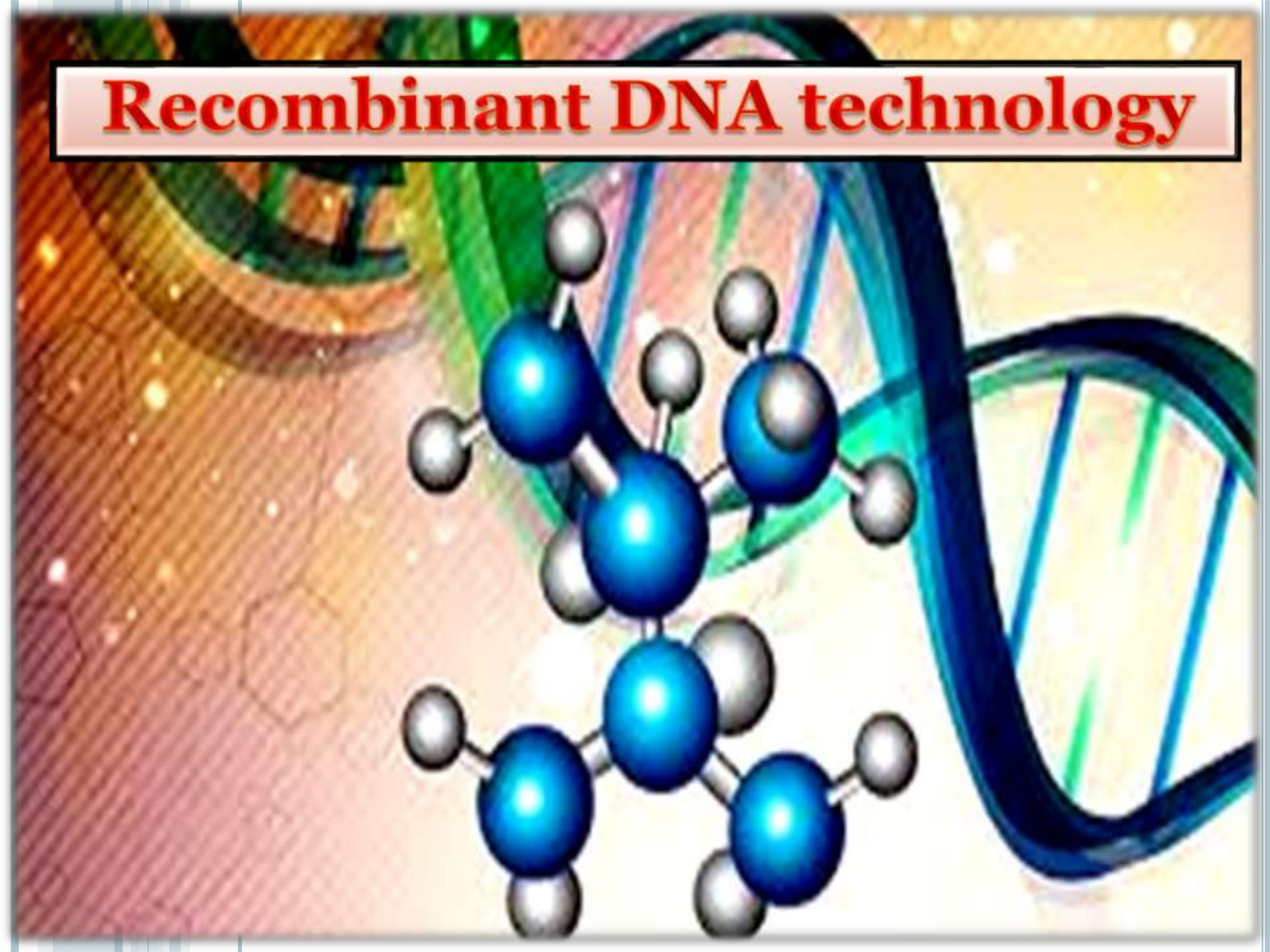
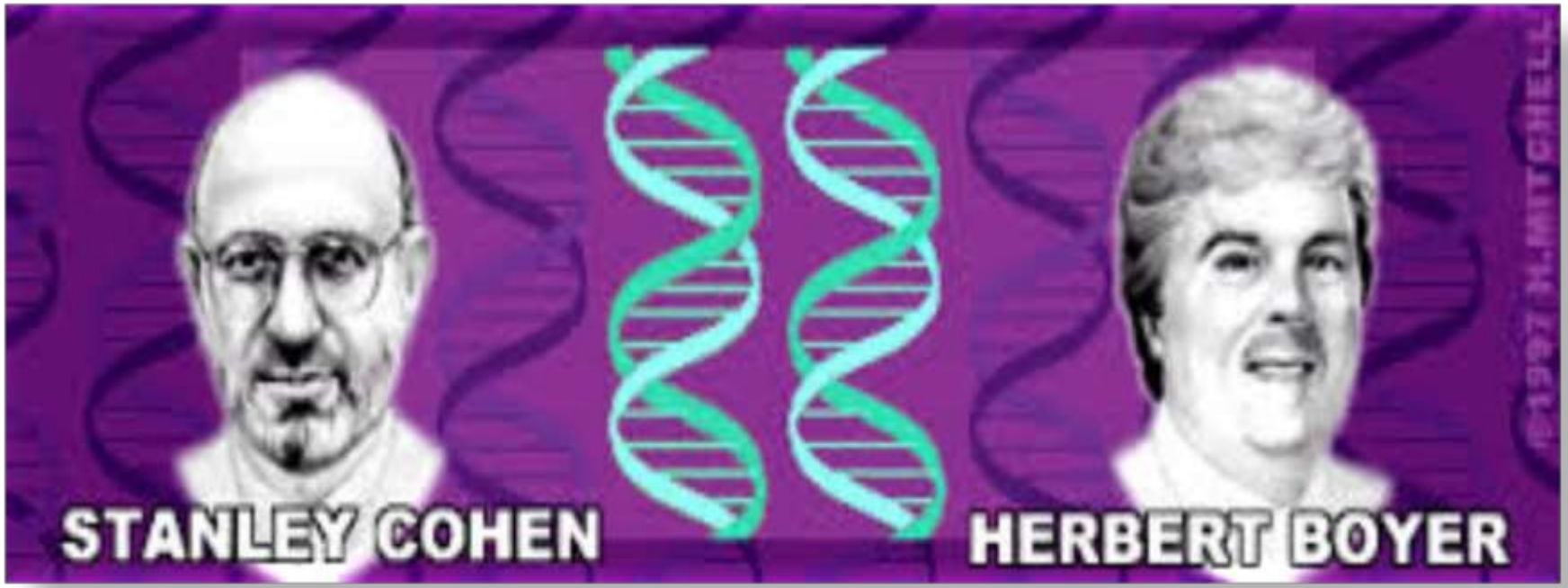
Recombinant DNA technology



HISTORY



Herbert Boyer (1936-) and Stanley N. Cohen (1935-) dev
recombinant DNA technology, showing that genetically
engineered DNA molecules may be cloned in foreign cel

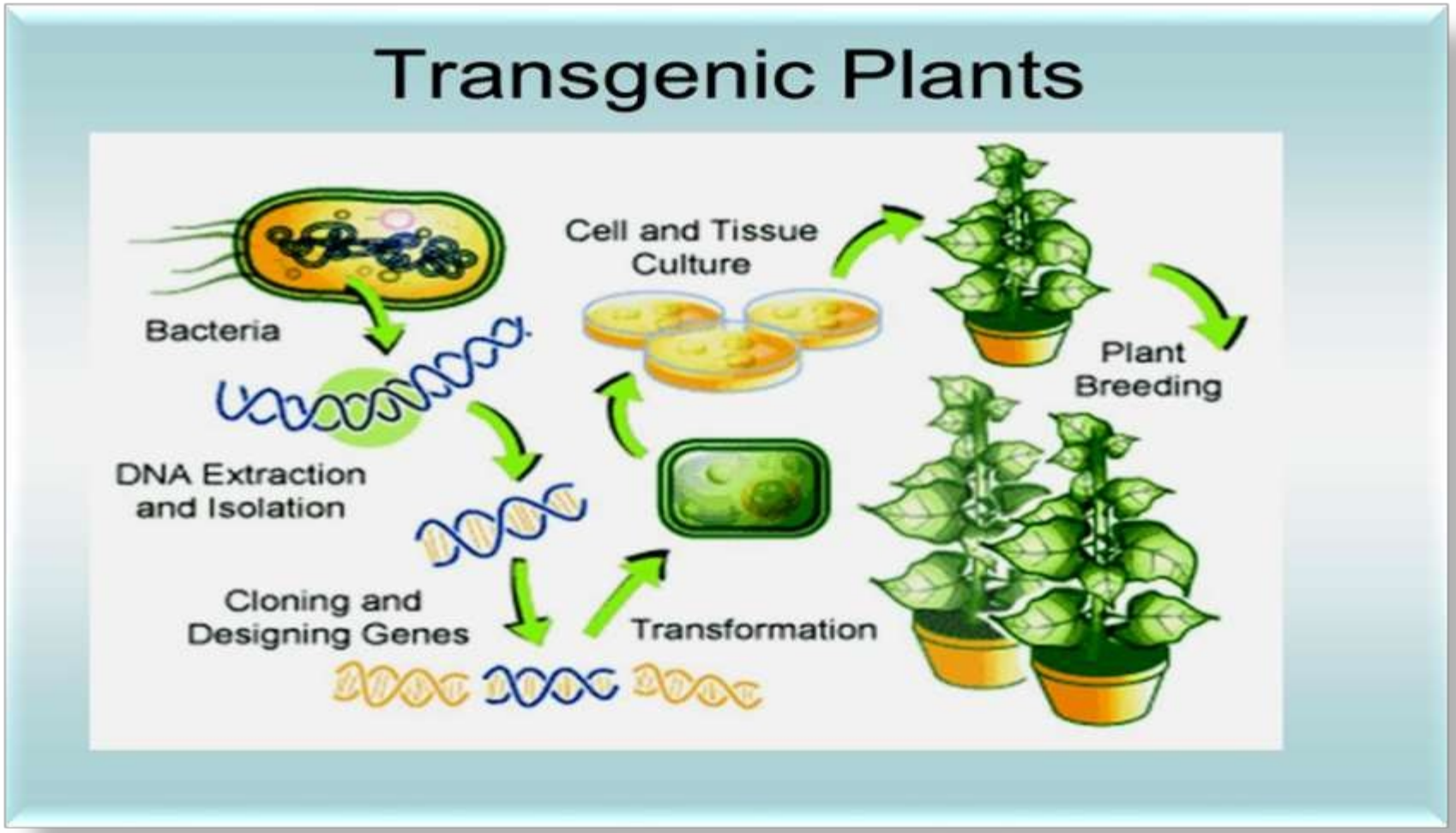
APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY

AGRICULTURE

INDUSTRIES

MEDICAL

APPLICATIONS IN CROP IMPROVEMENT



Resistance to diseases, insects and pests, herbicides, drought,metal toxicity tolerance…

DEVELOPMENT OF ROOT NODULES IN CEREAL CROPS

Leguminous plants have root-nodules which contain nitrogen fixing bacteria Rhizobium

The bacterial genes responsible for this nitrogen fixation can be transferred now to cereal crops like wheat, rice, maize, barley etc.

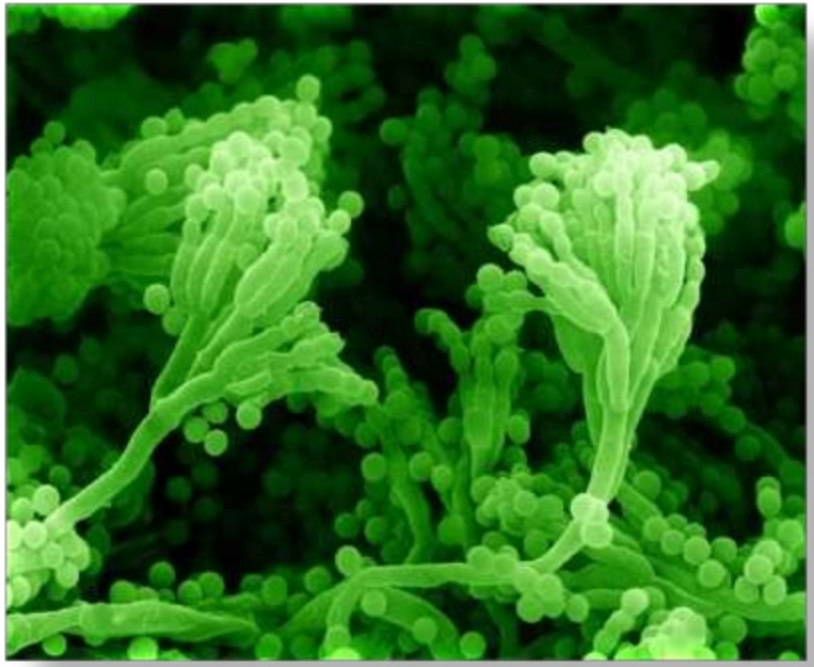
DEVELOPMENT OF C_4 PLANTS:

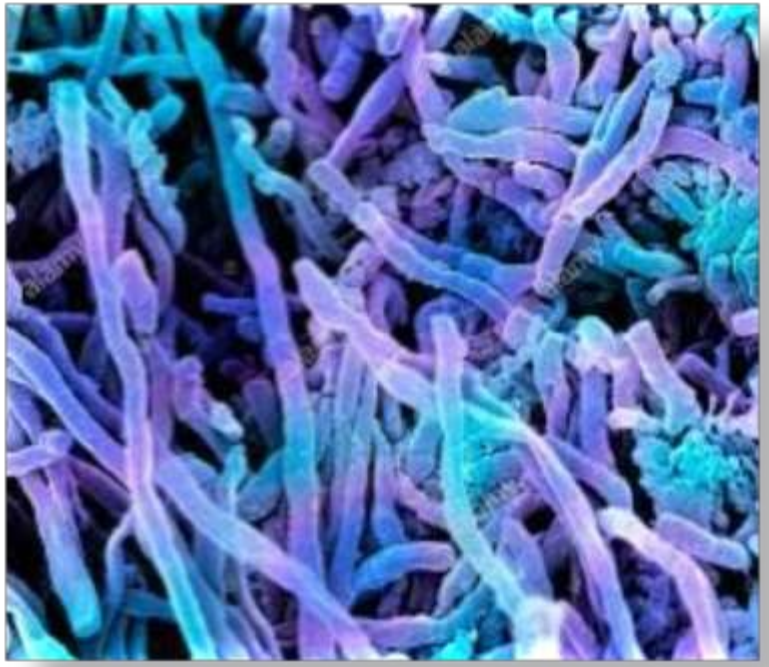
Improvement in yield can be achieved by improving the photosynthetic efficiency of crop plants.

