BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
Tamil Nadu, India

Programme : M.Sc., Biochemistry

Course Title : GENETIC ENGINEERING

Course Code: BC302CR

Unit – II

TRANSGENICS

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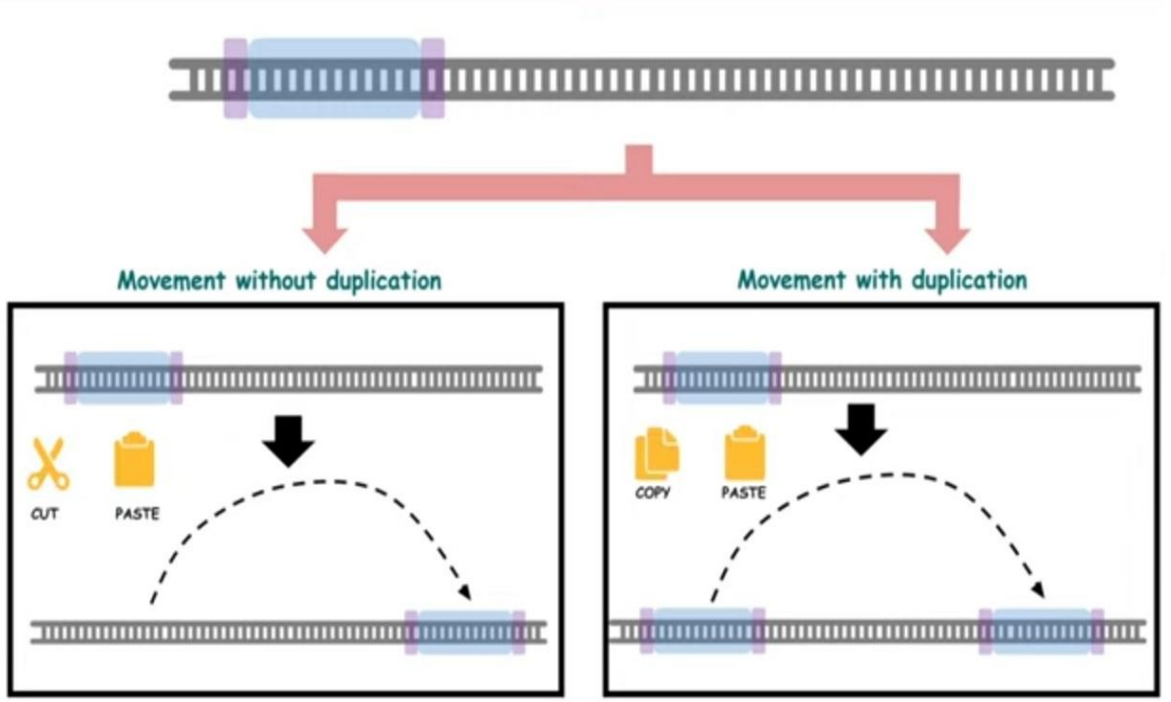
TRANSPOSONS

- Transposable elements are mobile genetic units that exhibit broad diversity in their structure and transposition mechanisms.
- Transposable elements occupy a large fraction of many eukaryotic genomes and their movement and accumulation represent a major force shaping the genes and genomes of an organisms.
- Barbara McClintoct first discovered transposable elements in corn in the 1940lmost all organisms.

General characteristics of TE

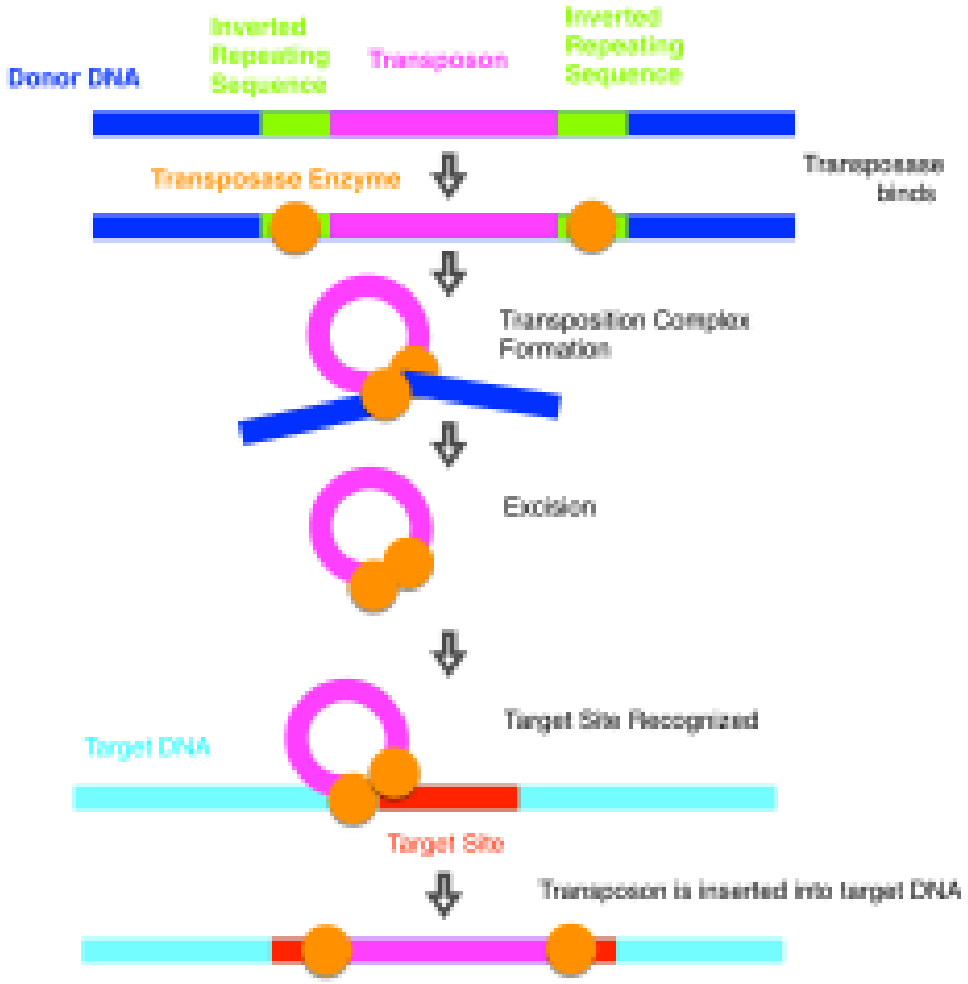
- They were found to be DNA sequences that code for enzymes, which bring about the insertion of an identical copy of themselves into a new DNA site.
- Transposition events involve both recombination and replication processes which frequently generate two daughter copies of the original transposable elements.
- One copy remains at the parent site and another appears at the target site.
- A transposable element is not a replicon. Thus, It cannot replicate apart from the host chromosome.



Different classes of transposons

DNA transposons

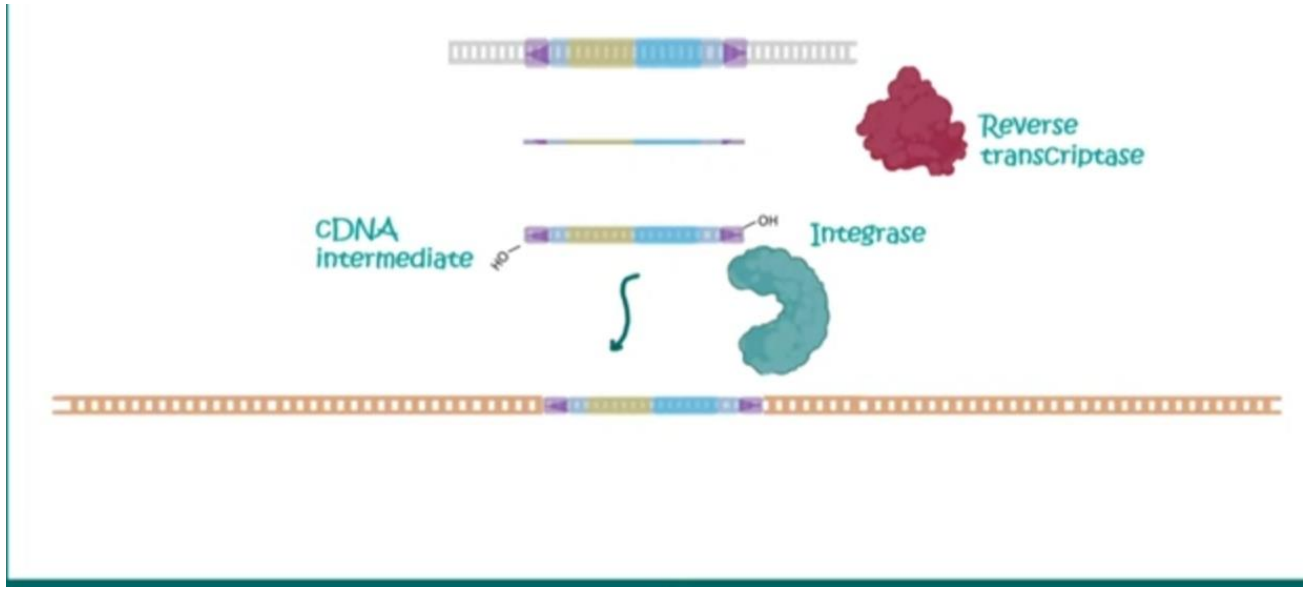
- DNA transposons move from one genomic location to another by a cut-and-paste mechanism. They are powerful forces of genetic change and have played a significant role in the evolution of many genomes.
- As genetic tools, DNA transposons can be used to introduce a piece of foreign DNA into a genome.

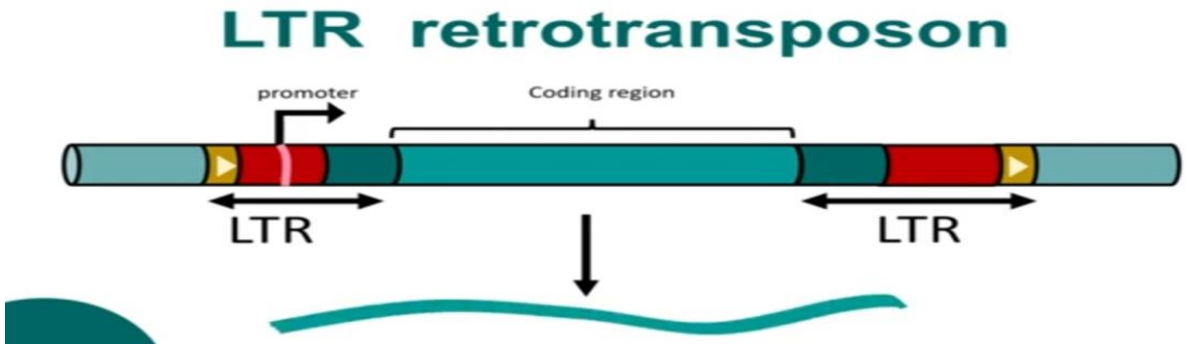


Viral like reterotransposons

•The viral-like retrotransposons, encode a reverse transcriptase and, often, an integrase.

•With these enzymes, these elements can be transcribed into RNA, reverse-transcribed into DNA, and then integrated into a new location within the genome.





Poly A tail reterotransposons

•SINE & LINEs are short and long interspersed retero transposable elements, respectively invades new genomic sites using RNA intermediates.

•Both are found in all eukaryotes.

