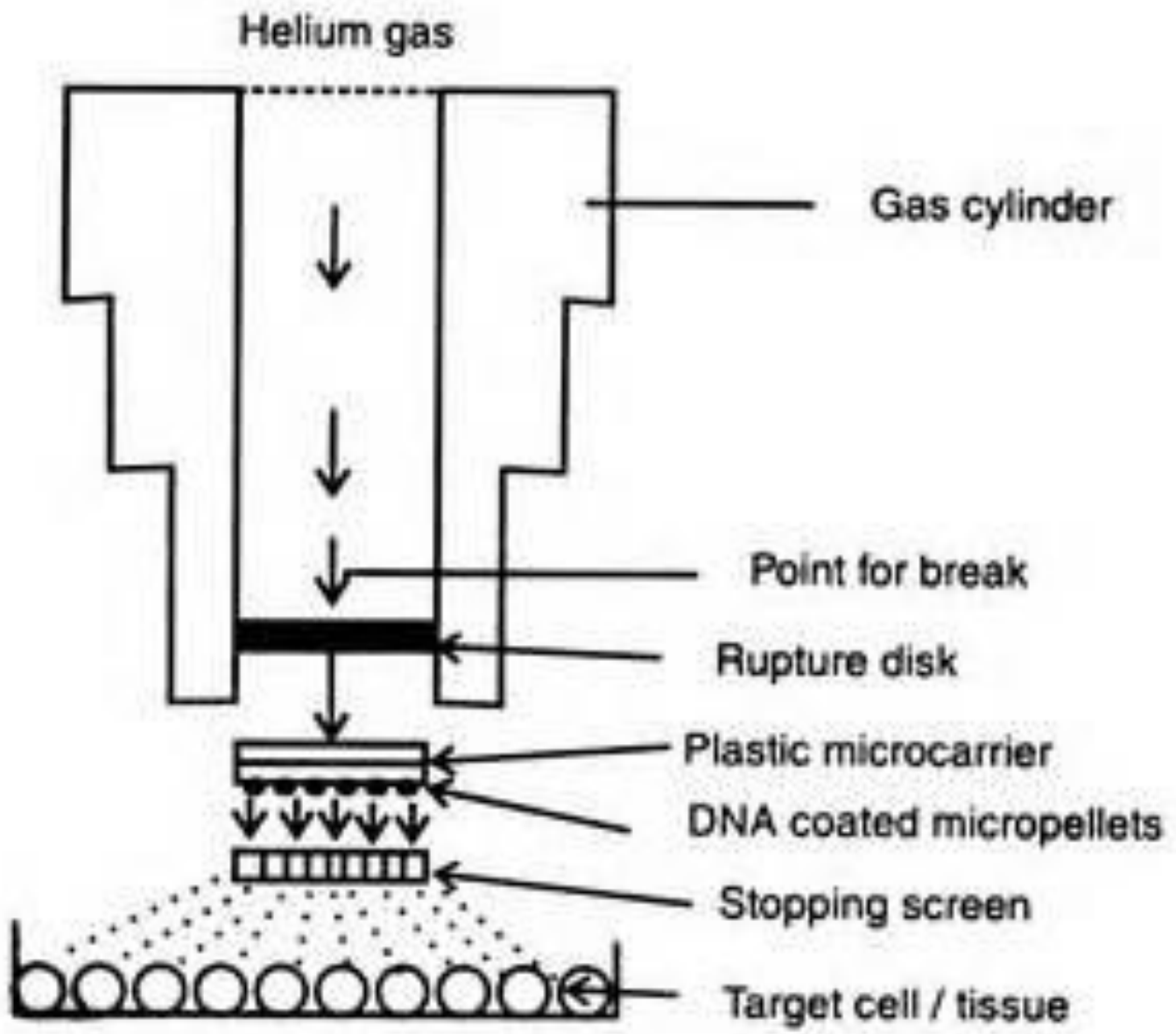
(i) *Micro projectile bombardment*

(ii) Particle bombardment

(iii) Particle acceleration

(iv) Biolistic process

- Gene gun: Prof. Stanford and co-workers of Cornel University (1987)
- Shoot foreign DNA into plant cells or tissues at a very high speed so that genetic material containing gene of interest can be transferred to the cell
- Consist a chamber connected to an outlet to creat vaccum


Helium gas
Gas cylinder
Point for break
Rupture disk
Plastic microcarrier
DNA coated micropellets
Stopping screen
Target cell / tissue

1. Gas cylinder containing He which is temporarily sealed off from the rest of the chamber with a plastic rupture disc

2. When the pressure of the cylinder exceeds the bursting point of the plastic disc, it get ruptured pushing the flying disc (Plastic microcarriers) at a rate of 300 to 600 meters/sec

3. The gene to be transferred is coated to flying disc using gold or tungsten beads approximately 0.4-1μm in diameter (microprojectiles or microcarriers). The required DNA coated beads are inserted onto the lower surface of the plastic disc

4. The projected flying disc is ceased by a Stopping screen containing microscopic holes which allow the microprojectiles carrying DNA to pass outside

5. Some of the DNA covered particles are sprayed through this holes into target cells or tissues held on a petri plate placed just below the holes