The photosynthetic rate can be increased by conversion of C_3 plants into C_4 plants

Applications in Medicines

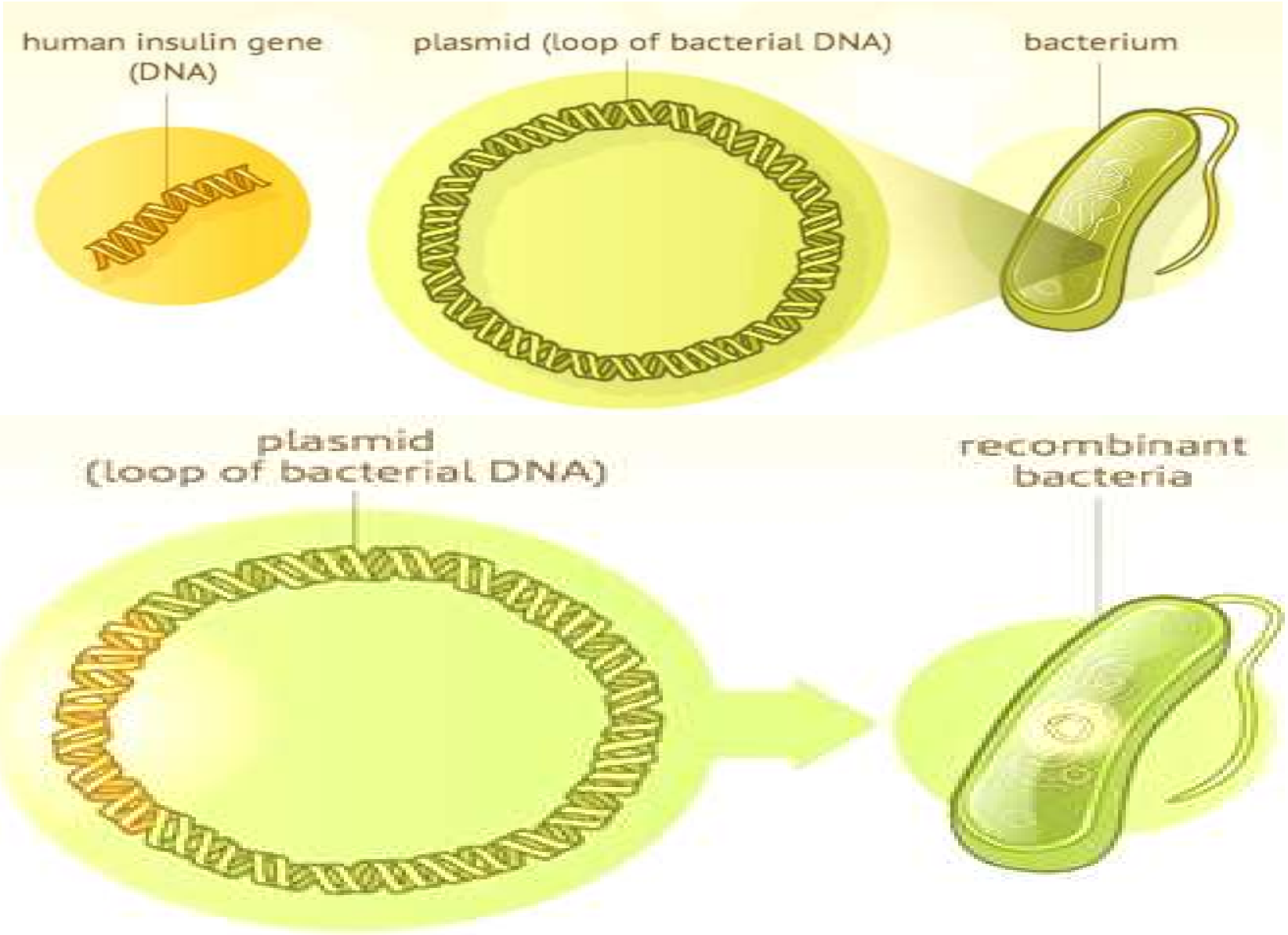
PRODUCTION OF ANTIBIOTICS:



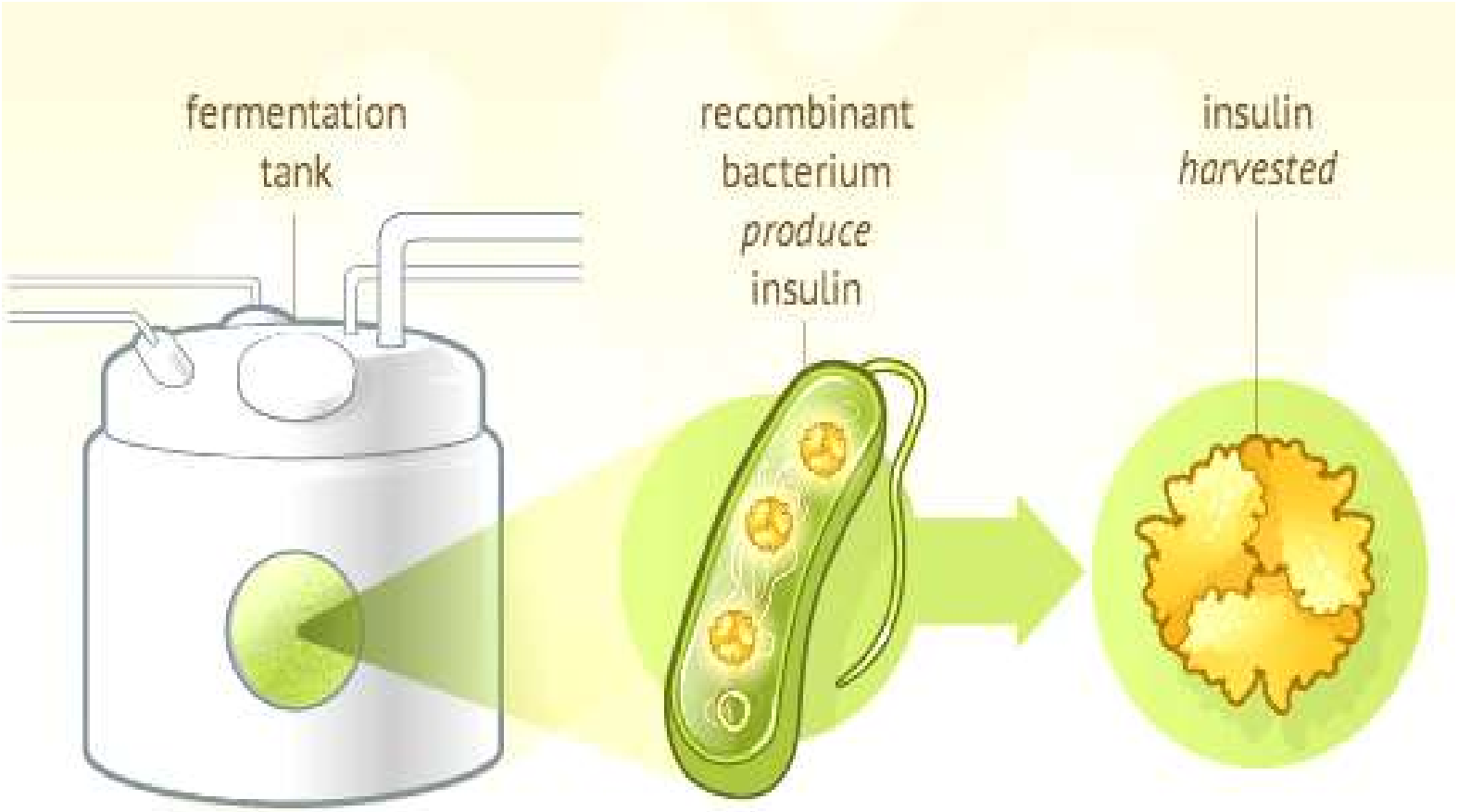


Penicillium and Streptomyces fungi are used for mass production of famou
antibiotics penicillin and streptomycin. Genetically efficient strains of thes
fungi have been developed to greatly increase the yield of these antibiotic

PRODUCTION OF HORMONE INSULIN:

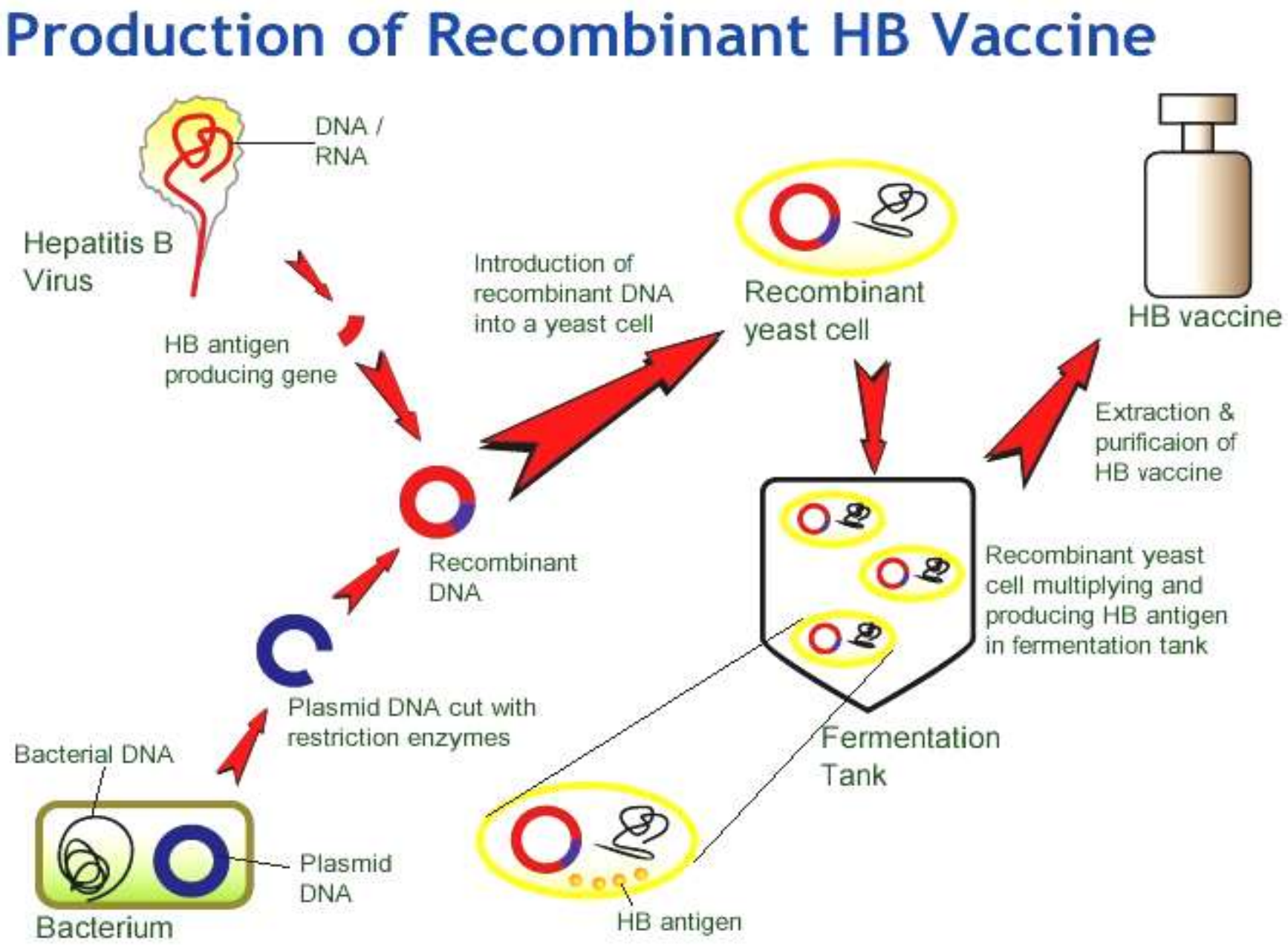


PRODUCTION OF HORMONE INSULIN:



Human gene for insulin production has been incorporated into bacteria DNA and such genetically engineered bacteria are used for large scale production of insulin. This insulin does not cause allergy .

PRODUCTION OF VACCINES



PRODUCTION OF INTERFERONS

- **Interferon** was named for its ability to interfere with viral proliferation.
- Interferon's are virus-induced proteins produced by virus-infected cells.
- Interferon are antiviral in action and act as first line of defense against viruses causing serious infections, including breast cancer and lymph nodes malignancy.
- Natural interferon is produced in very small quality from human blood cells.
- It is very costly.
- It is now possible to produce interferon by recombinant DNA technology at much cheaper rate.

INTERFERONS USED FOR HCV INFECTION

PRODUCTION OF ENZYMES

Used to dissolve blood clots

GENE THERAPY

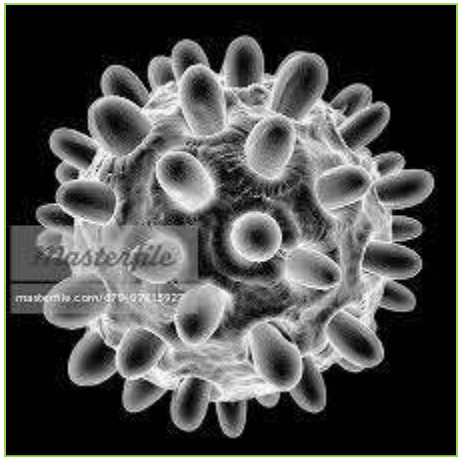
PKU

Genetic engineering may one day enable the medical scientists to replace the defective genes responsible for hereditary diseases (e.g., haemophilia, phenylketonuria, alkaptonuria) with normal genes. This new system of therapy is called gene therapy.

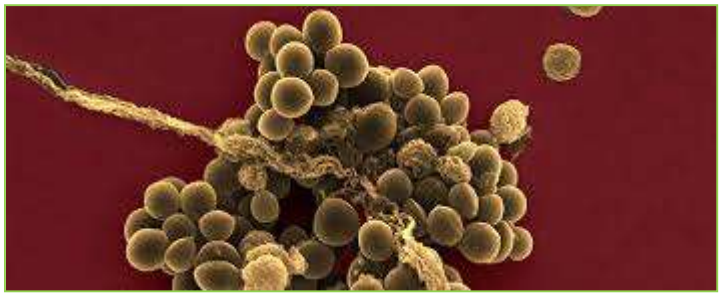
SOLUTION FOR DISPUTED PARENTAGE

DIAGNOSIS OF DISEASES

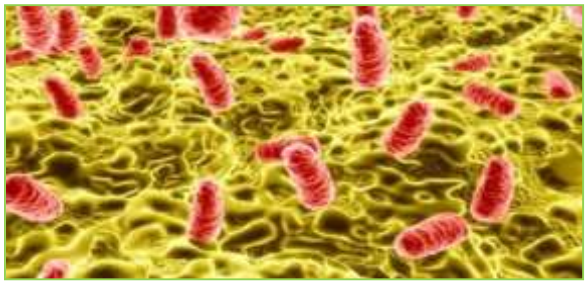
- Recombinant DNA technology has provided a broad range of tools to help physicians in the diagnosis of diseases.
- food poisoning Salmonella
- Pus forming Staphylococcus
- hepatitis virus
- HIV.