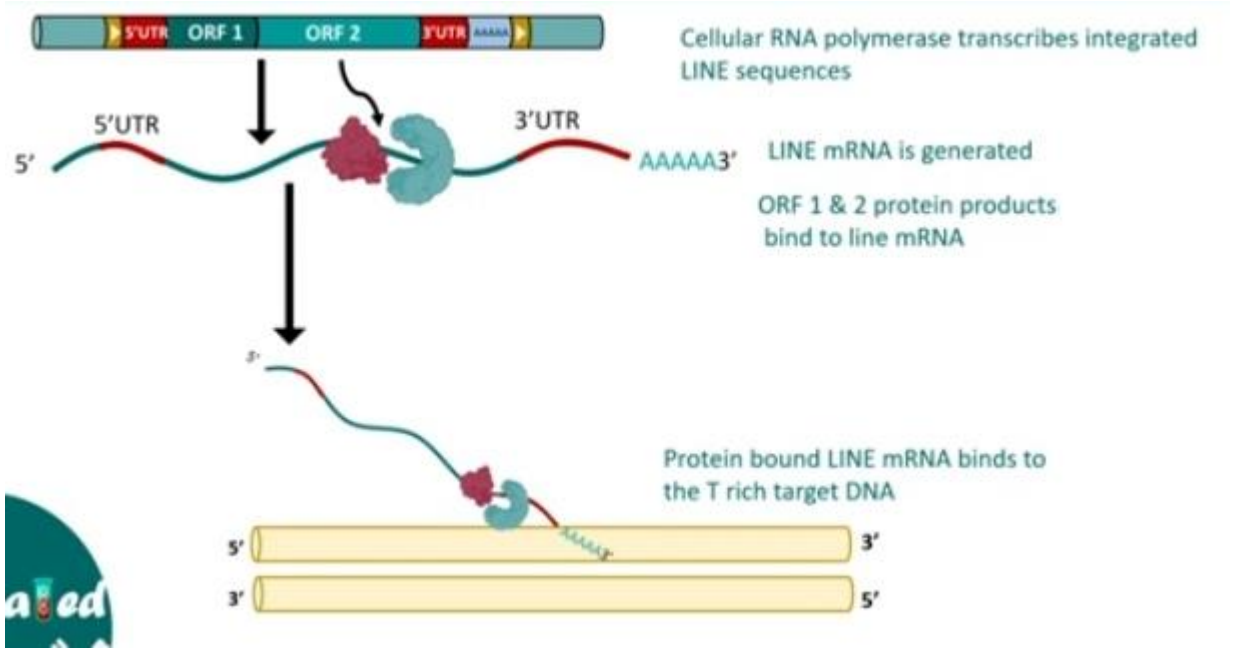
•LINEs are autonomous reteroelements, SINEs are their dependants.

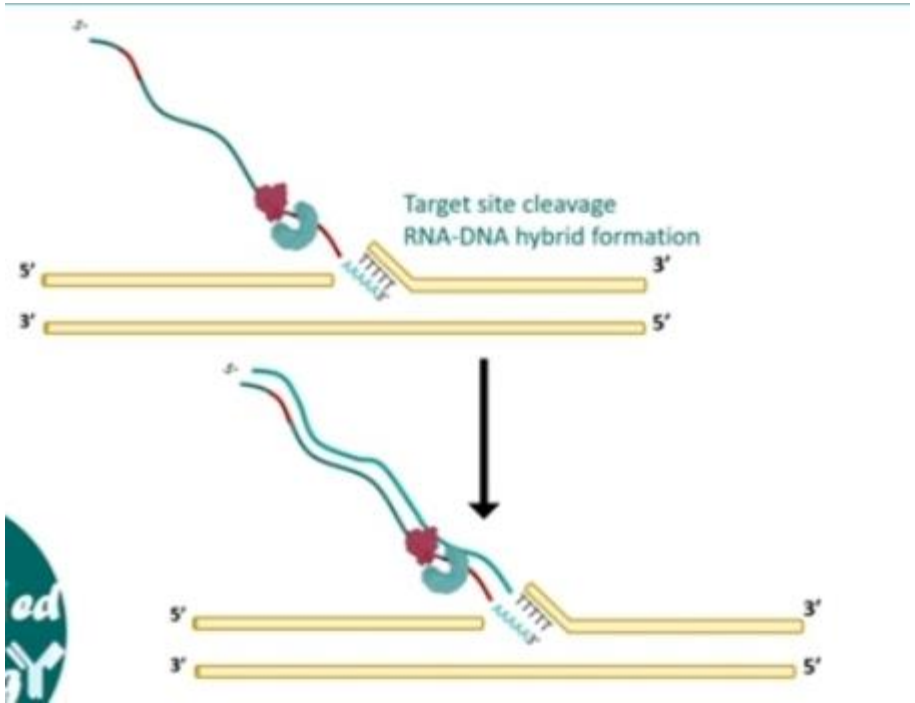
•LINE – 6000bp

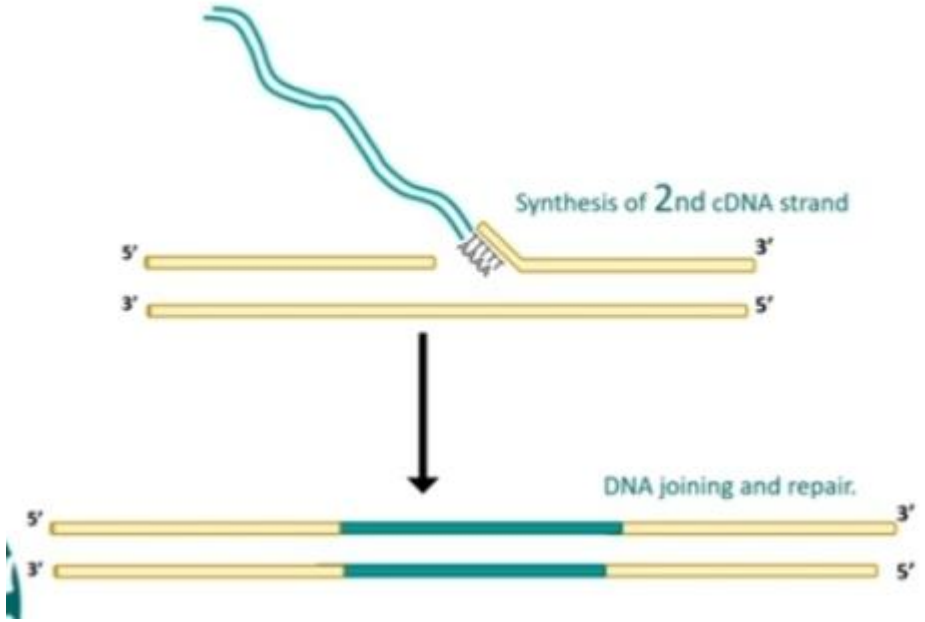
•SINE – 248bp

•ORF 1 – encodes RNA binding protein

•ORF 2 – encodes a protein with endonuclease & revere transcriptase


5'UTR
ORF 1
ORF 2
3'UTR
AAAAA
Cellular RNA polymerase transcribes integrated LINE sequences
5'UTR
3'UTR
5'
AAAAA3'
LINE mRNA is generated
ORF 1 & 2 protein products bind to line mRNA
Protein bound LINE mRNA binds to the T rich target DNA
5'
3'
3'
5'


Target site cleavage
RNA-DNA hybrid formation
5'
3'
3'
5'
5'
3'
3'
5'


Synthesis of 2nd cDNA strand
5'
3'
3'
5'
DNA joining and repair.
5'
3'
3'
5'

INTRODUCTION

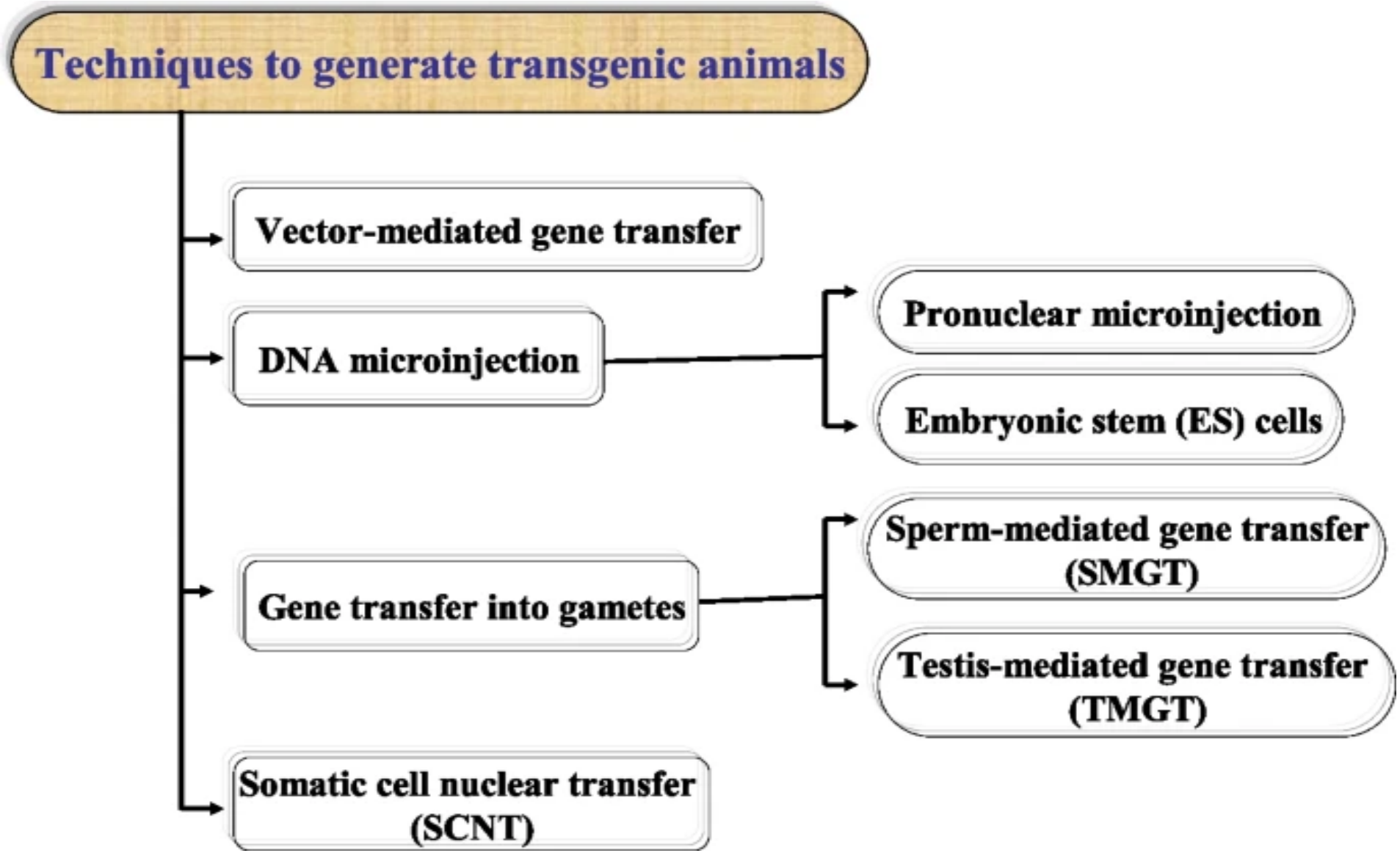
- A transgenic animal is one whose genome has been changed to carry genes from another species or to use techniques for animal genome editing for specific traits.
- A mouse was the first successful transgenic animal. Then pigs, sheep, cattle, and rabbits came a few years later.
- The foreign-interested genes that will be used in animal transgenic techniques are prepared using a variety of methods. The produced gene of interest is placed into a variety of vectors, including yeast artificial chromosomes, bacterial plasmids, and cosmids.

- Several techniques, including heat shock, electroporation, viruses, the gene gun, microinjection, and liposomes, are used to deliver the created vector, which includes the interesting gene, into the host cell.
- Transgenesis can be carried out in the gonads, sperm, fertilized eggs, and embryos through DNA microinjection, retroviruses, stem cells, and cloning.
- The most effective transgenic marker at the moment is fluorescent protein.
- Transgenesis success is confirmed by the integration of an antibiotic resistance gene, western and southern blots, PCR, and ELISA.
- If technology solves social and ethical problems, it will be the most promising in the future.