HBV

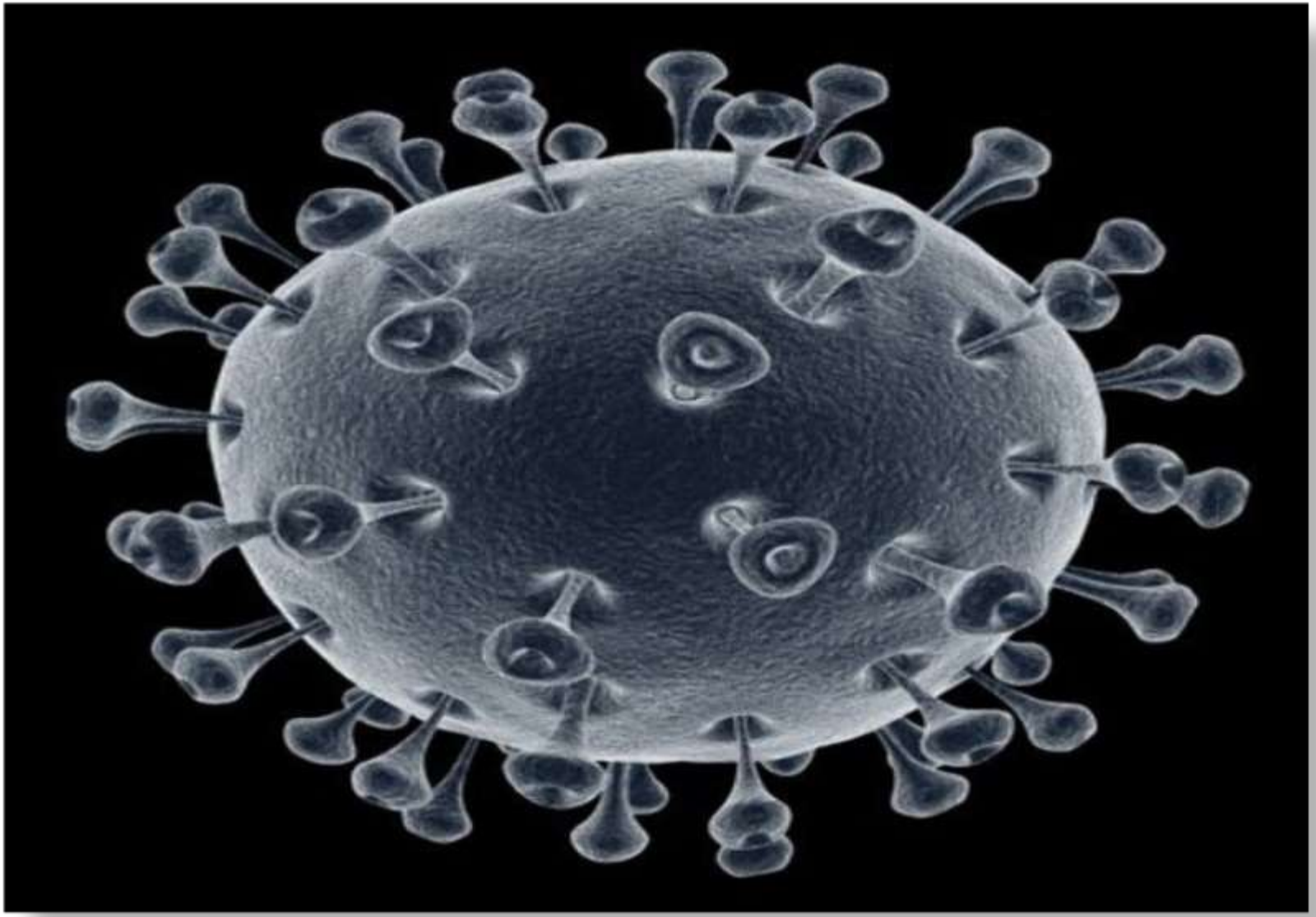


staphylococci

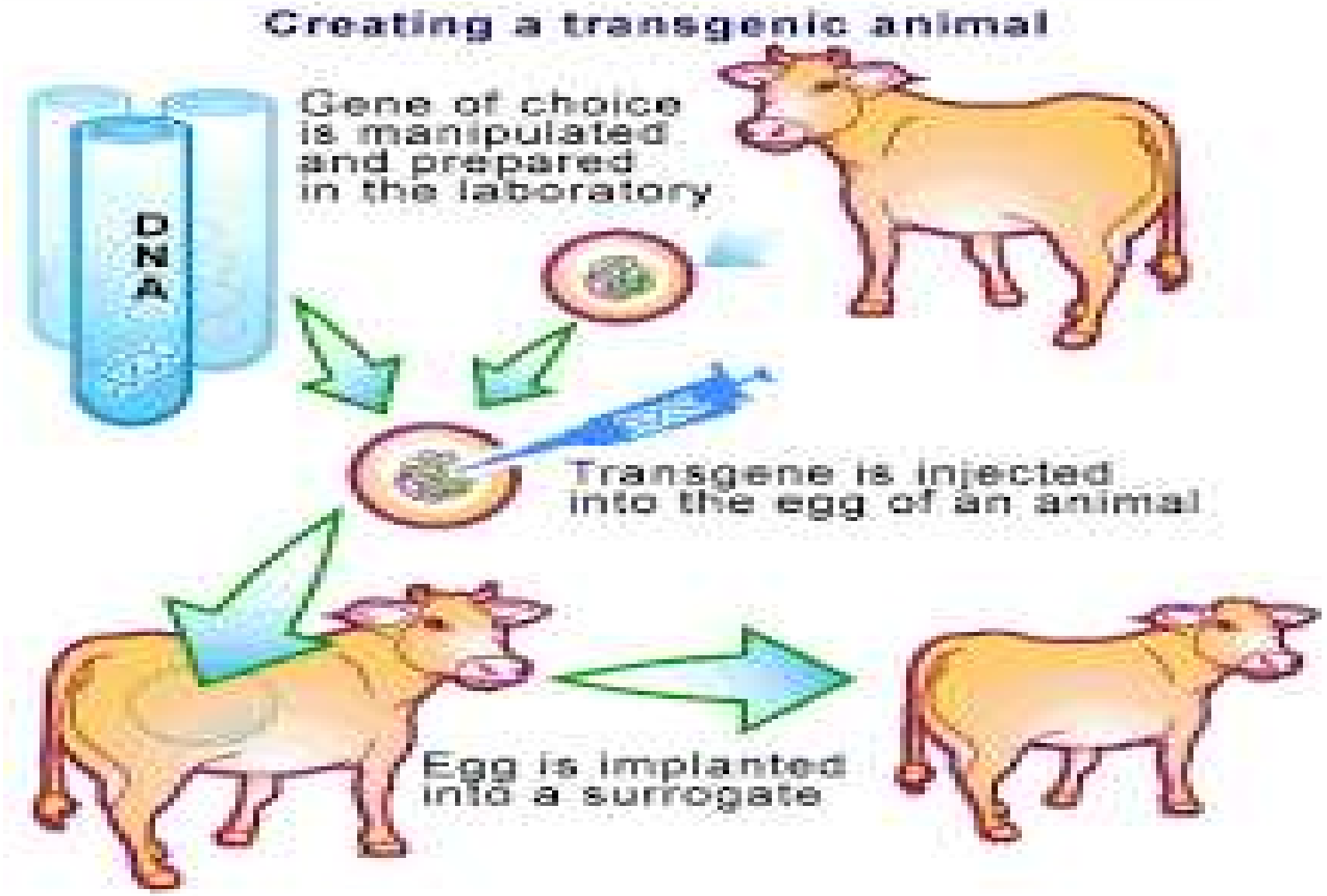


salmonella

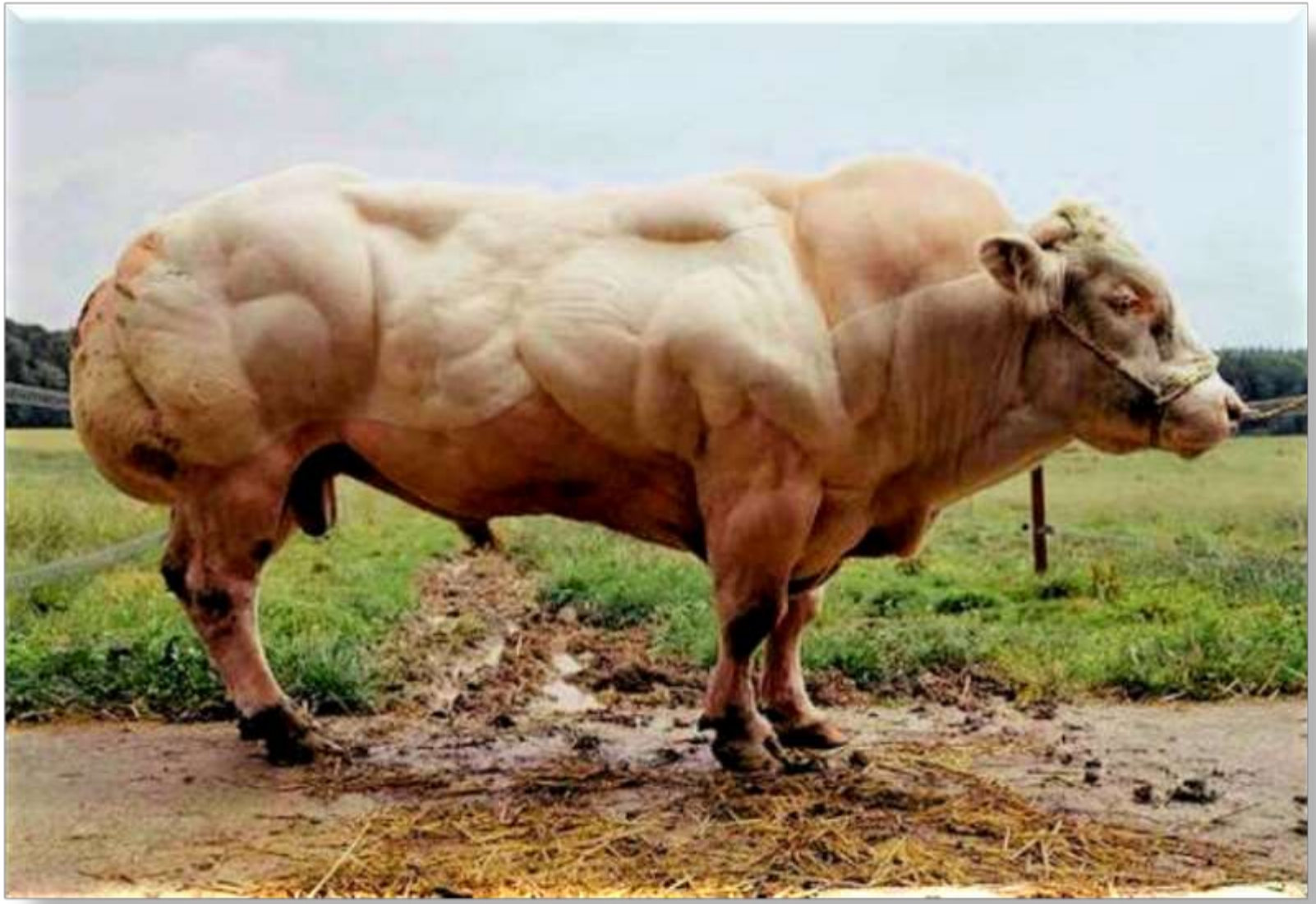
HIV UNDER ELECTRON MICROSCOPE



TRANSGENIC ANIMALS



TRANSGENIC ANIMAL- FOR MEAT



TRANSGENIC-CHICKEN

DOLION - TRANSGENIC ANIMAL

LOZEBRA – TRANSGENIC ANIMAL


Industrial
application

- In industries, recombinant DNA technique will help in the production of chemical compounds of commercial importance.
- Improvement of existing fermentation processes and production of proteins from wastes.
- This can be achieved by developing more efficient strains of microorganisms.
- Specially developed microorganisms may be used even to clean up the pollutants.
- Biotechnology, especially recombinant DNA technology has many useful applications in crop improvement, medicines and industry.

Basic principles- recombinant DNA technology

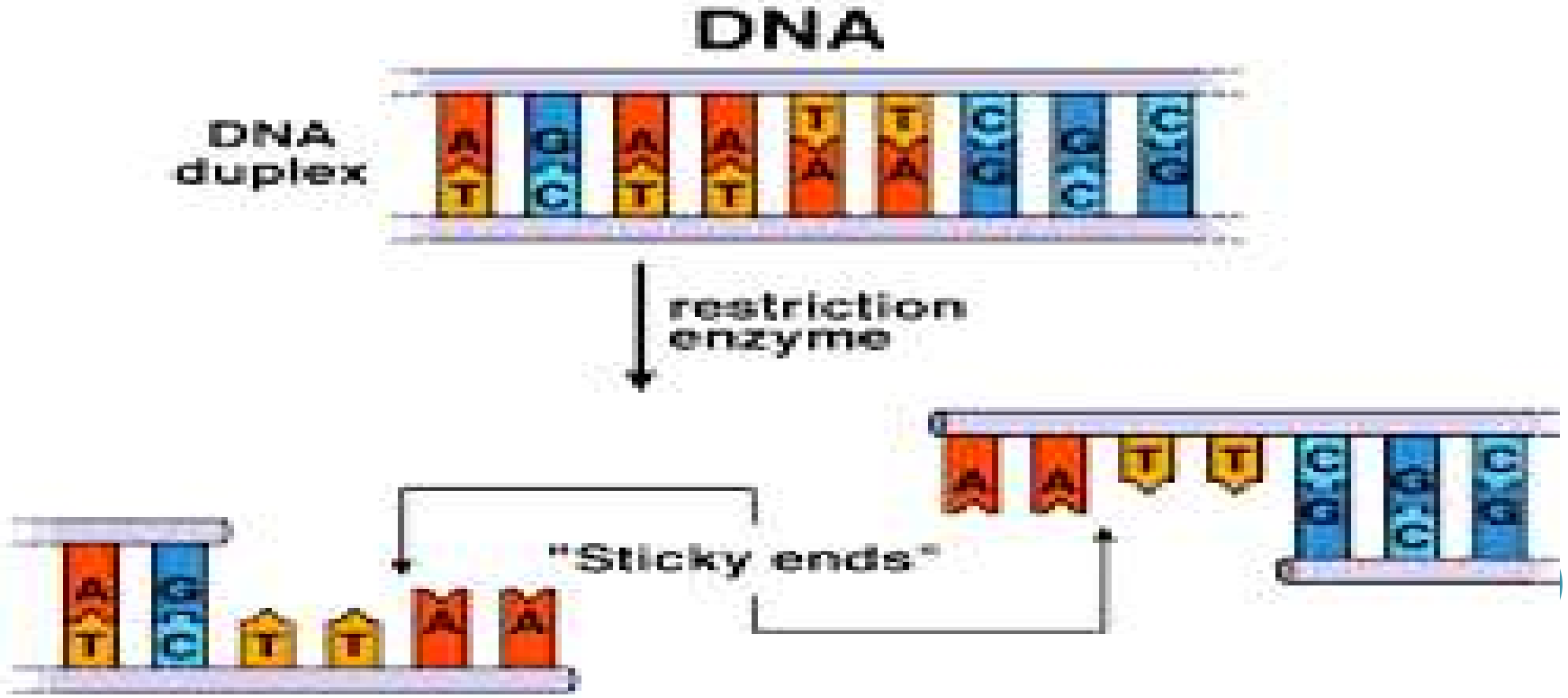
- Generation of DNA fragments and selection o the desired piece of DNA .
- Insertion of the selected DNA into a cloning vector (e.g. a plasmid) to create a recombina DNA or chimeric DNA
- Introduction of the recombinant vectors into host cells (e.g. bacteria).
- Multiplication and selection of clones containing the recombinant molecules.
- Expression of the gene to produce the desired product.

Molecular tools of genetic engineering

- Enzymes most commonly used in recombinant DNA experiment are molecular tools.

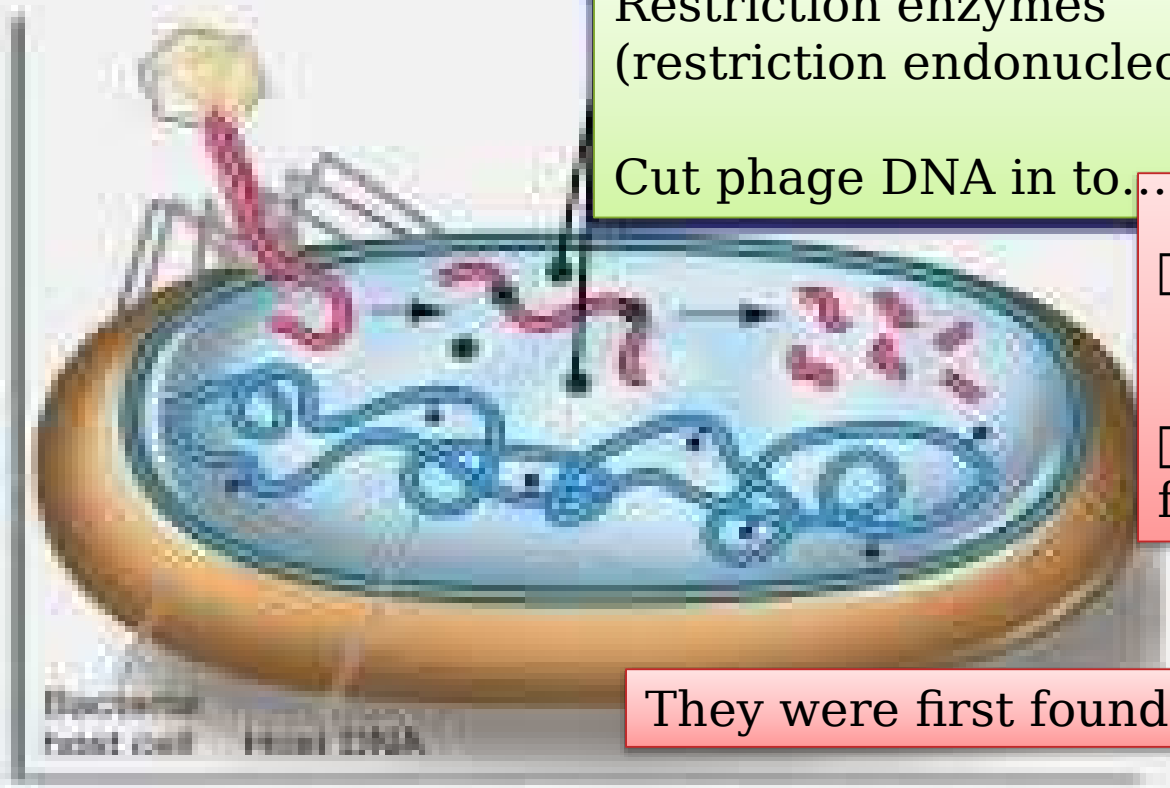
✓ **RESTRICTION ENDONUCLEASES -**

(DNA CUTTING ENZYMES.)



THIS IS HOW THEY GOT NAME....

Some bacteria defend themselves from attack by bacteriophages by producing :



Restriction enzymes
(restriction endonucleoses)

Cut phage DNA in to.....

- smaller
- NonInfectious fragment

They were first found in E.COLI

Restriction endonucleases

Over 3000 restriction enzymes have been studied in detail, and more than 600 of these are available commercially.

Restriction endonuclease	source	Type ends formed
EcoRI	Escherichia coli	Forms sticky ends
BamHI	Bacillus amyloliquifaciens	Forms sticky ends
HaeIII	Hemophyllus aegypticus	Forms blunt ends
HindIII	Hemophyllus influenza	Forms sticky ends
NotI	Nocardia otitidis	Forms sticky ends

Nomenclature for restriction endonucleases

- EcoRI
  - Escherichia (E) (genus)
  - coli (co) (specific epithet)
  - strain Ry13 (R) (strain)
  - first endonuclease (1) (order of identification)

- HindIII
  - Haemophilus (H) (genus)
  - influenzae (in) (specific epithet)
  - strain Rd (d) (strain)
  - third endonucleases (III)(order of identification)

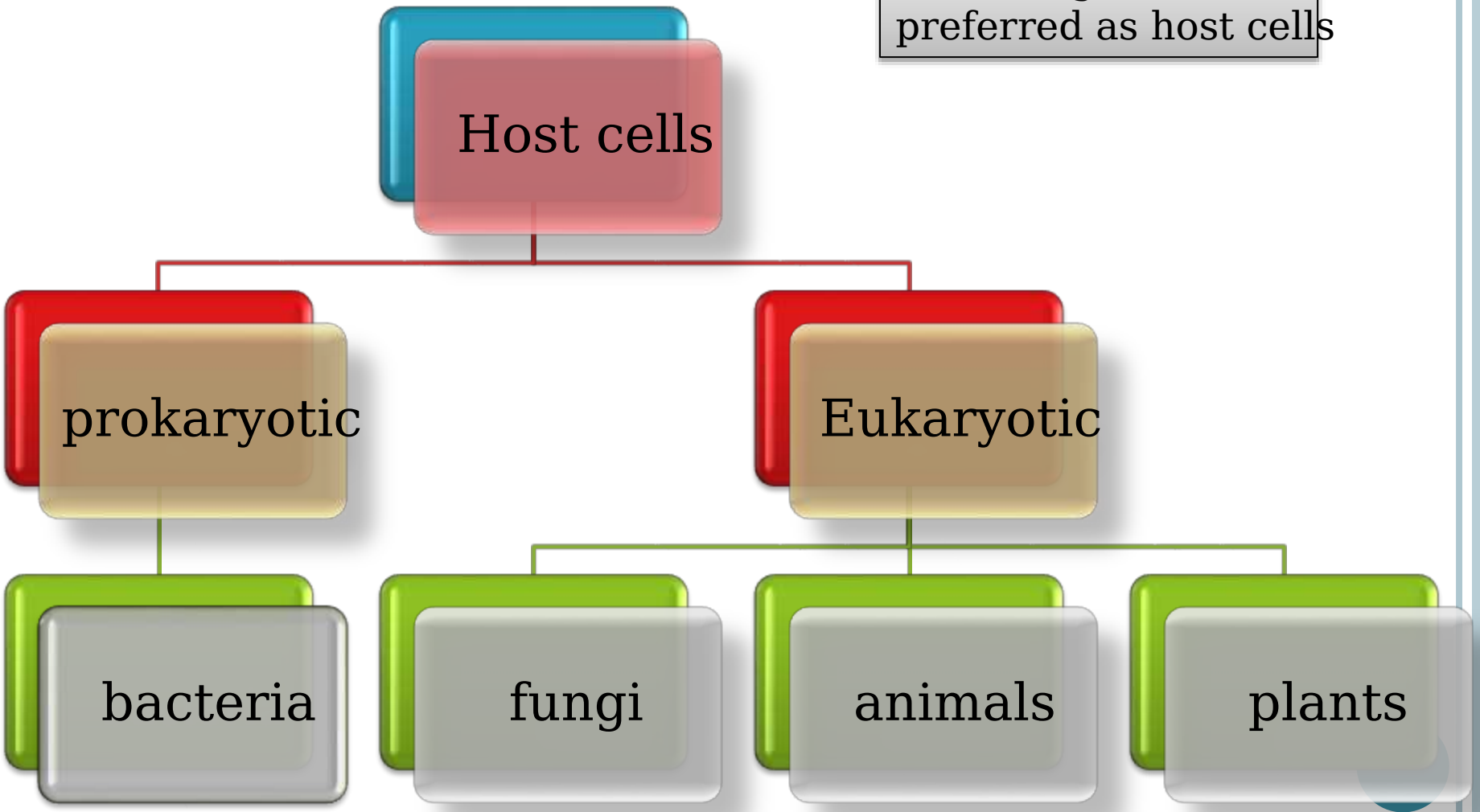
DNA ligases-DNA joining enzymes

- The cut DNA fragments are covalently joined together DNA ligase.
- These enzymes were originally isolated from viruses, E.coli and eukaryotic cells.
- DNA ligases actively participate in cellular DNA repa process.
- DNA Ligase joins (seals) the DNA fragments by formi phosphodiester bond between the phosphate group of carbon of one deoxyribose with the hydroxyl group of carbon of another deoxyribose.

HOST CELLS-THE FACTORIES OF CLONING

- The hosts are the living systems or cells in which the carrier of recombinant DNA molecule or vector can he propagated.

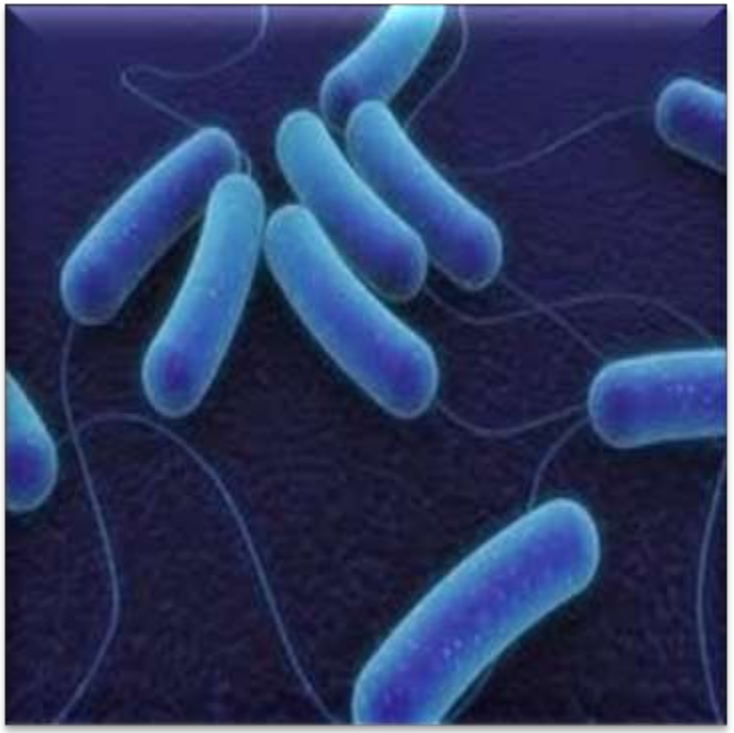
micro organisms are preferred as host cells



- The bacterium, Escherichia coli was the first organism in the DNA technology experiments and continues to be the host of choice.
- Major drawback – cannot perform post-translational modifications.
- Bacillus subtilis - non-pathogenic bacterium used as a in industry for the production of enzymes, antibiotics, insecticides etc.
- Bacillus subtilis considered as an alternative to E.coli.

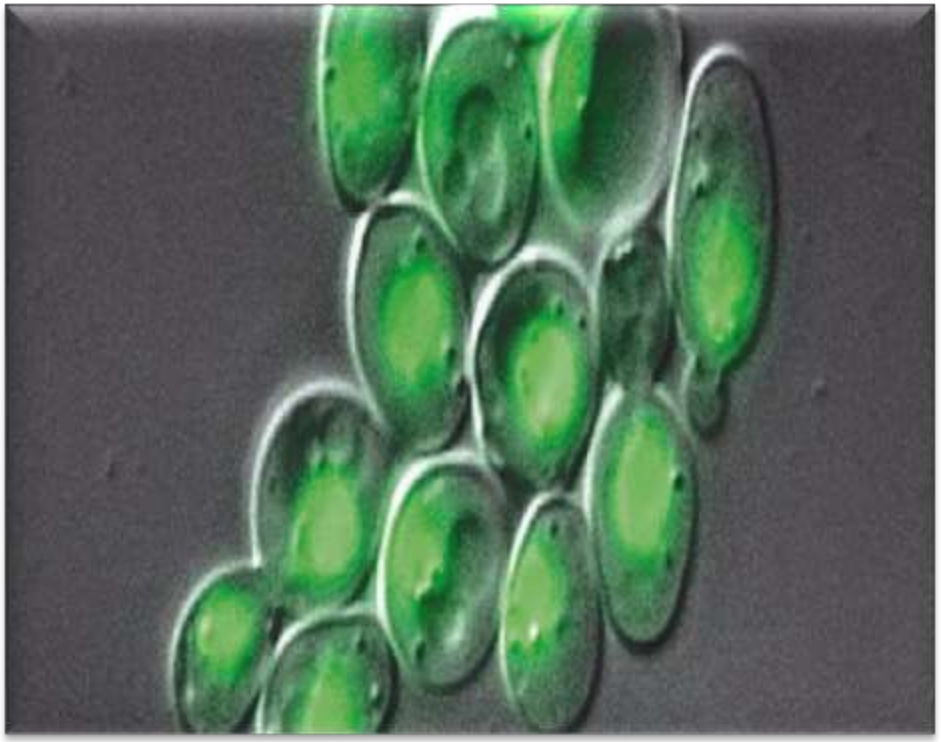
ESCHERICHIA COLI AND BACILLUS SUBTILIS

Escherichia coli

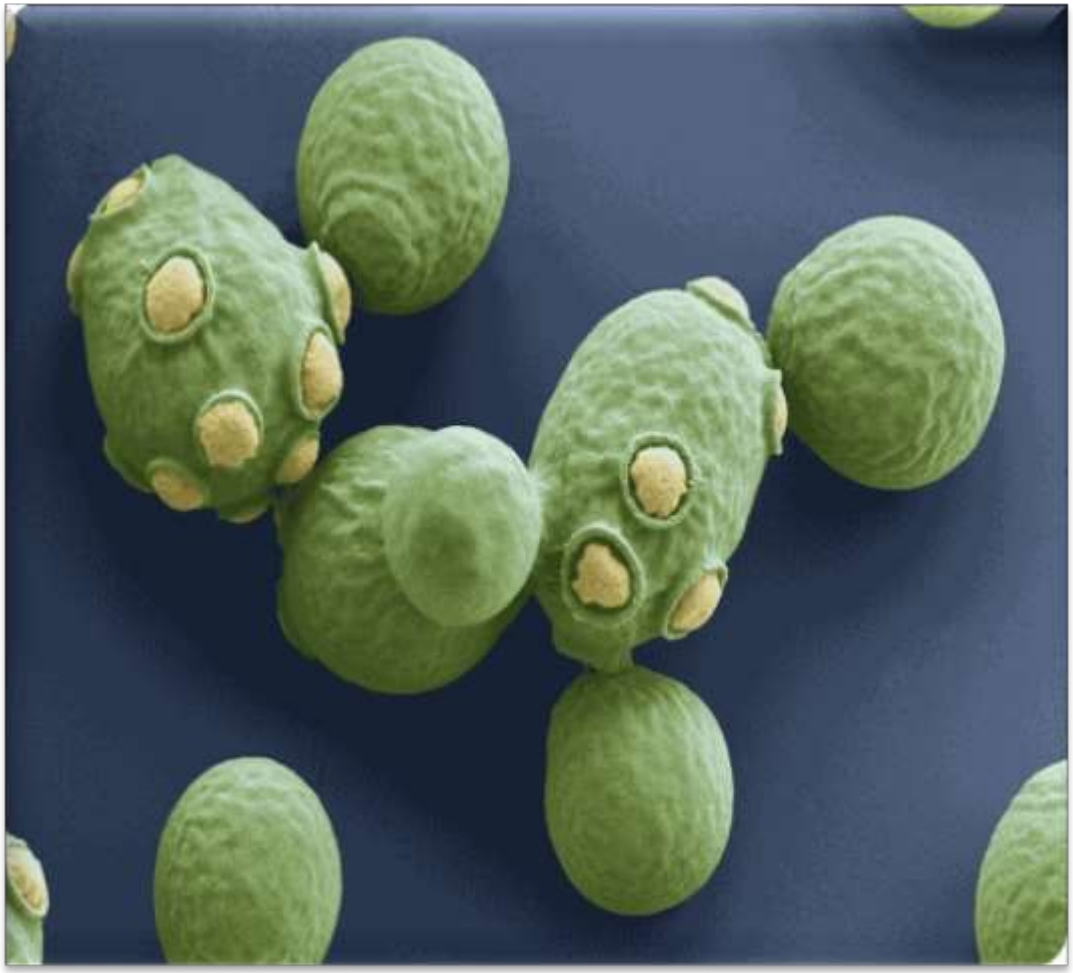


Bacillus subtilis

Eukaryotic hosts- yeast



SACCHAROMYCES CEREVISIAE



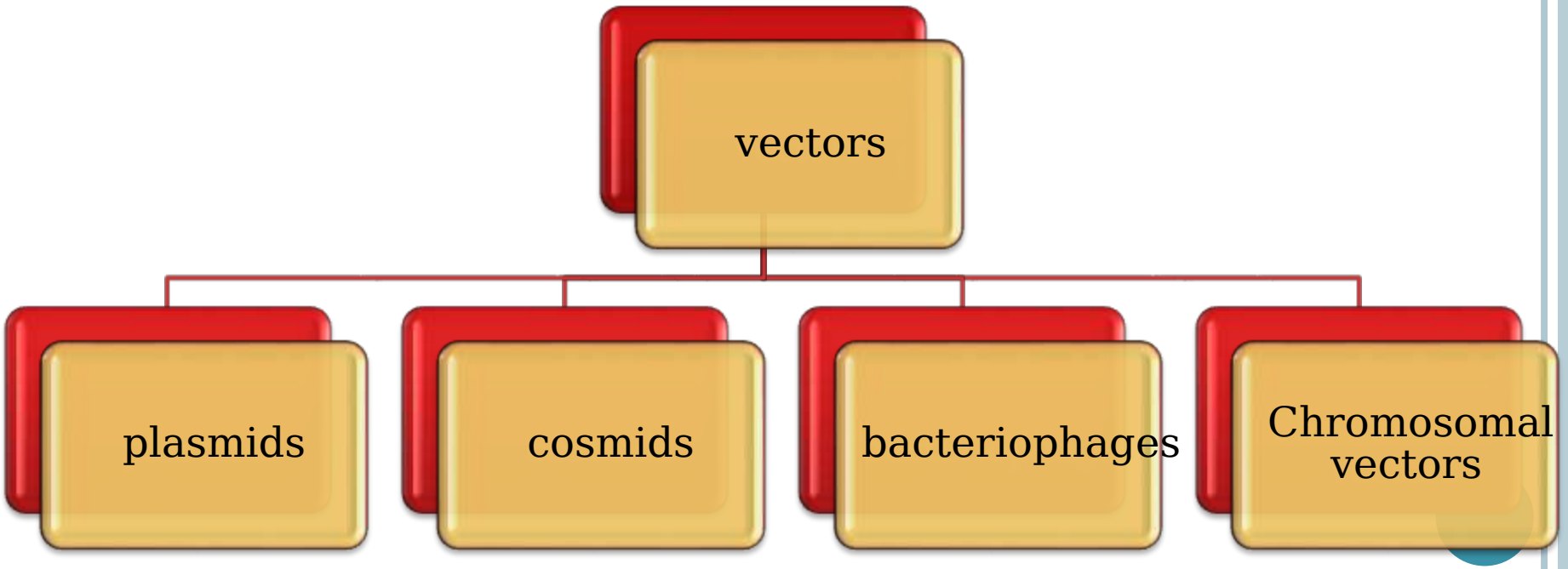
MAMMALIAN CELLS

Mammalian cells possess the machinery to modify the protein to the active form (post-translational modifications).