Transgenesis

The transgenesis technique involves the introduction of foreign DNA sequences into the genome of transfected cells and ensuring that the DNA sequences are integrated and transmitted to the offspring. Some of the practical applications of transgenesis in animal production.

- Greater prolificacy and reproductive performance
- improved feed utilization and growth rate
- improved carcass composition
- improved milk production
- increased disease resistance


Techniques to generate transgenic animals
Vector-mediated gene transfer
DNA microinjection
Pronuclear microinjection
Embryonic stem (ES) cells
Gene transfer into gametes
Sperm-mediated gene transfer (SMGT)
Testis-mediated gene transfer (TMGT)
Somatic cell nuclear transfer (SCNT)

VECTOR MEDIATED GENE TRANSFER

- Vectors increase the probability of gene expression.
- Plasmids, cosmids, the P1 phage, BACs (bacterial artificial chromosomes), and YACs (yeast artificial chromosomes) may each hold 20 kilobytes (kb), 40 kb, 90 kb, 200 kb, and 1000 kb of DNA.
- Viruses have the ability to deliver their genome into cells efficiently.

Retroviral vectors

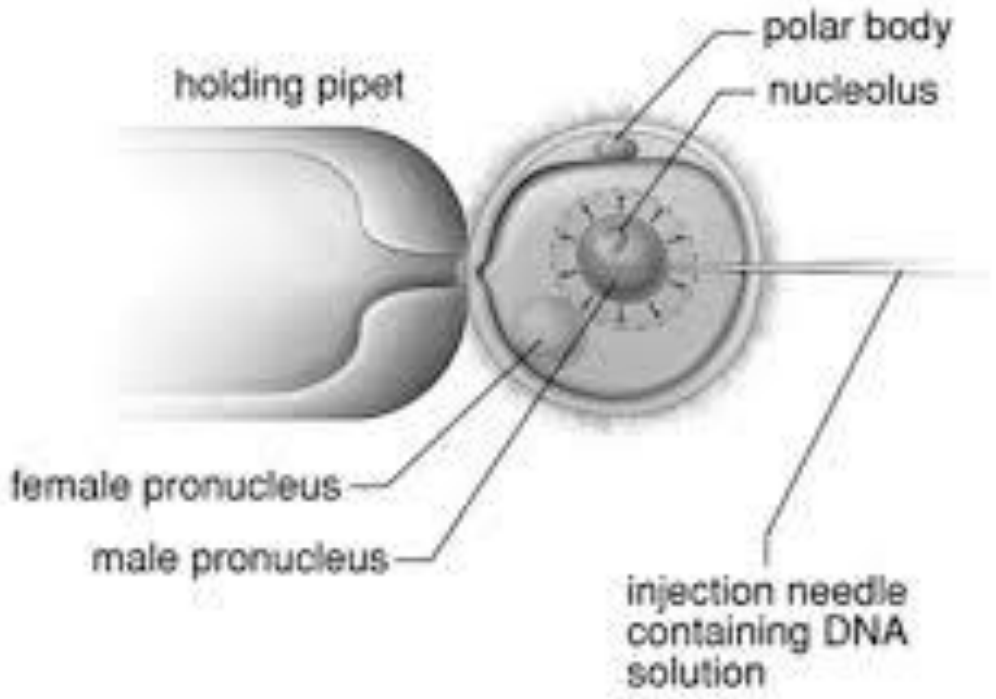
- They are RNA viruses that can generate DNA from RNA using reverse-transcriptase enzymes.
- They can copy themselves when a cell divides by integrating into the host DNA .
- Recently, retroviral vectors were used to allow for the integration of a foreign gene into the host genome.
- They can carry up to 7 to 8 kb from foreign genes, but at the same time, this may not be enough for long genes or structures that require extensive regulatory sequences for transcription.

Adenoviral vectors

- Adenoviral vectors are double-stranded DNA vectors that are not enveloped. Adenoviral vectors are extensively utilized as research tools in vitro and in small animal models due to their relatively easy manufacture and high levels of transgene expression.
- Adenovirus vectors (AdV) are extremely strong gene transfer vehicles, with applications capable of holding up to 10 kb of foreign DNA.
- The elimination of structural genes such as gag, pol, and env, which aid in the assembly of viral particles by the retrovirus, is a common change in this type of vector.

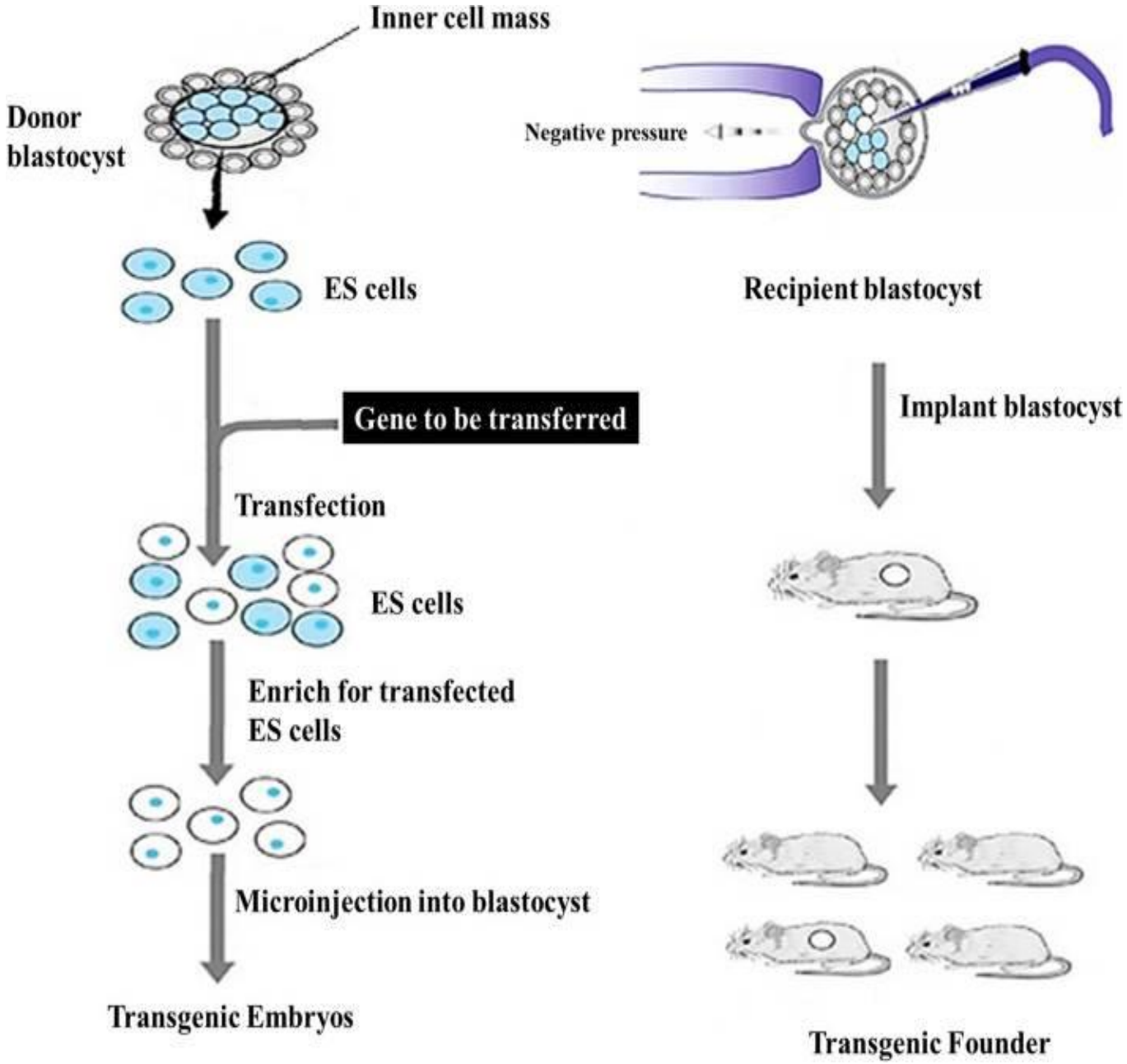
Pronuclear DNA microinjection

- Microinjection of genes into the pronuclei of zygotes.
- The major drawback of this method is that some copies of the foreign gene are randomly integrated into the host genome, causing transgene and host gene expression to be disrupted.
- The experiment requires a large number of embryos in the pronucleus stage.

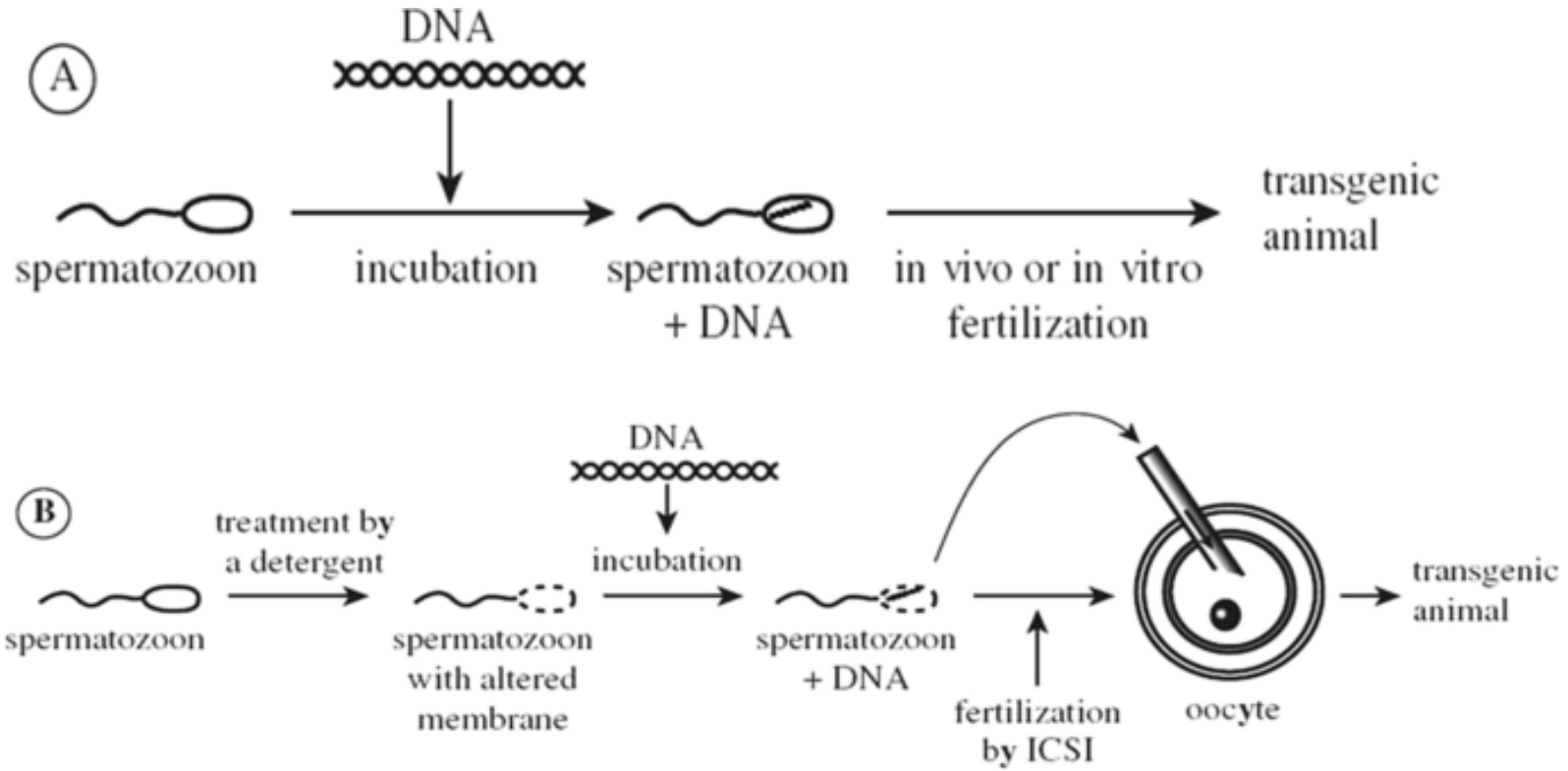


Embryonic stem (ES) cells

- The appropriate DNA sequence is inserted into an in vitro culture of embryonic stem (ES) cells using homologous recombination.
- Foreign DNA can be introduced into ES cells, and utilizing a selection gene, clones carrying the foreign gene can be generated.