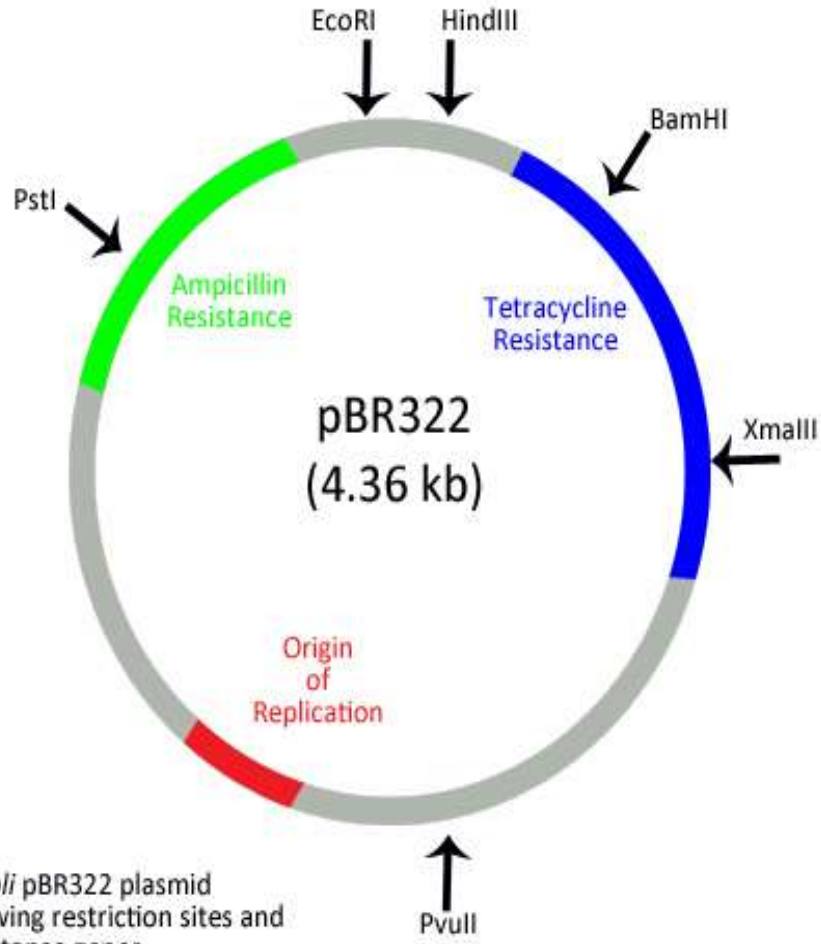
The advantage is that certain complex proteins which cannot be synthesized by bacteria can be produced by mammalian cells e.g. tissue plasminogen activating factor

vectors

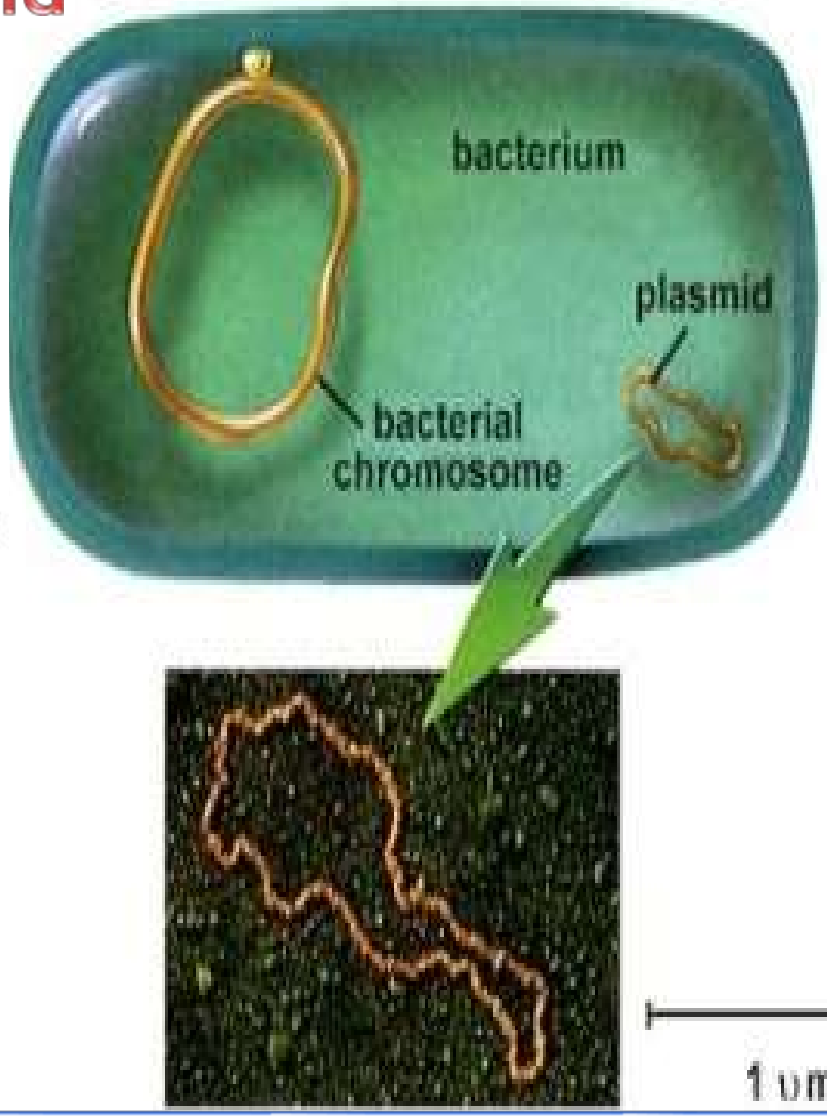
- Vectors are the DNA molecules, which can carry a foreign DNA fragment to be cloned.
- Self-replicating in an appropriate host cell.
- Most important vectors are ……..



Plasmid

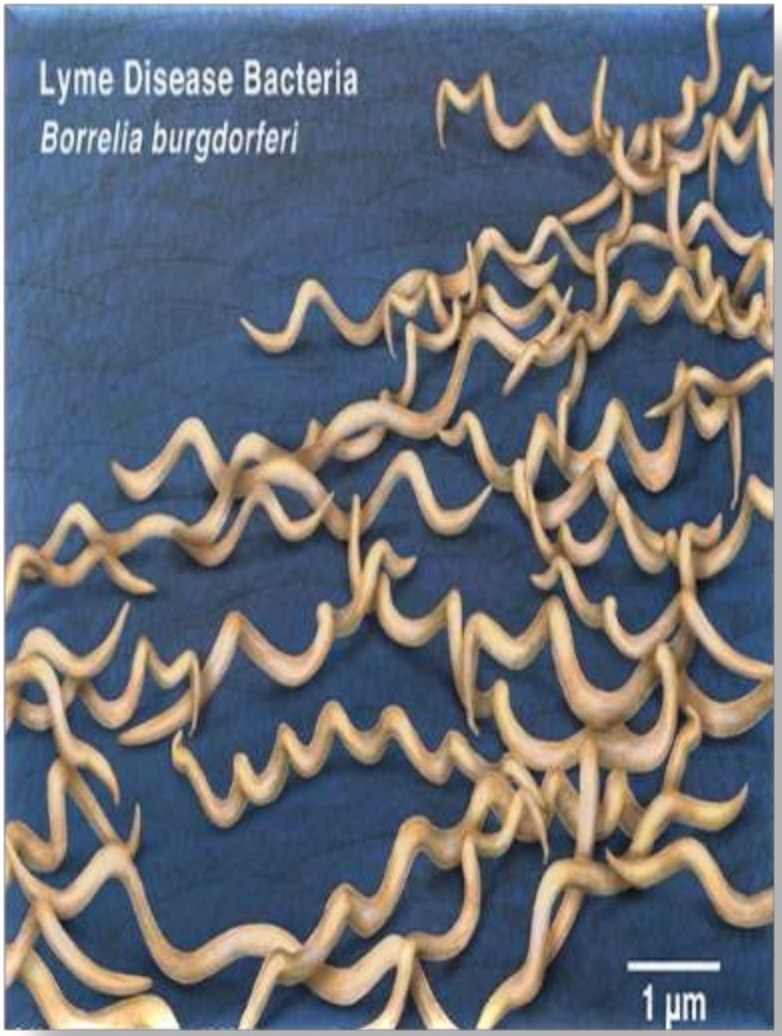


E. coli pBR322 plasmid showing restriction sites and resistance genes.

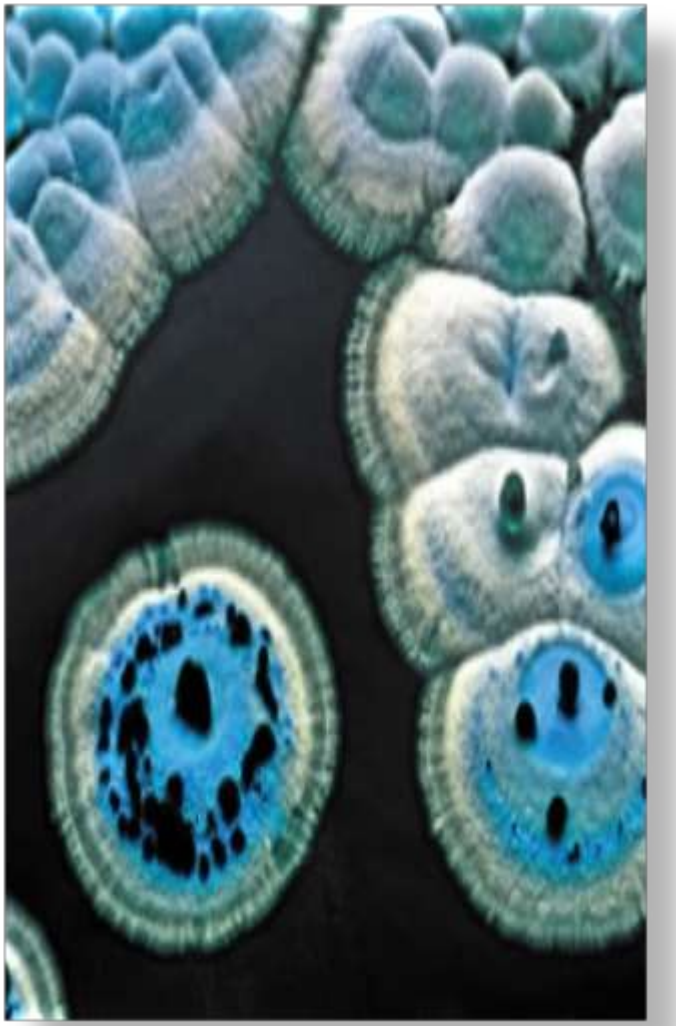


pBR322 ol E.coli is **the most popular and widely used plasmid vector** and is appropriately regarded as the **parent or grand parent of several other vectors. (others pBR325,pBR328 and pBR329)**

linier plasmid - bacteria

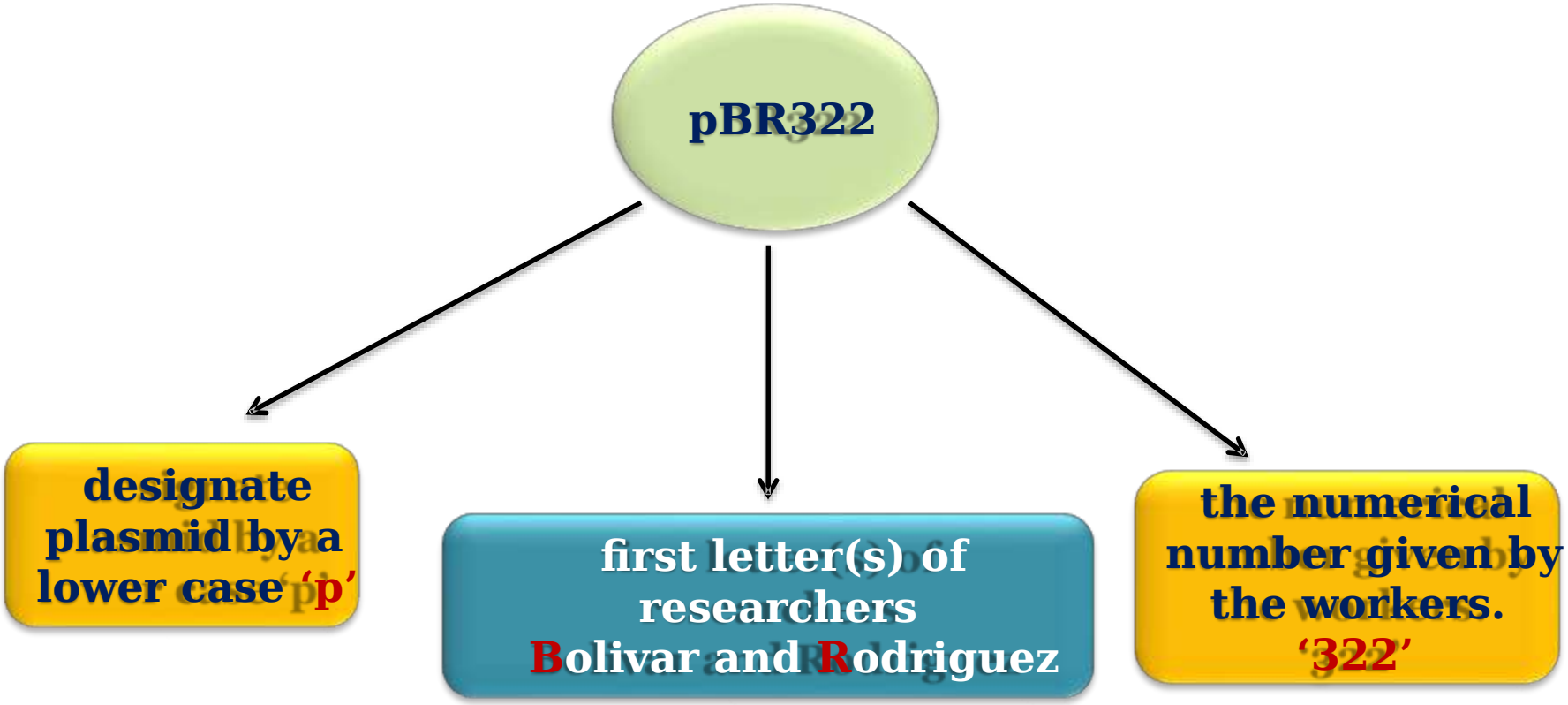


Borrelia burgdorferi



streptomyces

Nomenclature of plasmids



Some plasmids are given **names of the places** where they are discovered e.g.

(**pUC** is plasmid from **U**niversity of **C**alifornia.)