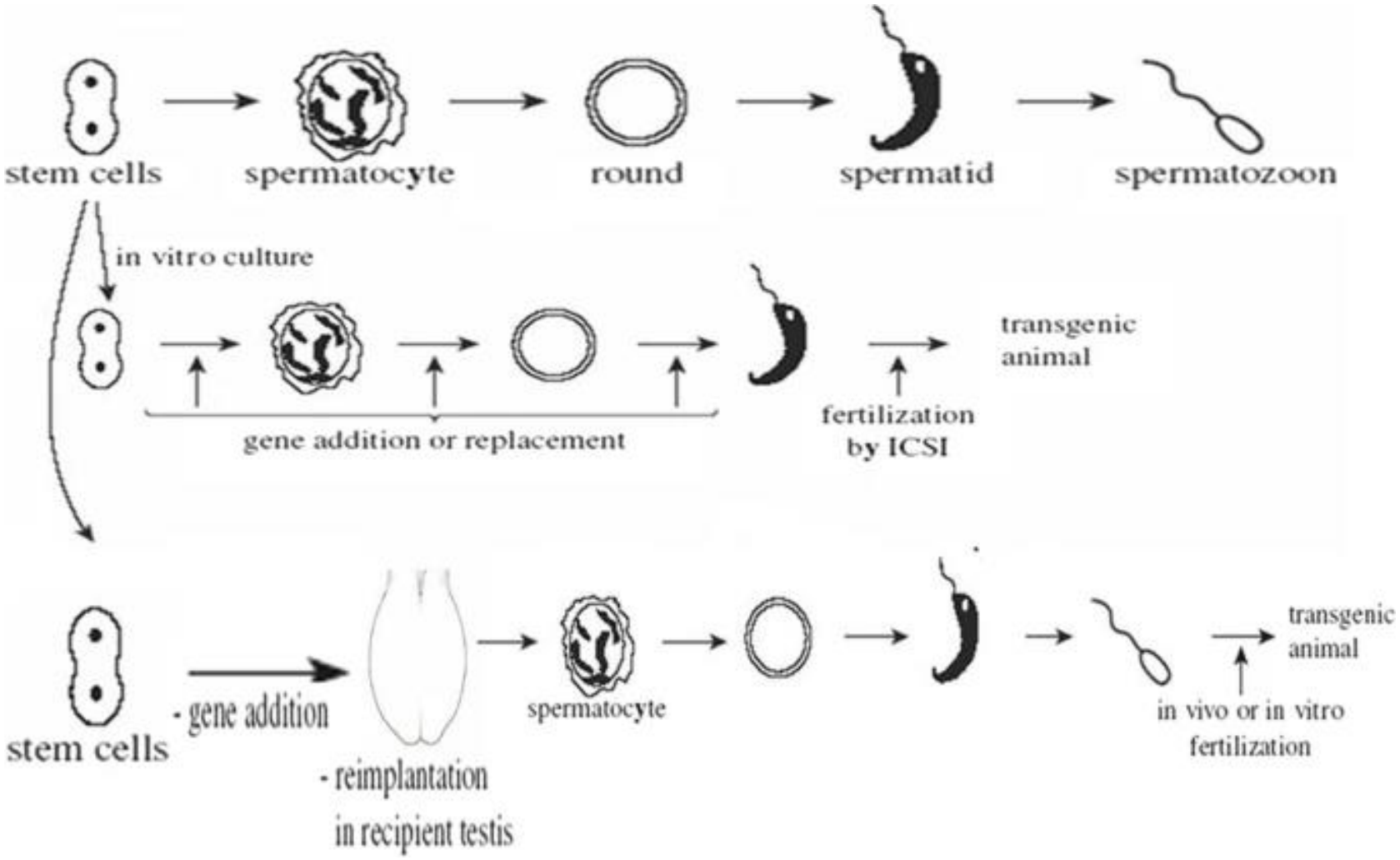


Sperm-mediated gene transfer technique (SMGT)



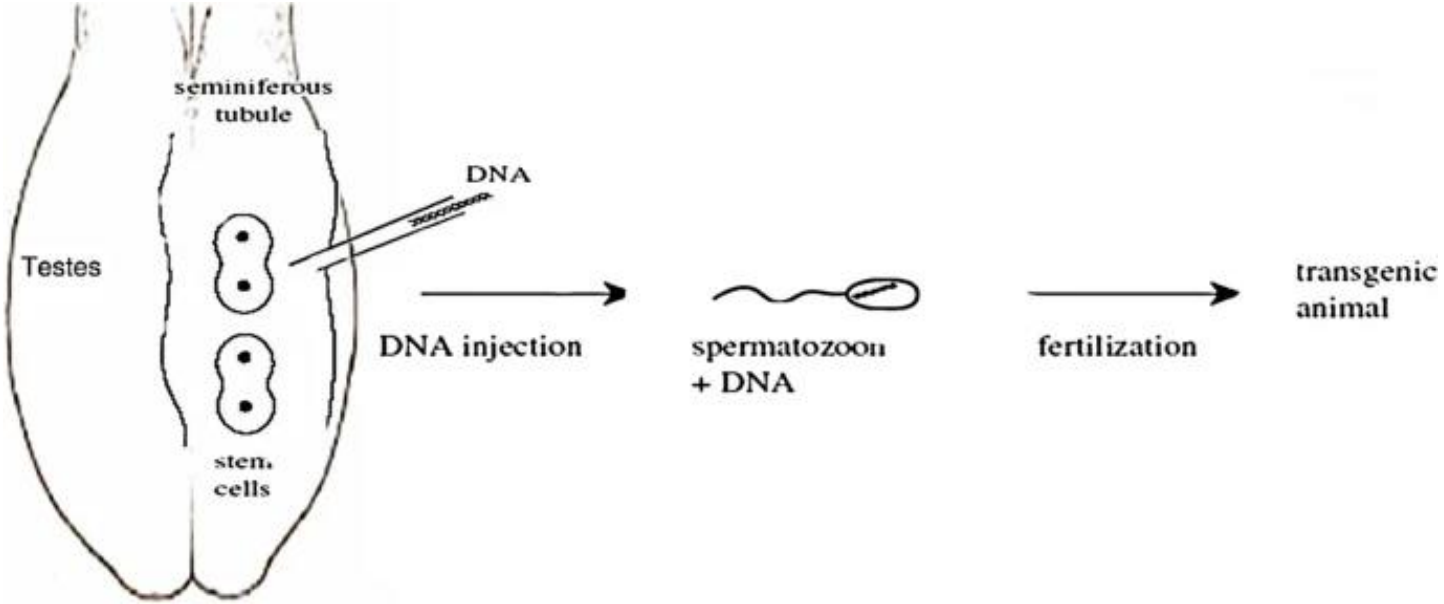
In vitro sperm precursors

- Sperm stem cells can be extracted, grown in vitro for a brief time, and then transferred into an adoptive testis.
- The transplanted cells continue to differentiate, eventually producing functioning sperm.



Testis-mediated gene transfer technique (TMGT)

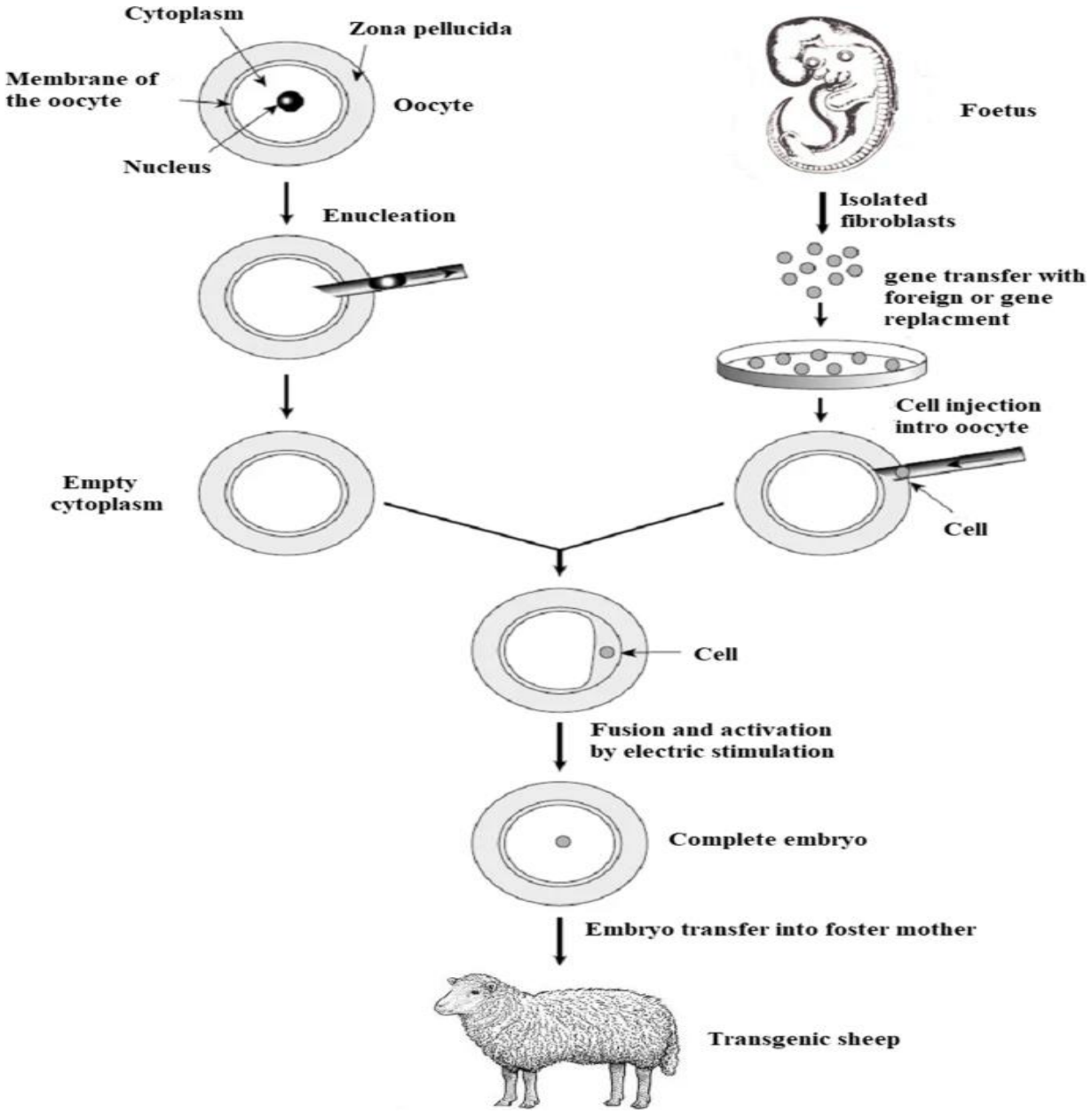
Adenovirus-mediated gene transfer may be effective for transfecting testicular somatic cells, and that this approach may be applicable for in vivo gene therapy for male infertility in the future, despite the fact that spermatogenesis is slightly impaired.



Somatic cell nuclear transfer (SCNT)

•The technique involves the transfer of a somatic cell nucleus to an enucleated egg's cytoplasm where it will be reprogrammed by egg cytoplasmic components to become a zygote

• In mammals, the zygote needs to be artificially implanted into a surrogate mother's uterus



Applications of transgenic animals

- **Animal production**

Animal	Genes introduced or deleted	Performance criteria (consumer benefit)
Bovine	*β* and *κ* casein	Casein protein expression has increased (improved protein content of milk)
Bovine	Intestinal lactase	Lactose in milk is being reduced (lactose intolerant people)

- **Environmental pollution** - Bioremediation
- **Industry**

Nexia Biotechnologies Inc. has developed a strain of dwarf goats from West Africa that naturally breed and lactate early (BELE®), decreasing transgenic protein production time compared to sheep, cows, and conventional goats.

- **Medicine**

Drug	Disease/target	Animal
Alpha-lactalbumin	Anti-infection	Cow
Human protein C	Thrombosis	Pig, sheep
Fibrinogen	Wound healing	Cow &sheep
Glutamic acid decarboxylase	Type 1 diabetes	Mouse, goat

Recombinant selection and screening

- **DIRECT SELECTION OF RECOMBINANTS**

1. Blue white colony selection
2. Direct antibiotic resistance screening

- **INDIRECT SELECTION OF RECOMBINANTS**

1. Screening by nucleic acid hybridization
2. Screening by colony hybridization
3. Screening by immunological assay
4. Screening by protein enzyme assay

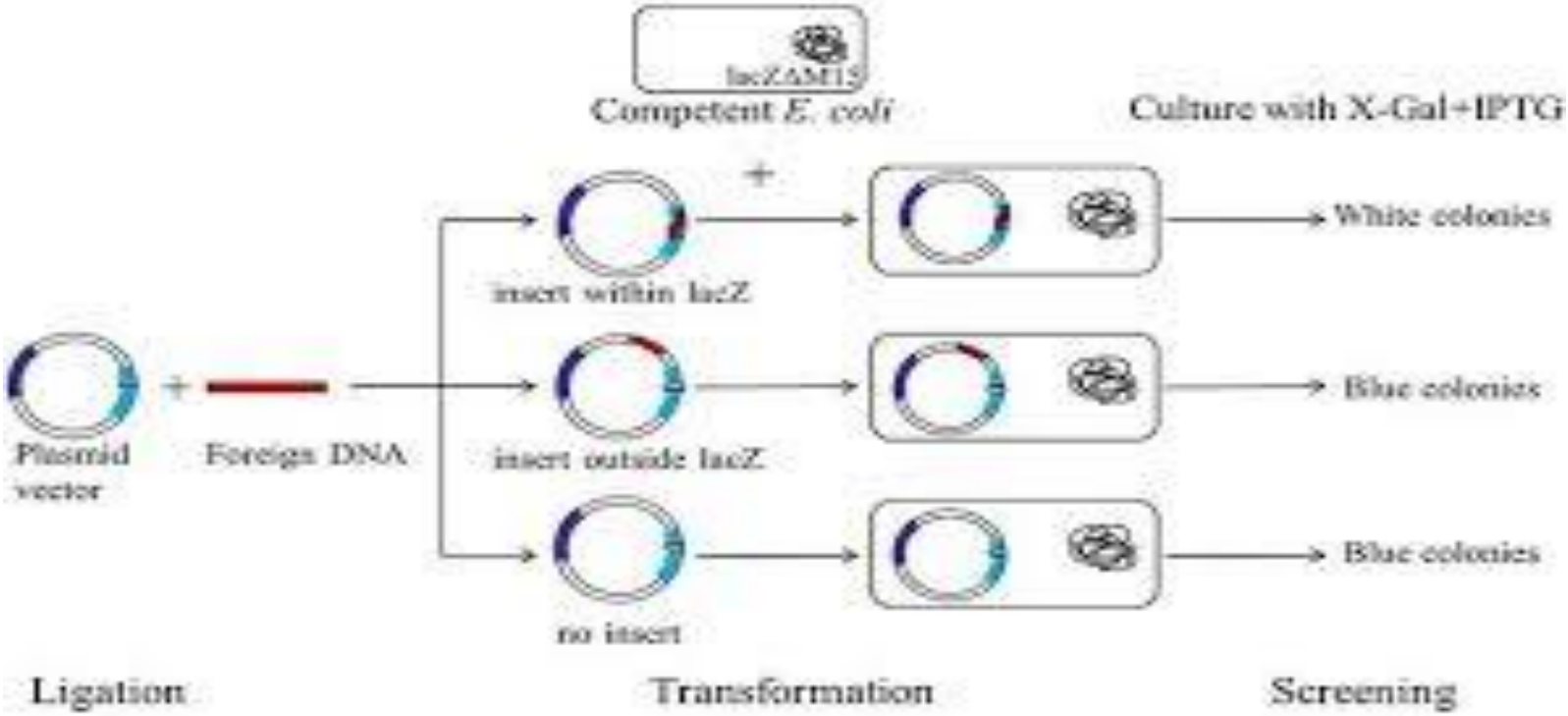
BLUE-WHITE SCREENING

- BLUE-WHITE SCREENING

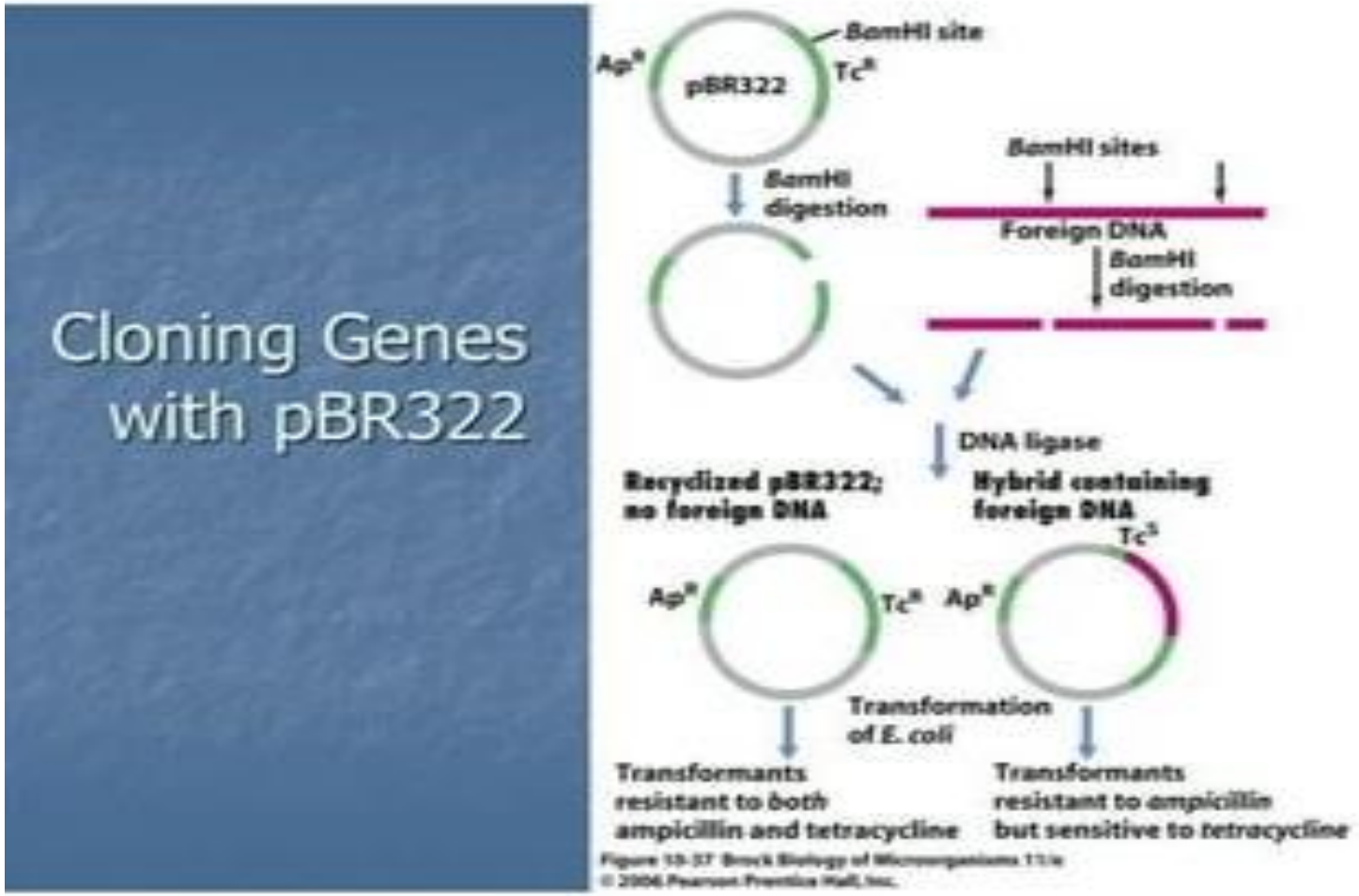
- The use of chromogenic substrate to detect a particular enzymatic activity is the basis to screen the desired clone. The colourless compound X-gal or 5-bromo-4-chloro-3-indolyl-β-D-galactoside used in this screening method is a substrate for β-galactosidase.
- The enzyme β-galactosidase is the product of lacZ gene of the lac operon. In this system, host contains lacZ gene without the initial region where as vector contains α-peptide to complement the defect to form active enzyme.

- As a result, if a vector containing α-peptide will be transformed into the host containing remaining lacZ, the two fragment will reconstitute to form active enzyme
- In addition the α-peptide region in vector contains multiple cloning site and as a result of insertion of gene fragment, consequently α-peptide will not be synthesized to give fully active β-galactosidase. The enzyme β-galactosidase oxidizes x-gal to form 5-bromo-4-chloro-indoxyl and galactose.
- The indoxyl derivative is oxidized in air to give a blue colored dibromo-dichloro derivative. Hence, blue colored colonies indicate the absence of insert where as colorless colonies indicate presence of an insert.