BACTERIOPHAGE

- Bacteriophages or simply phages are the viruses that rep within the bacteria
- Phages can take up larger DNA segments than plasmids

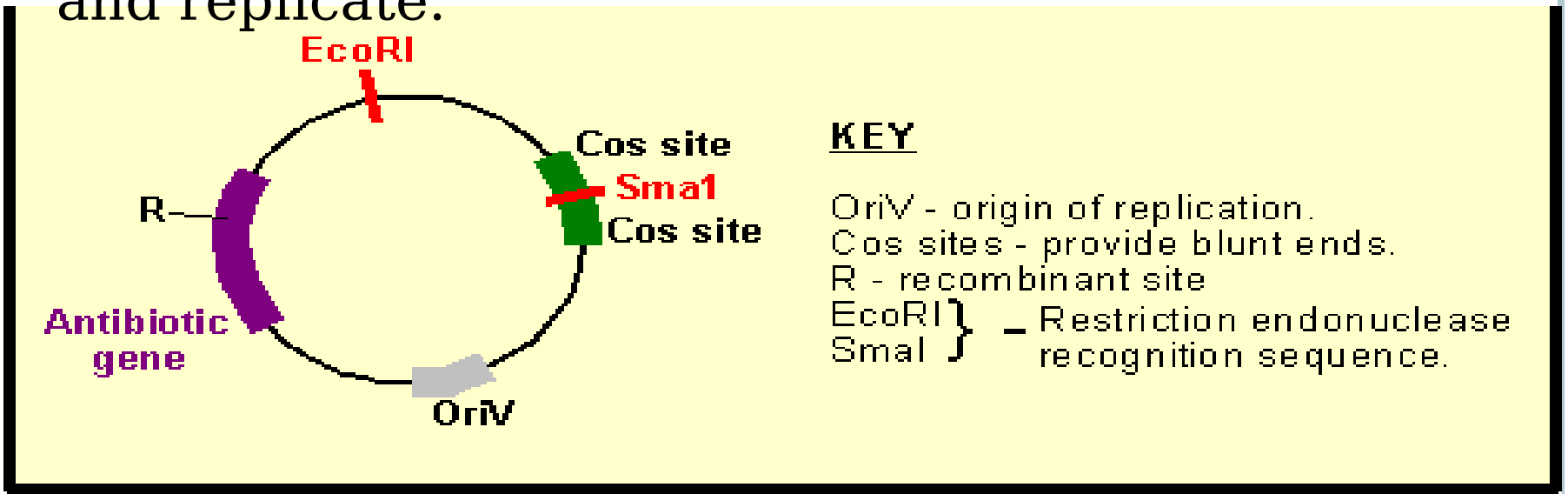
Most commonly used phages are bacteriophage λ (phage λ) and bacteriophage M13 (phage M13)

COSMIDS

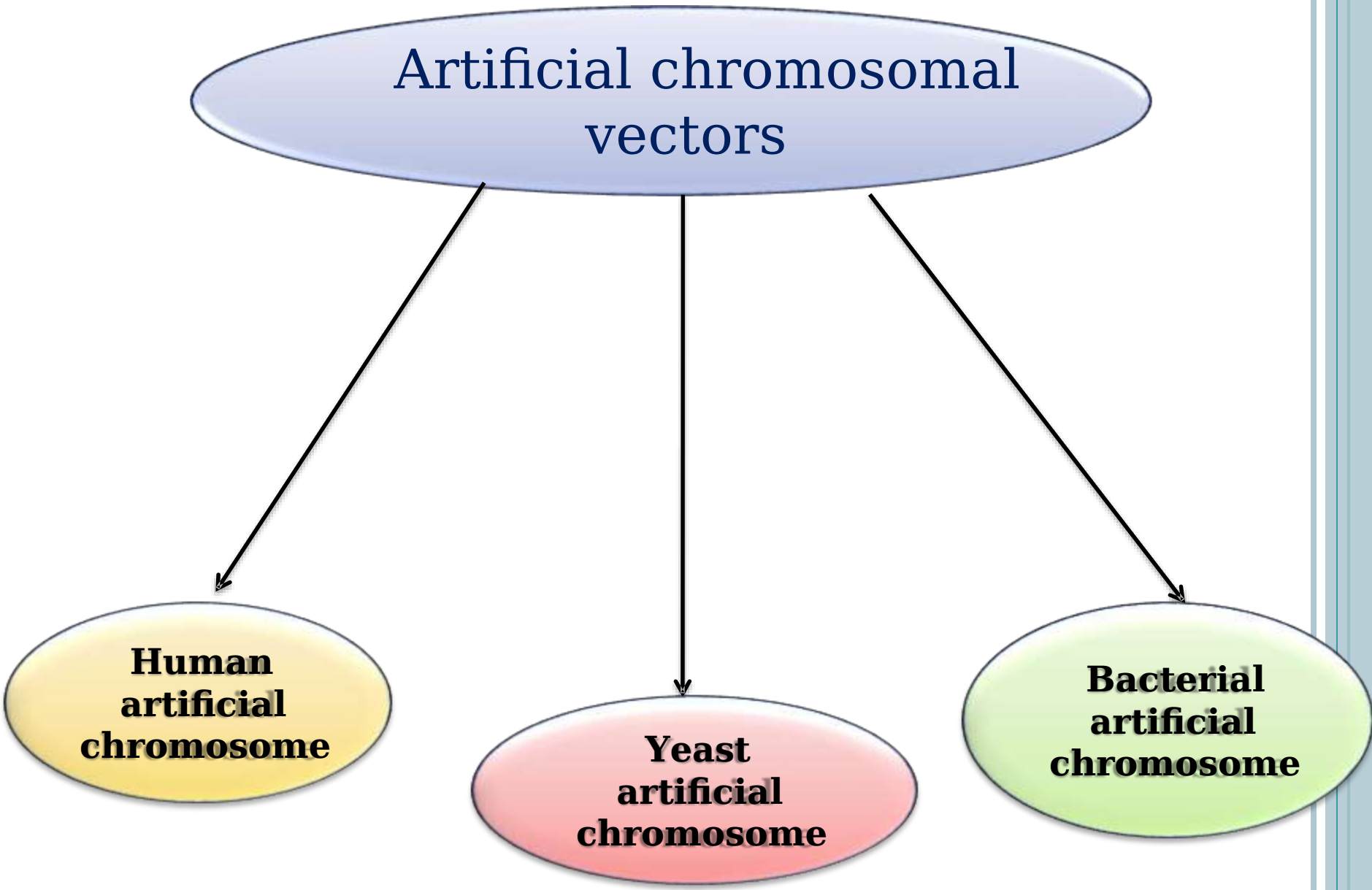
- Cosmids are the vectors having characteristics of both **plasmid and bacteriophage** .

Fragment of phage DNA including COS site + plasmid = cosmid

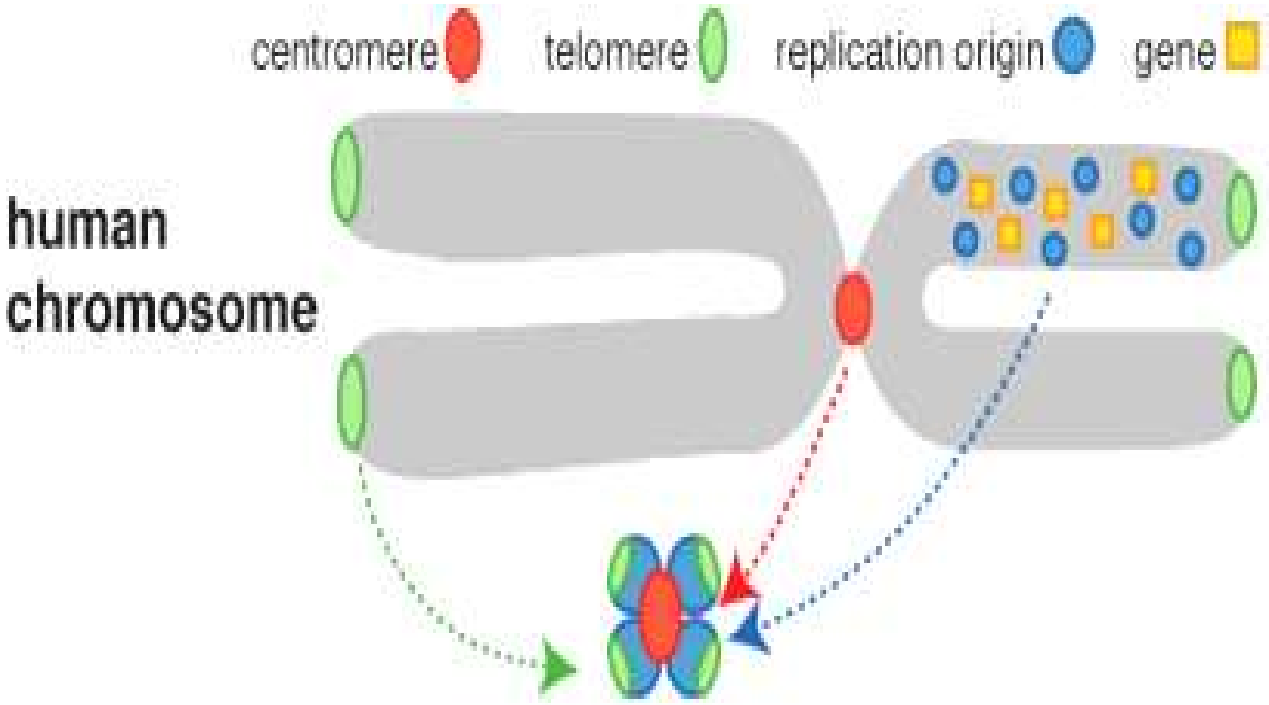
- Cosmids can carry larger fragments of foreign DNA tha plasmids.
- A foreign DNA of 40 kb can be inserted in to cosmids.
- Once inside the host cell, cosmids behave just like plasn and replicate.



Artificial chromosomal vectors



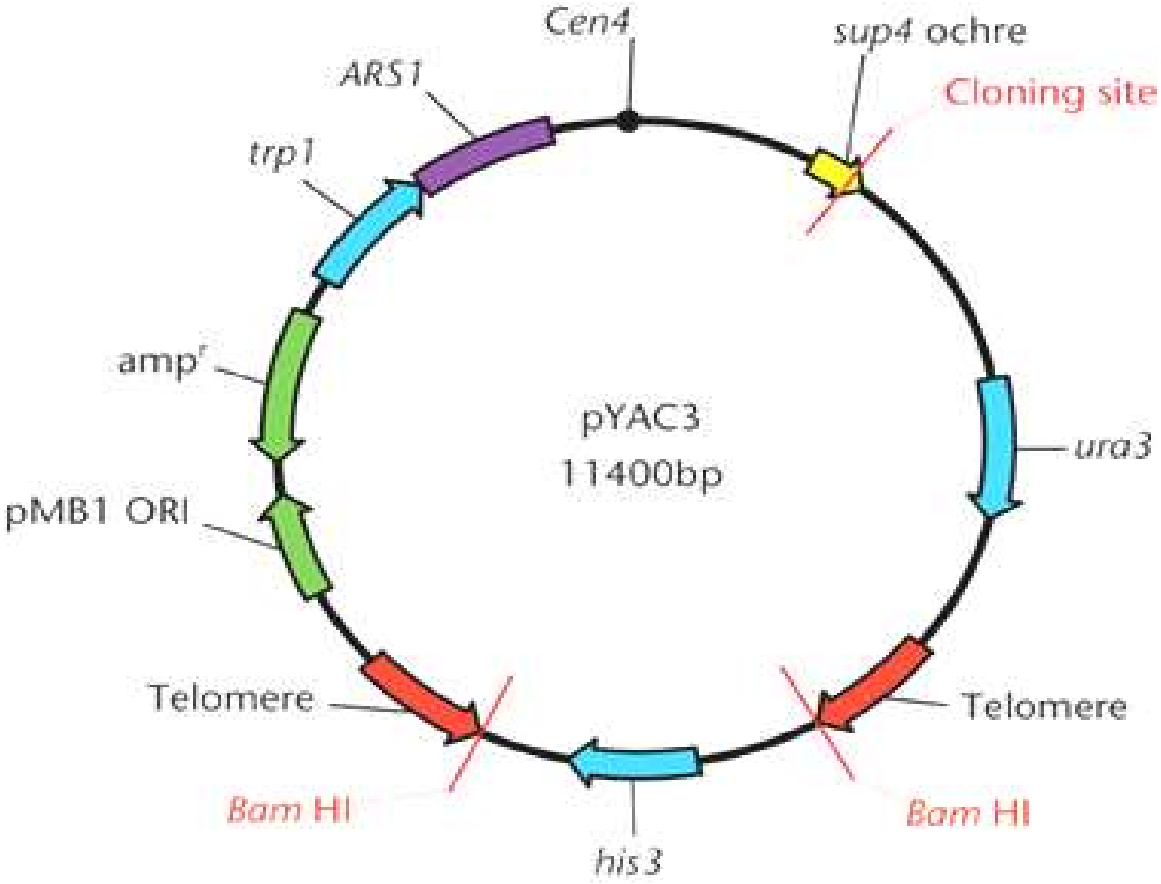
HUMAN ARTIFICIAL CHROMOSOME (HAC)



H. Willard 1997

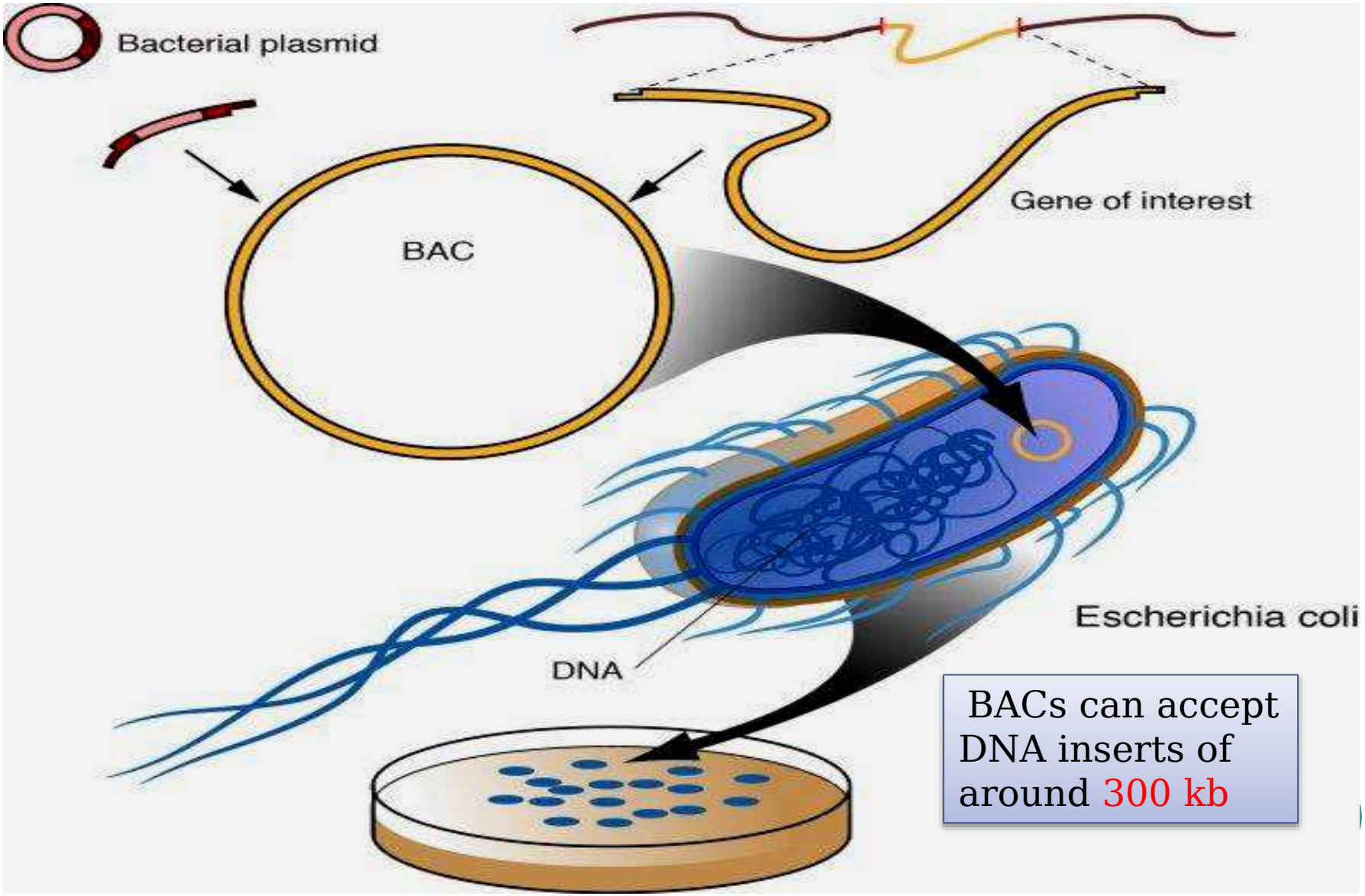
- **synthetically produced vector DNA, possessing the characteristics of human chromosome**
- **Advantage with HAC is that it can carry human genes that are too lon**
- **HAC can carry genes to be introduced into the cells in gene therapy.**

YEAST ARTIFICIAL CHROMOSOME



- Yeast artificial chromosome (YAC) is a synthetic DNA that can acce large fragments of foreign DNA (particularly human DNA).