Blue - white colony selection

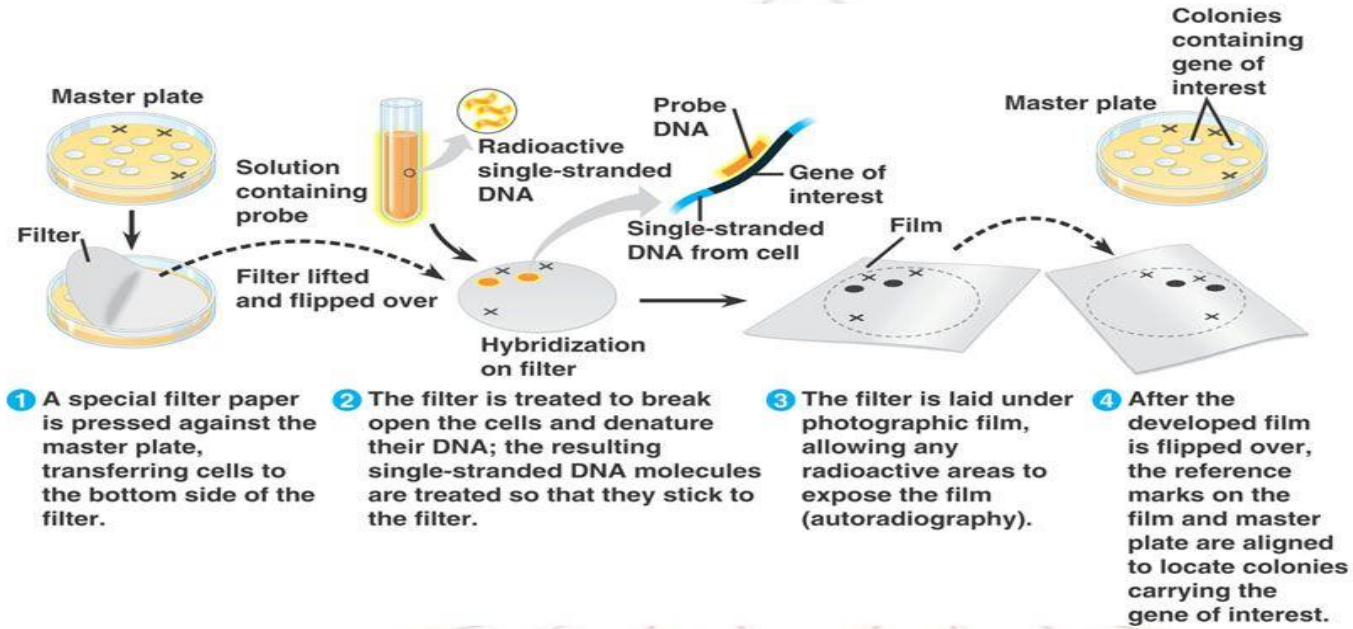


Direct antibiotic resistance screening

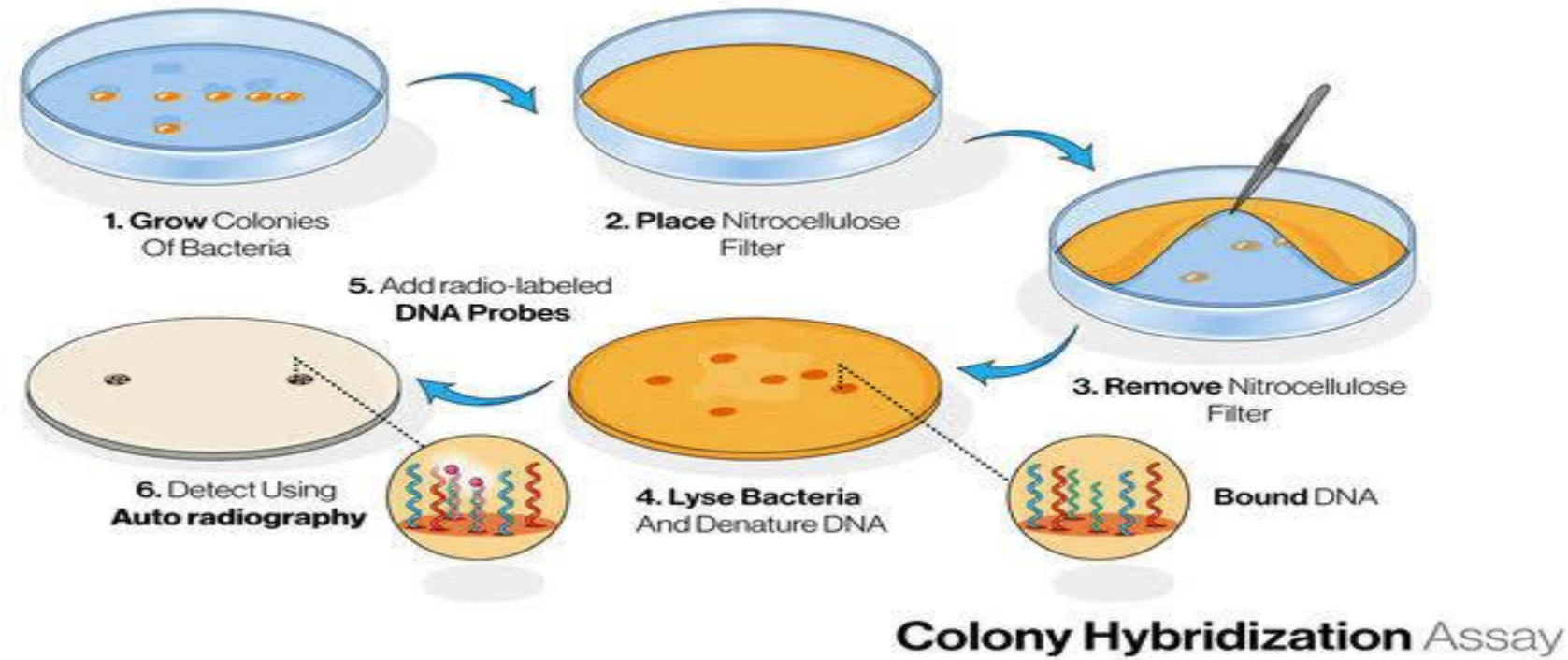


Nucleic acid hybridization

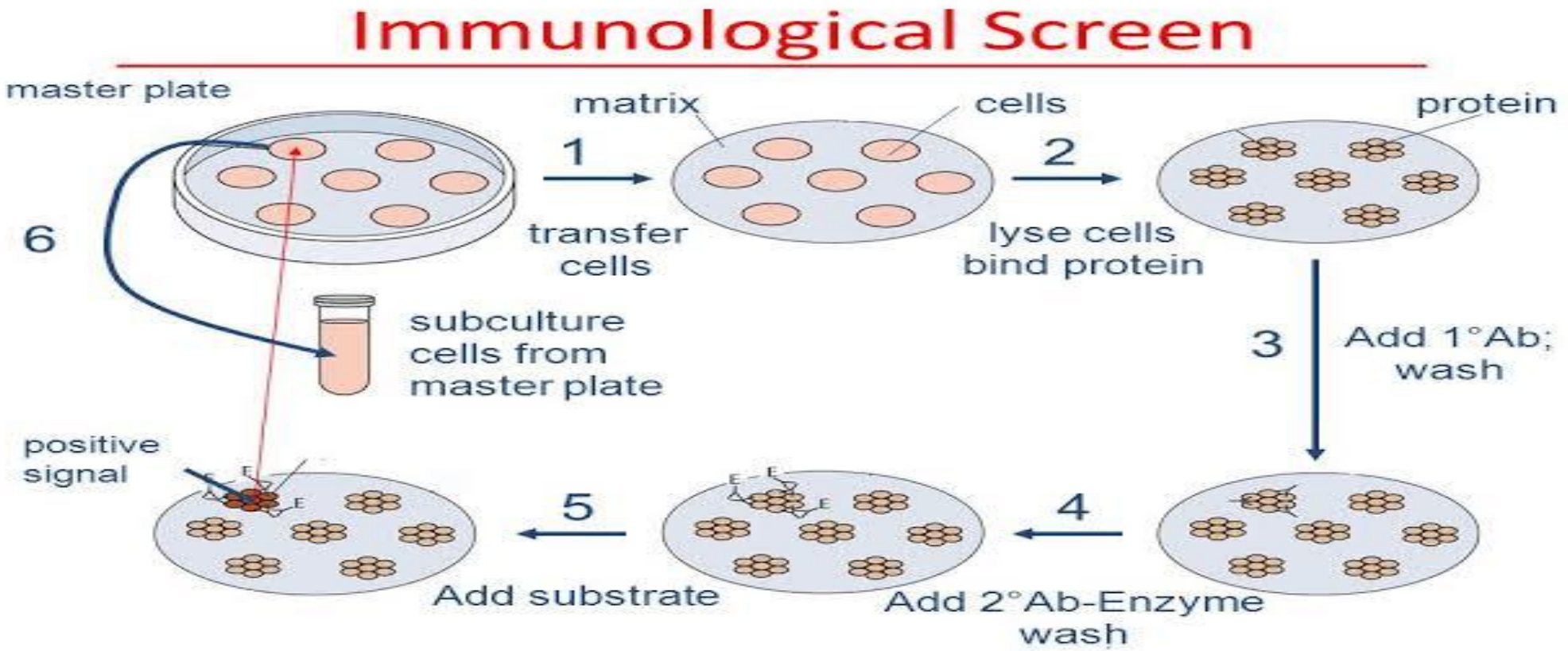
Figure 20.5 Nucleic acid probe hybridization



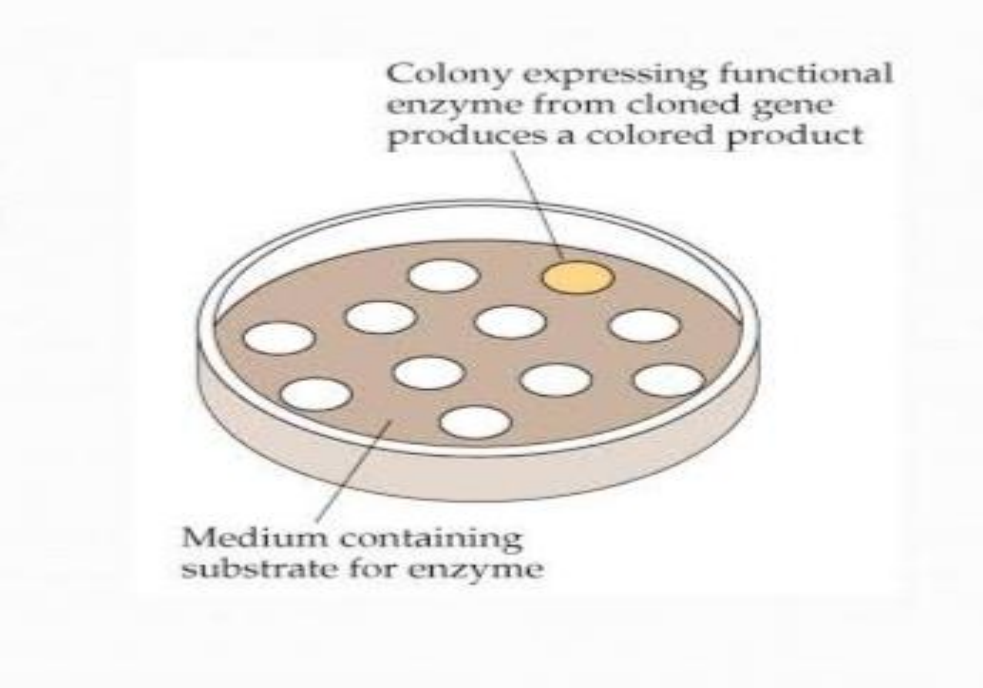
Colony hybridization



Immunological assay



Protein / enzyme assay



References

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2874221/
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10169938/
- https://www.slideshare.net/slideshow/screening-and-selection-of-recombinants/250946180
- Genetics- analysis of genes and genome by Daniel L. Harti

TRANSFORMATION

• Transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane(s).

• For transformation to take place, the recipient bacteria must be in a state of competence, which might occur in nature as a time-limited response to environmental conditions such as starvation and cell density, and may also be induced in a laboratory.

COMPETENT CELLS

• Bacterial cells that are able to take up DNA from the environment are called competent cells.

• In the laboratory, bacterial cells can be made competent and DNA subsequently introduced by a procedures.

ELECTROPORATION:

- Electroporation is a method of transformation via direct gene transfer.

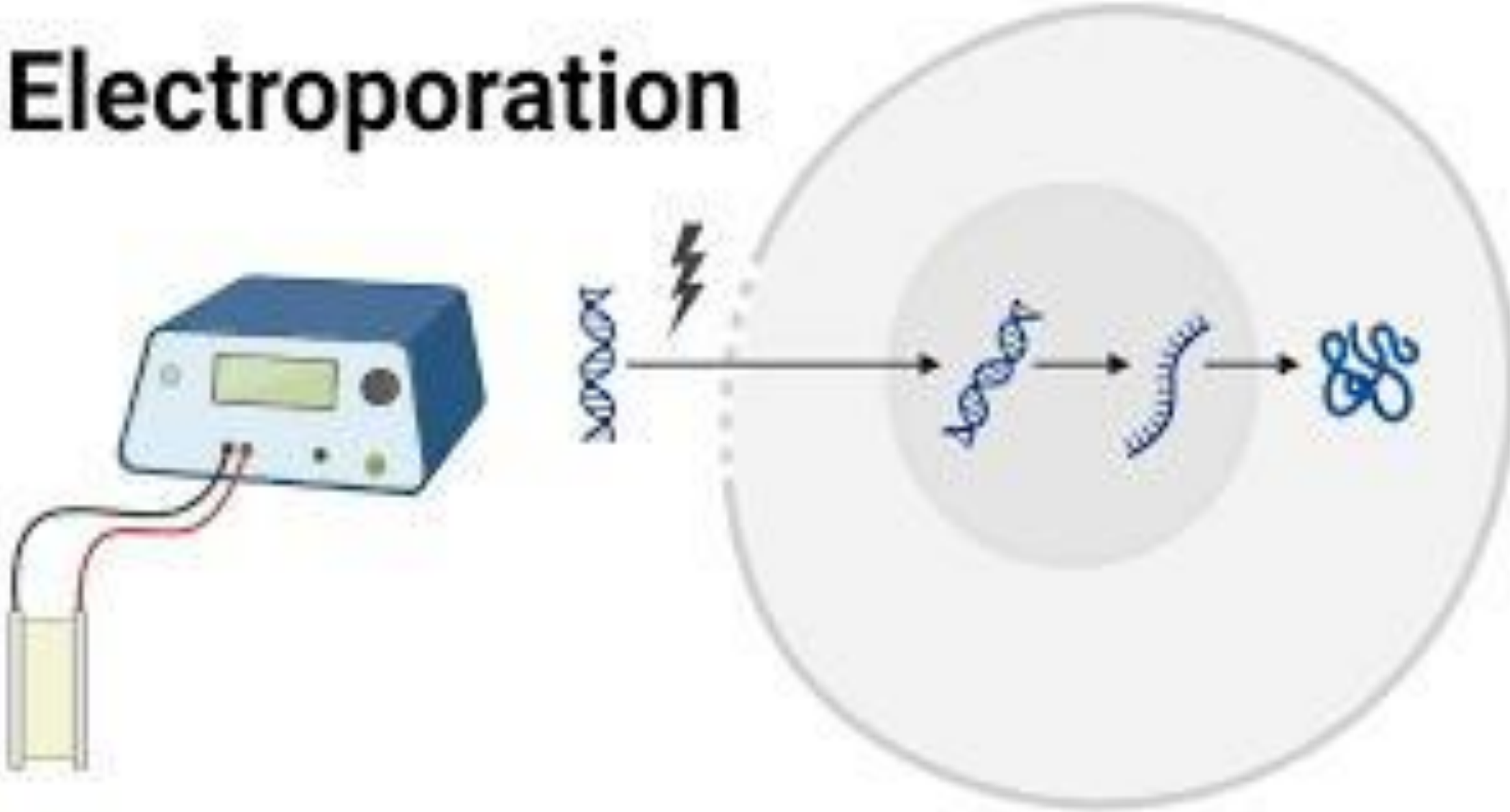
- In this technique mixture containing cells and DNA is exposed to very high voltage electrical pulses (4000-8000 V/cm) for very brief time periods (few milliseconds).

- It results in formation of transient pores in the plasma membrane, thorough which DNA seems to enter inside the cell.

- Electroporation is highly efficient for the introduction of foreign genes into tissue culture cells, to transform bacteria, yeast, or plant protoplasts by introducing new coding DNA.

- The process of introducing foreign DNA into eukaryotic cells is known as transfection.

Electroporation



MICROINJECTION:

- Direct injection of DNA into plant protoplast or cell using fine tipped (0.5-10um diameter) pipette.
-
- Protoplasts are immobilised on the agarose or held with a micropipette under suction.
- DNA is injected into the cytoplasm or nucleus.

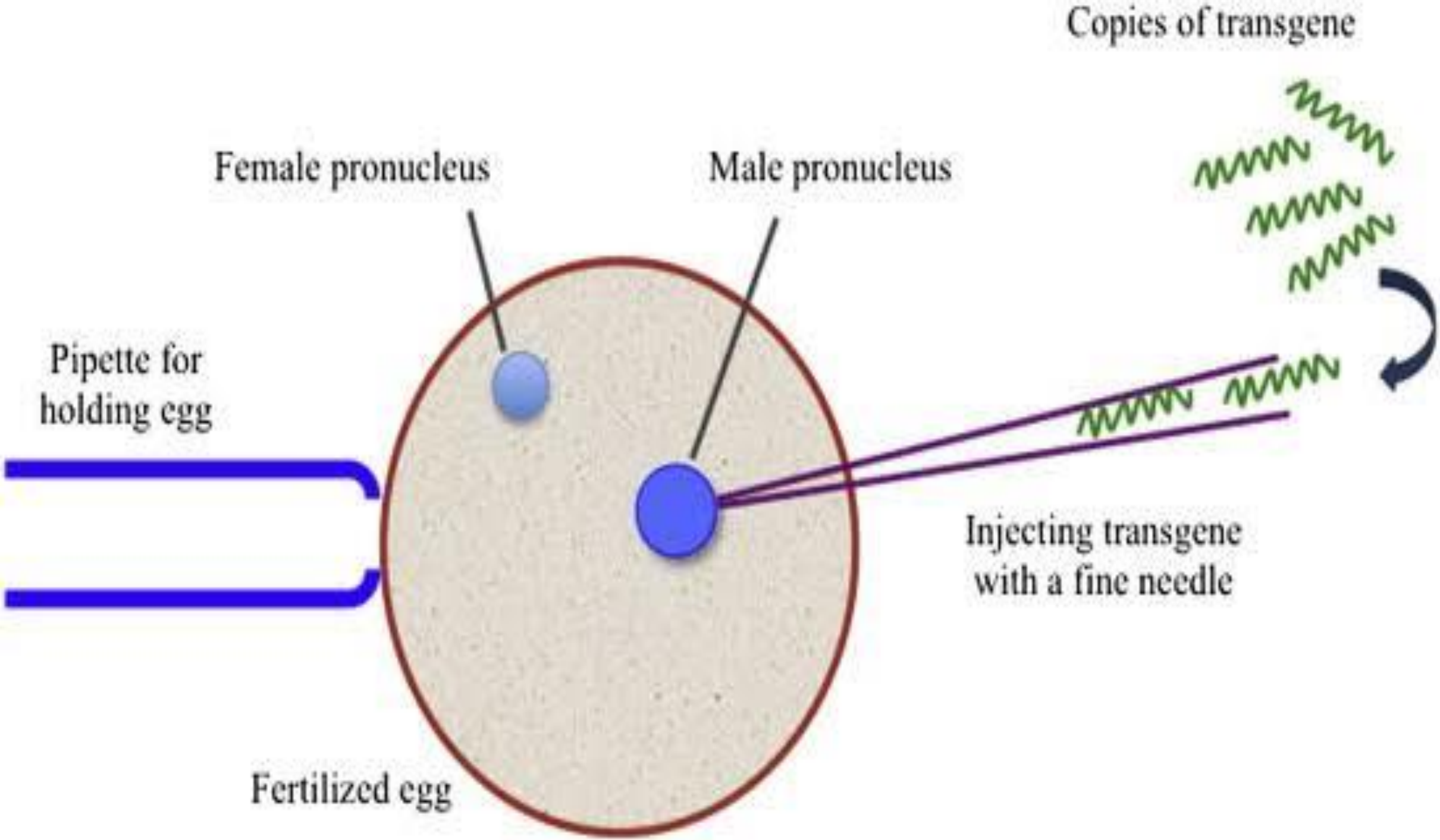
- Frequency of transformation:

Nucleus (14%)

Cytoplasm(6%)

- Successful transformation achieved in tobacco, alfalfa, Brassica sp.

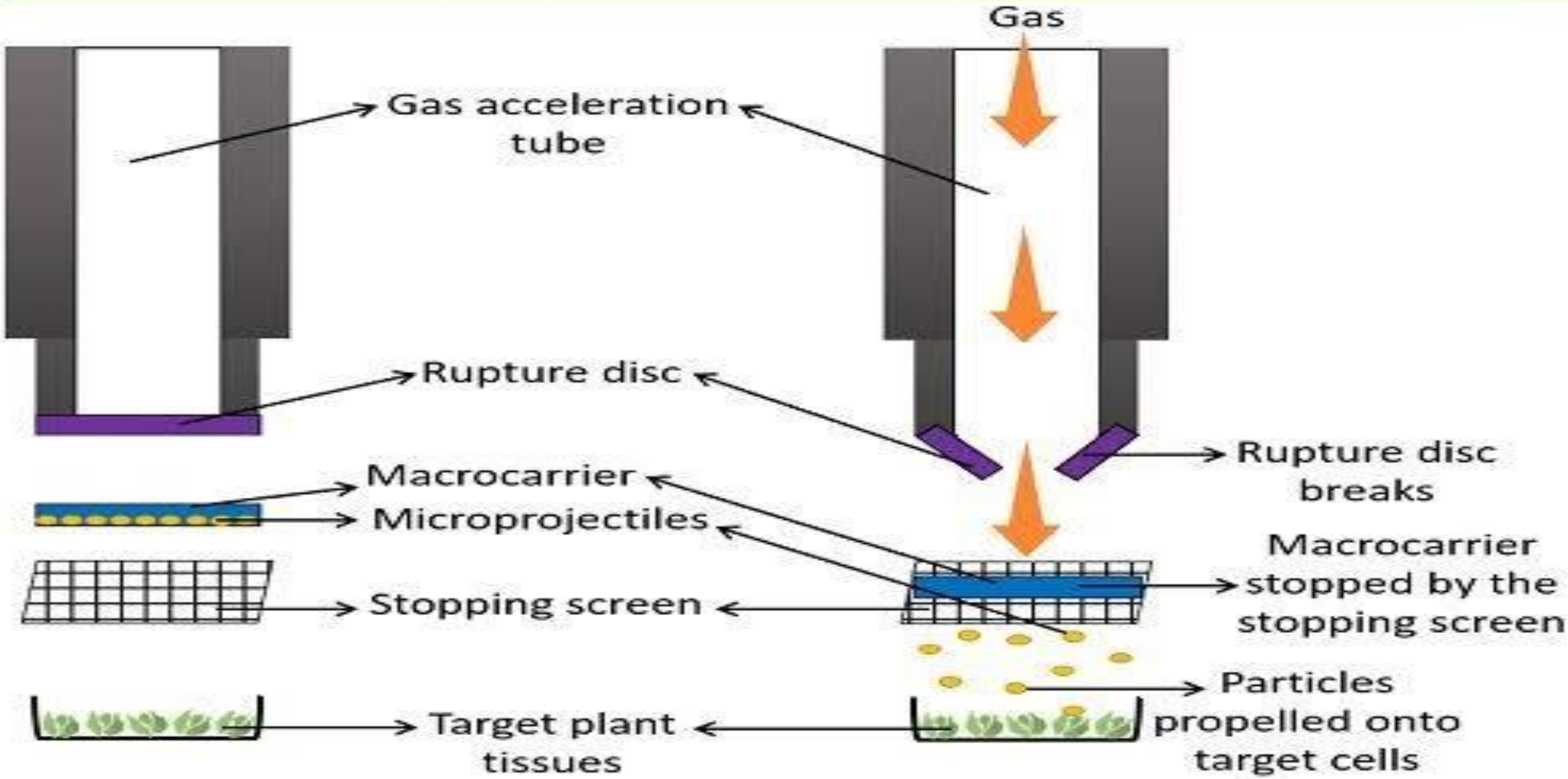
Transformation frequency ranging from 14-60%


Copies of transgene
Female pronucleus
Male pronucleus
Pipette for
holding egg
Injecting transgene
with a fine needle
Fertilized egg

PARTICLE BOMBARDMENT:

- The process of transformation employees foreign DNA coated with minute 0.2-0.7 um gold (or) are tungsten particles to deliver into target plant cells
- Two procedures have been used to accelerate the minute
- By electro static energy released by a droplet of water exposed to a high voltage.