BACTERIAL ARTIFICIAL CHROMOSOME



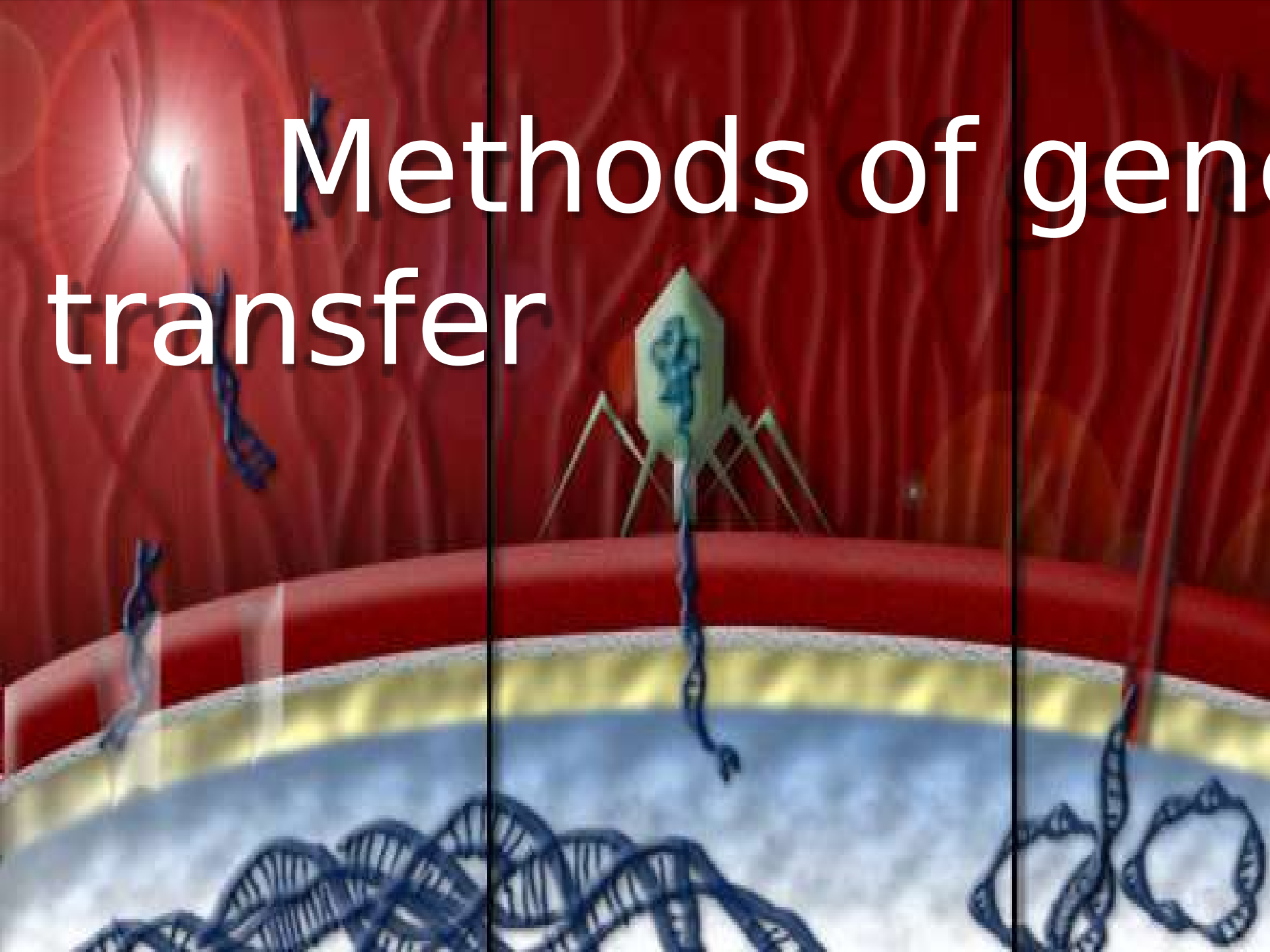
BACs can accept DNA inserts of around 300 kb

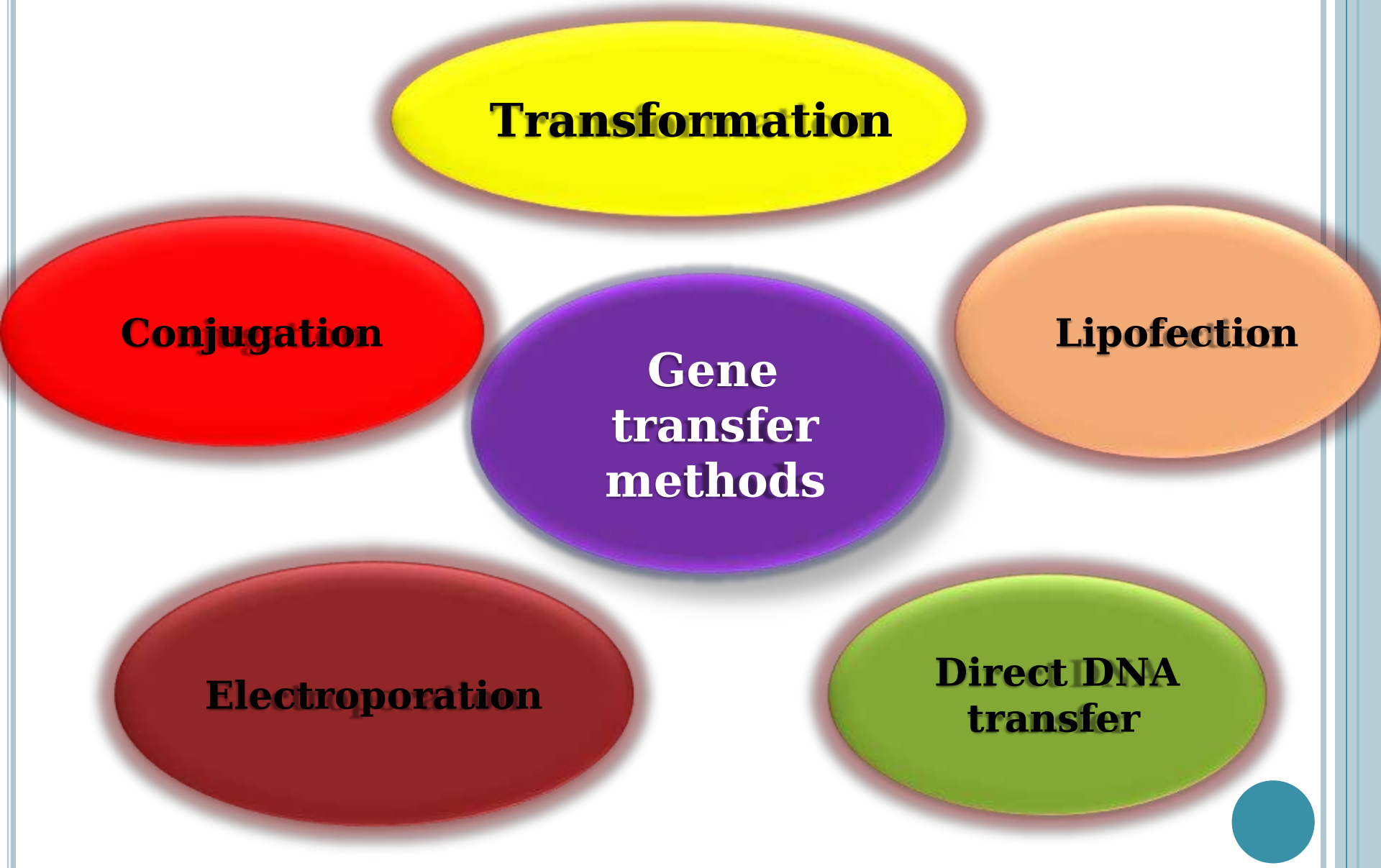
Choice of vector

- The size of the foreign DNA is very important in the choice of vect
- The efficiency of this process is often crucial for determining the success of cloning.

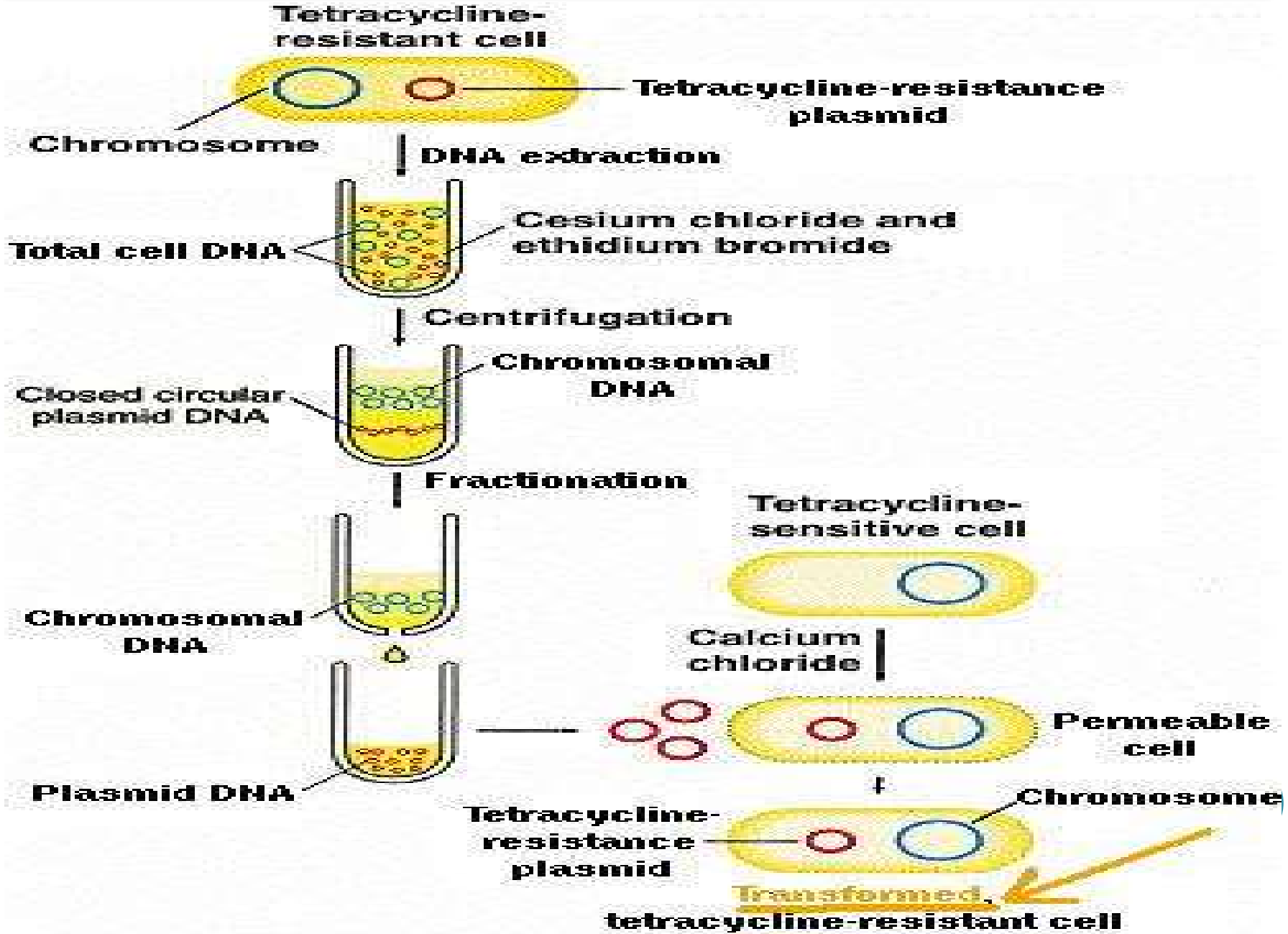
- Phage λ → E.Coli (5-25kb)
- Cosmid λ → E.Coli (35-45kb)
- Plasmid artificial chromosome (PAC) → E.Coli (100-300kb)
- Bacterial artificial chromosome (BAC) → E.Coli (100 -300kb)
- Yeast chromosome → **S. cerevisiae (200-2000kb)**

Methods of gene transfer

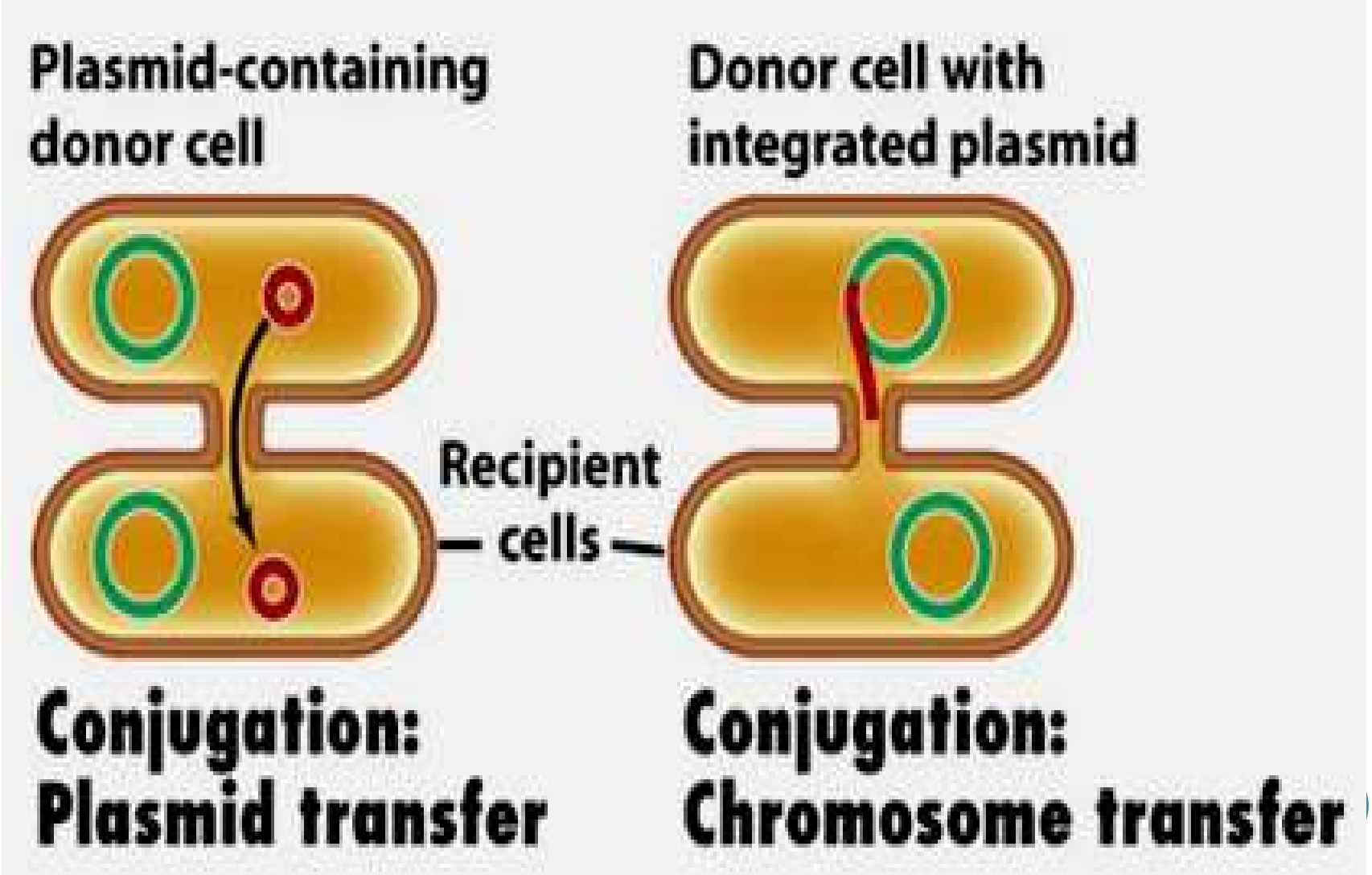



Transformation
Conjugation
Gene transfer methods
Lipofection
Electroporation
Direct DNA transfer

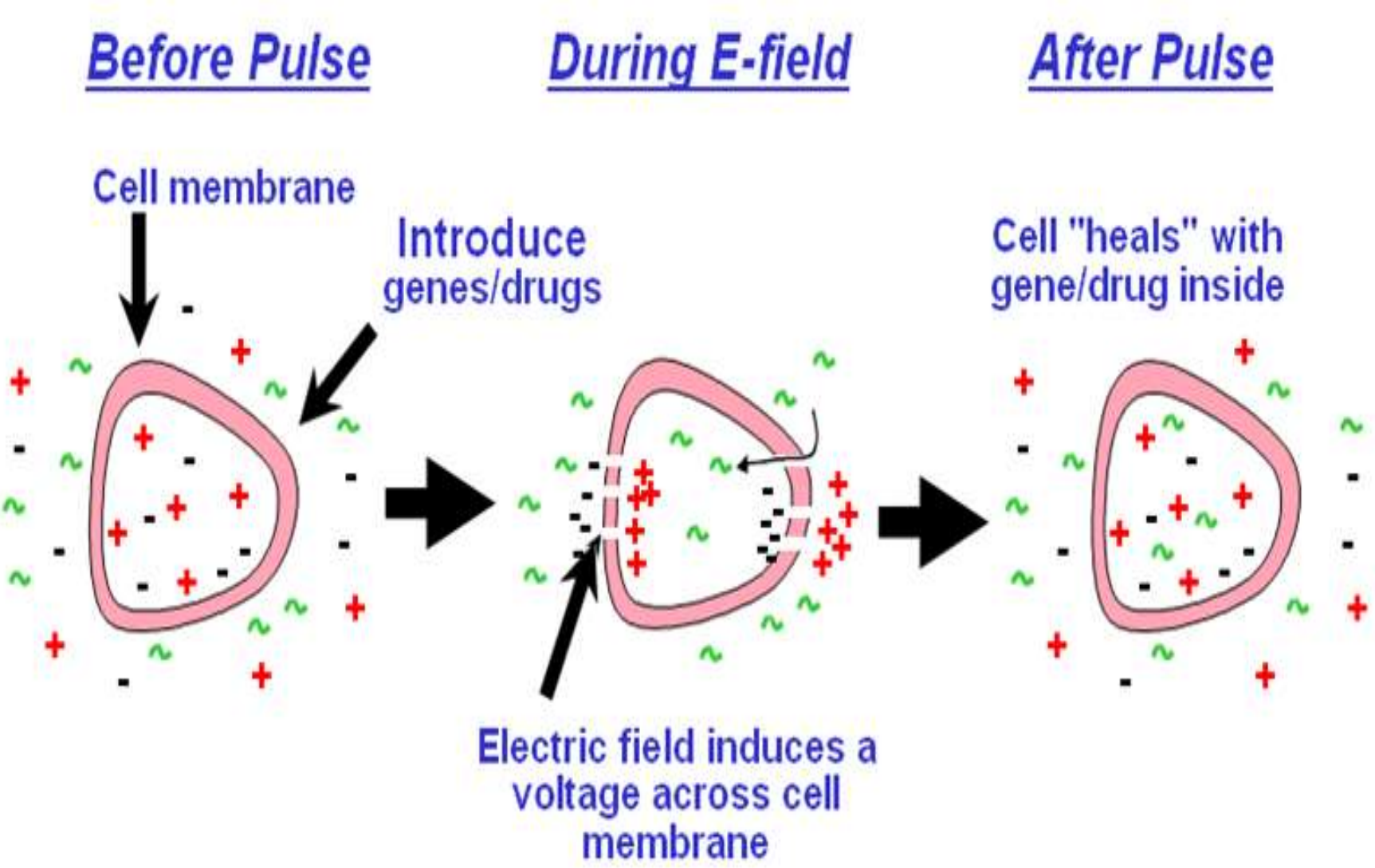
TRANSFORMATION



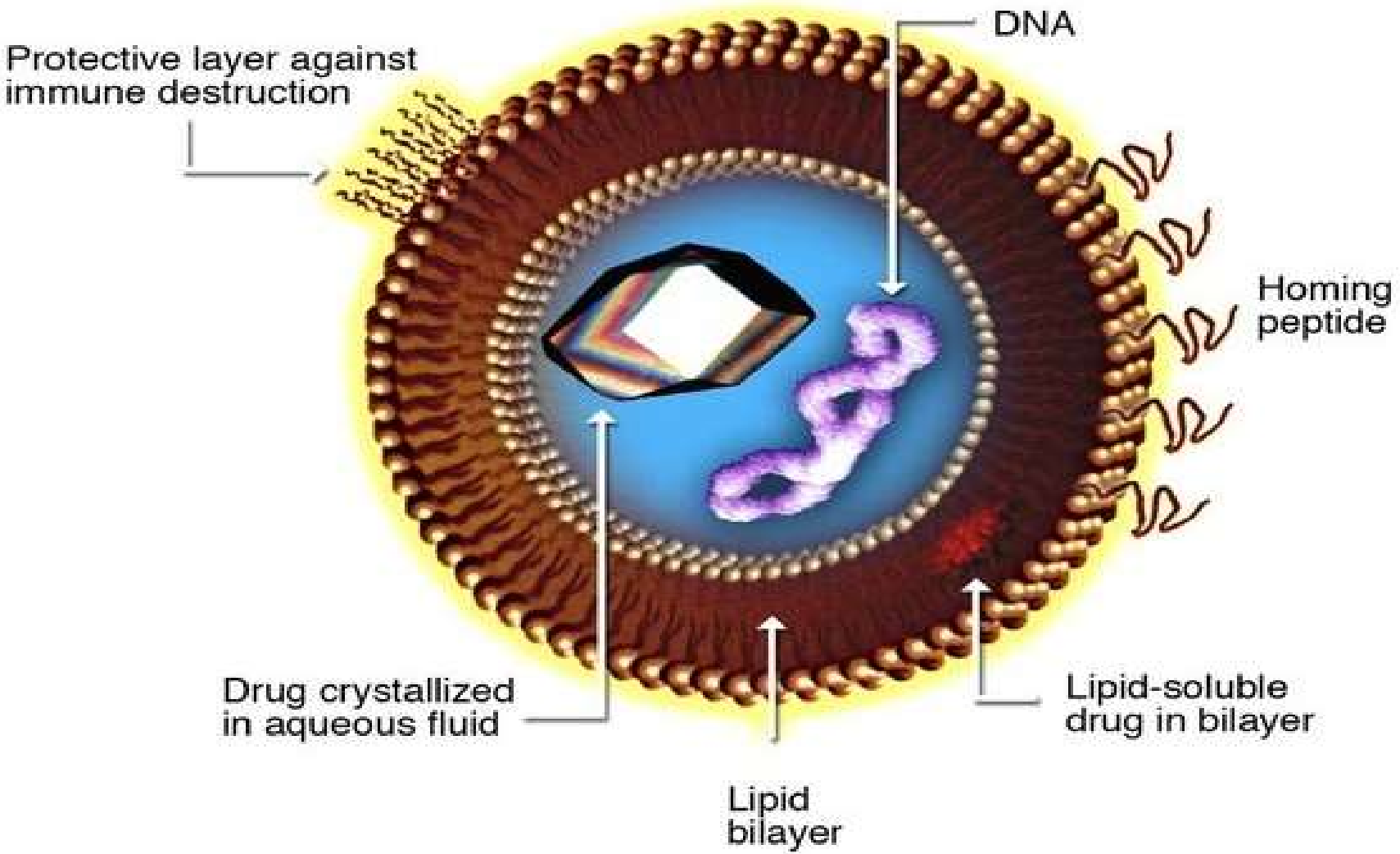
CONJUGATION



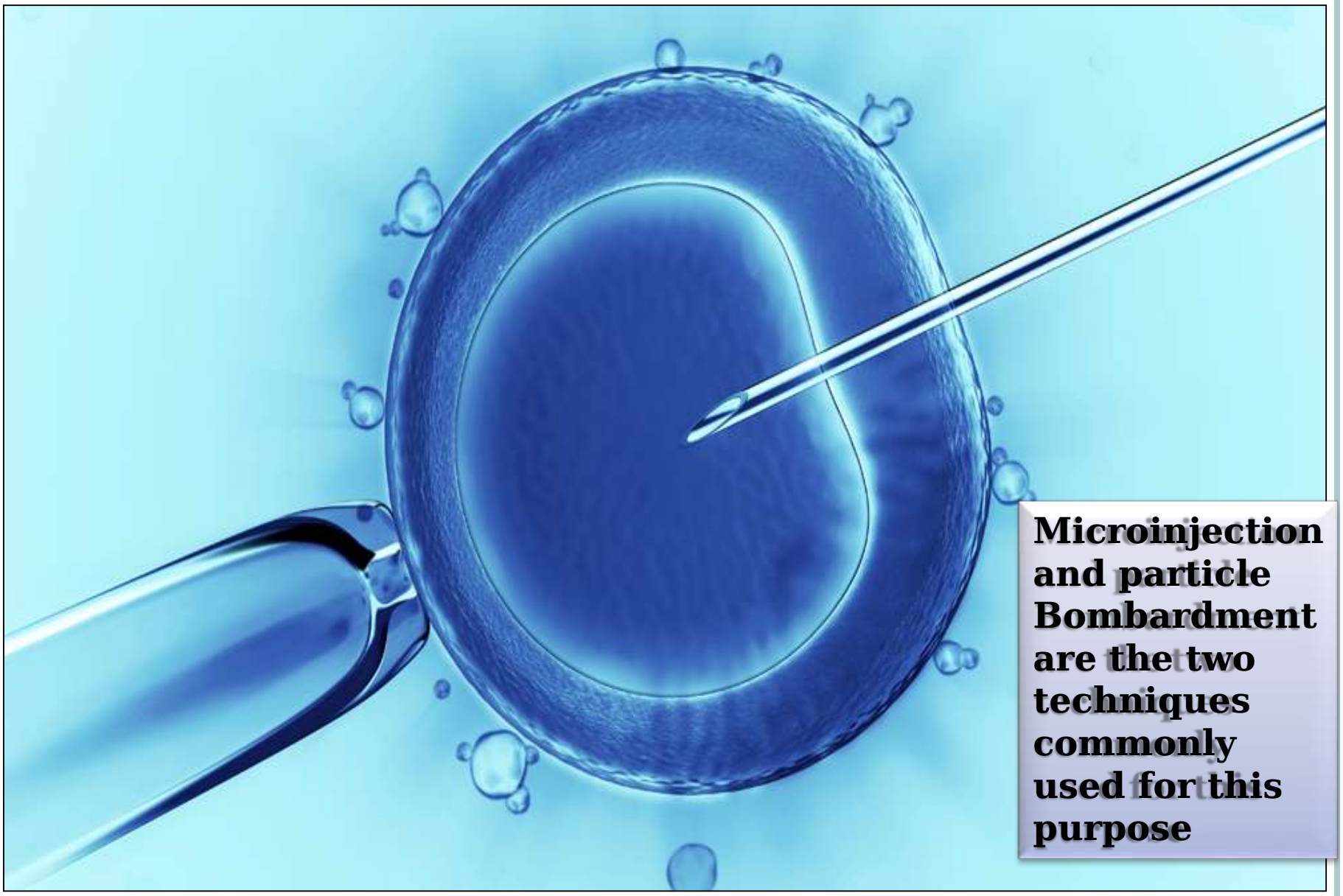
ELECTROPORATION



LIPOFECTION

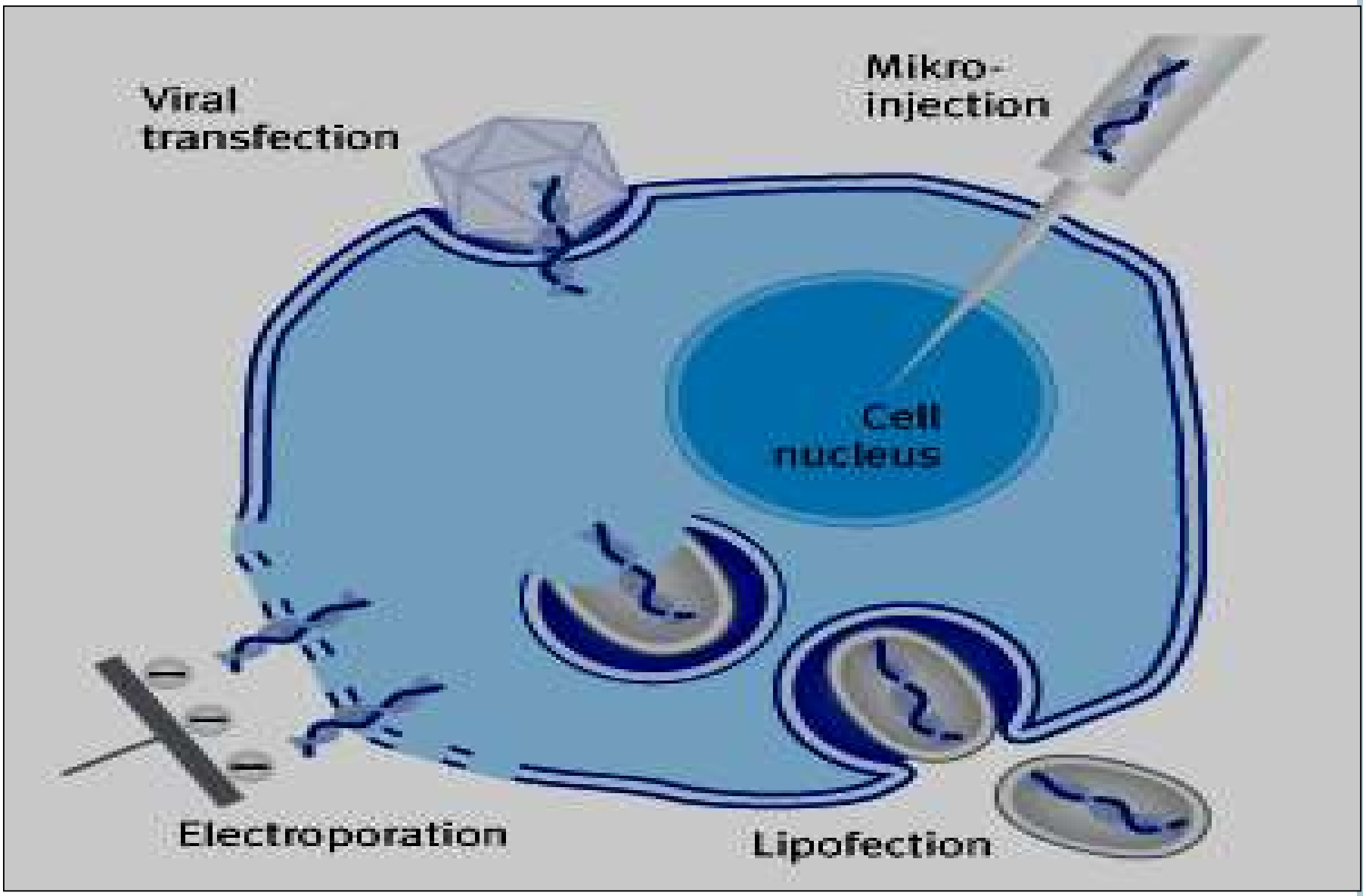


Micro injection of genetic material



Microinjection and particle Bombardment are the two techniques commonly used for this purpose

GENE TRANSFER AT A GLANCE



Gene cloning strategies

Generation of DNA fragments

Insertion in to cloning vectors

Introduction in to host cells

Selection and screening

Cloning from genomic DNA or mRNA

Genomic DNA

mRNA

GENOMICDNA

- DNA represents the complete genetic material of an organism which is referred to as genome.
- Theoretically speaking, cloning from genomic DNA is supposed to be ideal.
- But the DNA Contains non-coding sequences (introns),control regions and repetitive sequences This complicates the cloning strategie hence DNA as a source material is not preferred.

MessengerRNA

- The use of mRNA in cloning is preferred for following reasons…….
- mRNA represents the actual genetic informatic being expressed.
- Selection and isolation mRNA are easy.
- As introns are removed during processing , mR reflects the coding sequence of the Gene.
- The synthesis of recombinant protein is much easier with mRNA cloning.

Thank

YOU