Microprojectile Bombardment



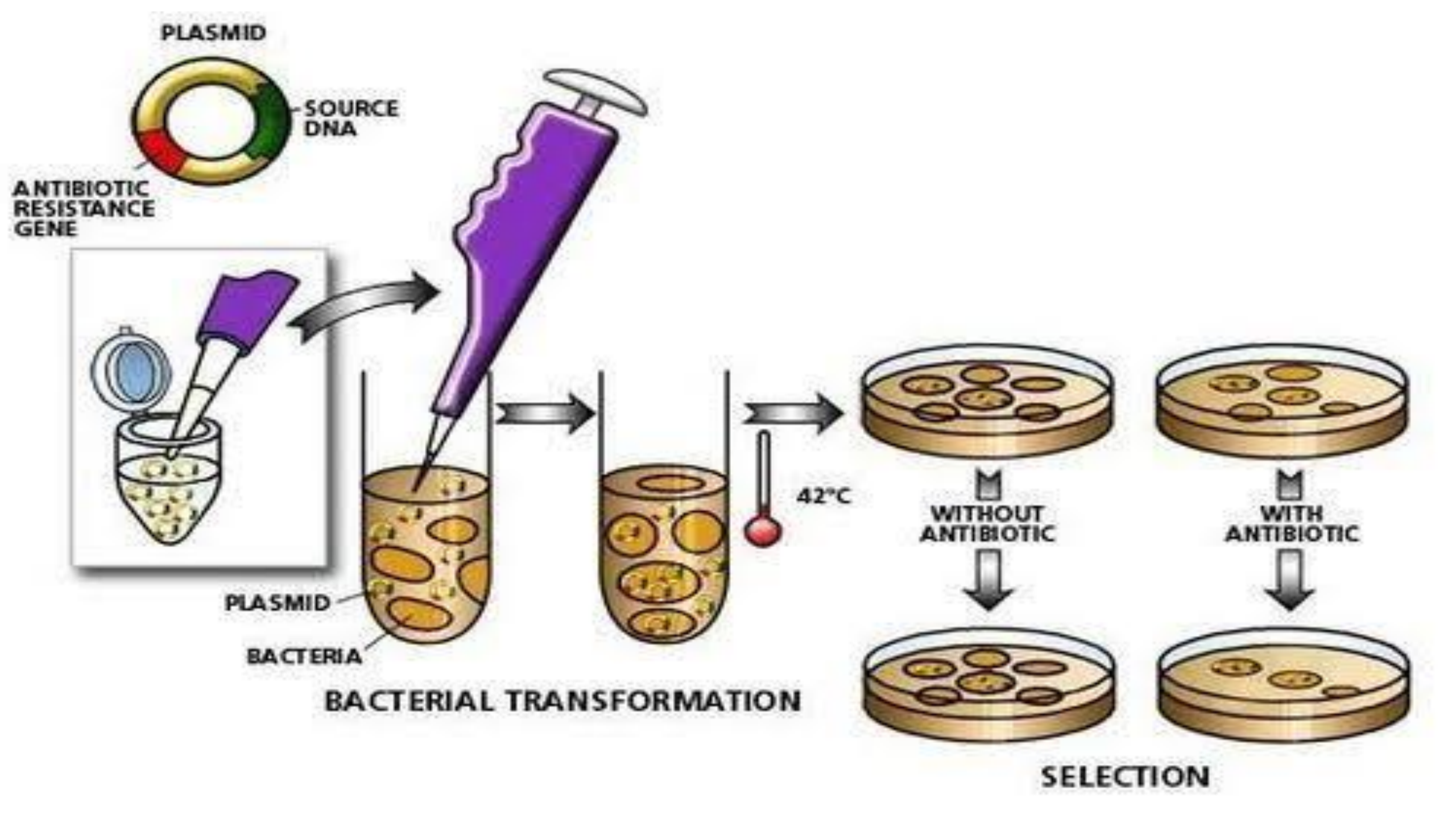
SELECTION OF TRANSFORMANTS

- Genetic alteration of a cell by the incorporation of exogenous genetic material (exogenous DNA)

- Transformation occurs naturally in some species of bacteria

- It can also be done by artificial means in other cells

For transformation we use competent bacteria:


PLASMID
SOURCE DNA
ANTIBIOTIC RESISTANCE GENE
PLASMID
BACTERIA
42°C
WITHOUT ANTIBIOTIC
WITH ANTIBIOTIC
BACTERIAL TRANSFORMATION
SELECTION

TERMS TO KNOW

- ***TRANSGENE***- It is a foreign gene or genetic material that has been transferred naturally or by any of a number of genetic engineering techniques from one organism to another.
- ***TRANSGENESIS***- The phenomenon of introduction of exogenous DNA into the genome to create and maintain a stable and heritable character.
- ***TRANSGENIC PLANTS***- The plant whose genome is altered by adding one or more transgenes are known as transgenic plants.

HISTORY

- The first genetically modified crop plant was produced in **1982**, an **antibiotic-resistant tobacco plant**.
- In **1987**, Plant Genetic Systems ,founded by Marc Van Montagu and Jeff Schell, was the first company to genetically engineer **insect-resistant** (**tobacco**) plants by incorporating genes that produced insecticidal proteins from Bacillus thuringiensis (Bt).
- The first genetically modified crop approved for sale in the U.S, in **1994**, was the **FlavrSavr tomato** as it had a **longer shelf life**.
- In **1994**, the European Union approved tobacco engineered to be resistant to the **herbicide bromoxynil**, making it the first commercially genetically engineered crop marketed in Europe.

- In **1995**, **Bt Potato** was approved by the US Environmental Protection Agency, making it the country's first pesticide producing crop.
- In **2000**, **Vitamin A**-enriched golden rice, was the first food with increased nutrient value.

GENE TRANSFER METHODS

- **BIOLOGICAL METHODS**
  - Agrobacterium mediated gene transfer
  - Plant virus vectors
- **PHYSICAL METHODS**
  - Electroporation
  - Microprojectile
  - Microinjection
  - Liposome Fusion
- **CHEMICAL METHODS**
  - Polyethylene glycol mediated
  - Diethylaminoethyl dextran mediated

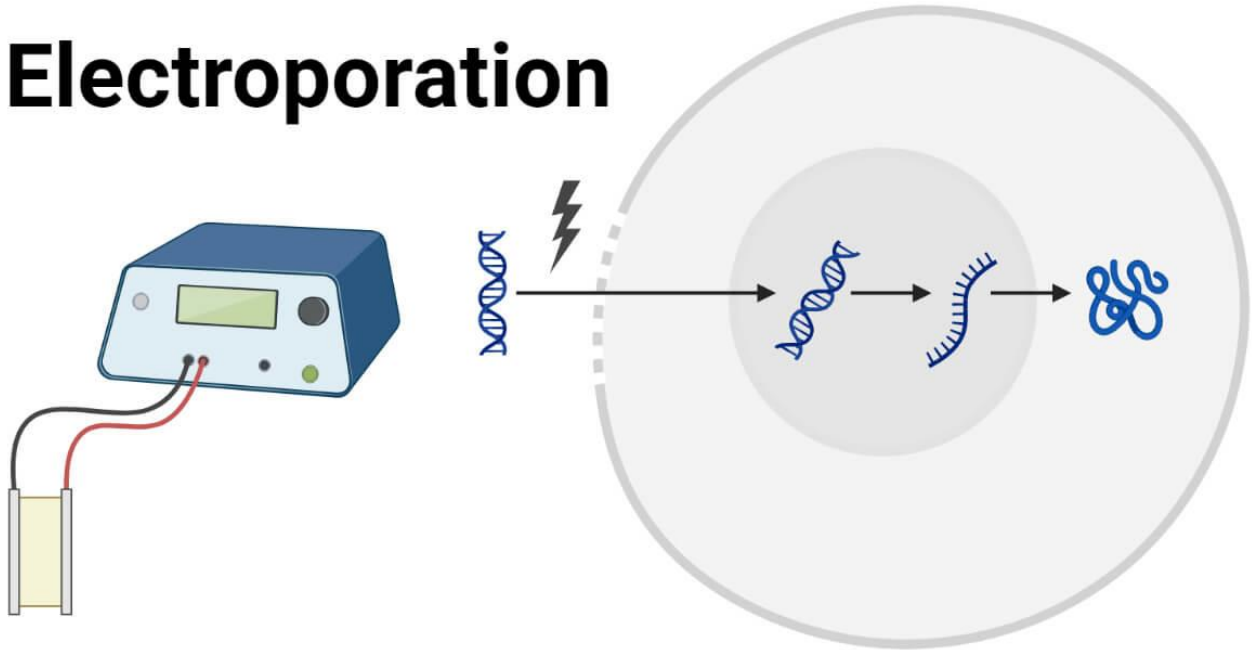
BIOLOGICAL METHODS

- Plant viruses are considered as efficient gene transfer agents as they can infect the intact plants and amplify the transferred genes through viral genome replication.
- Two classes of DNA viruses are known to infect higher plants, **caulimovirus** and **geminivirus**

PHYSICAL TRANSFER METHODS

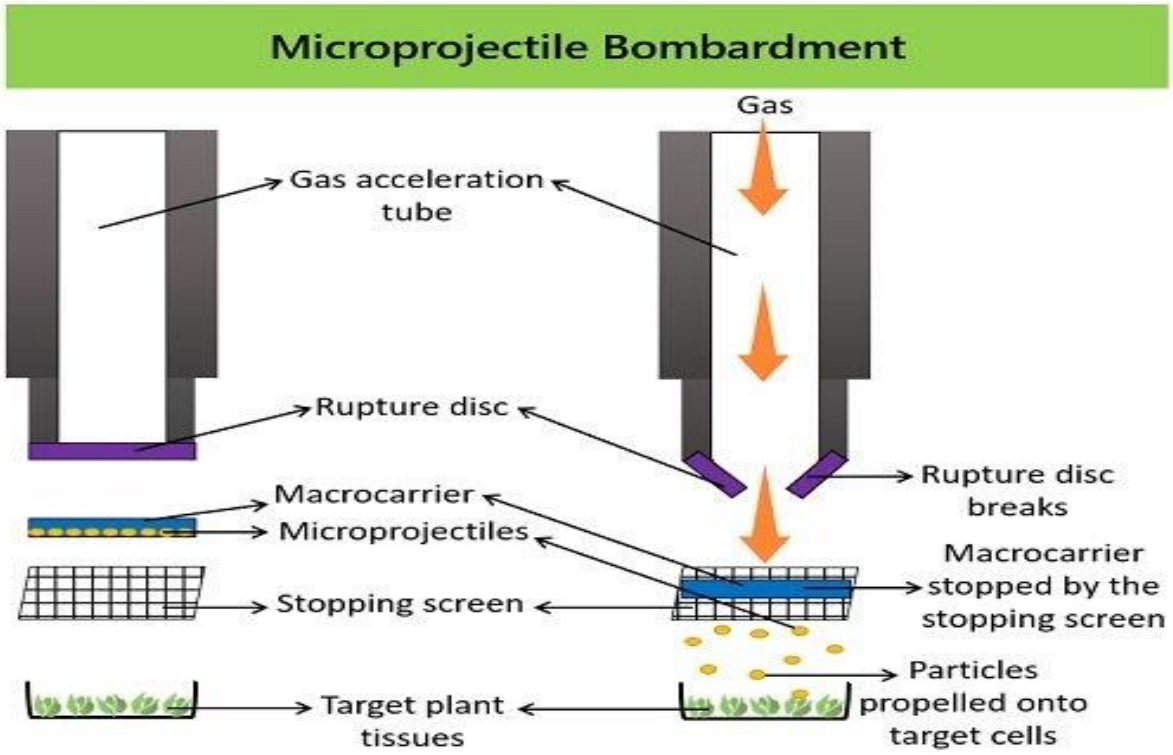
Electroporation:

- Electroporation involves the creation of pores in the cell membrane using electric pulse of high field strength.

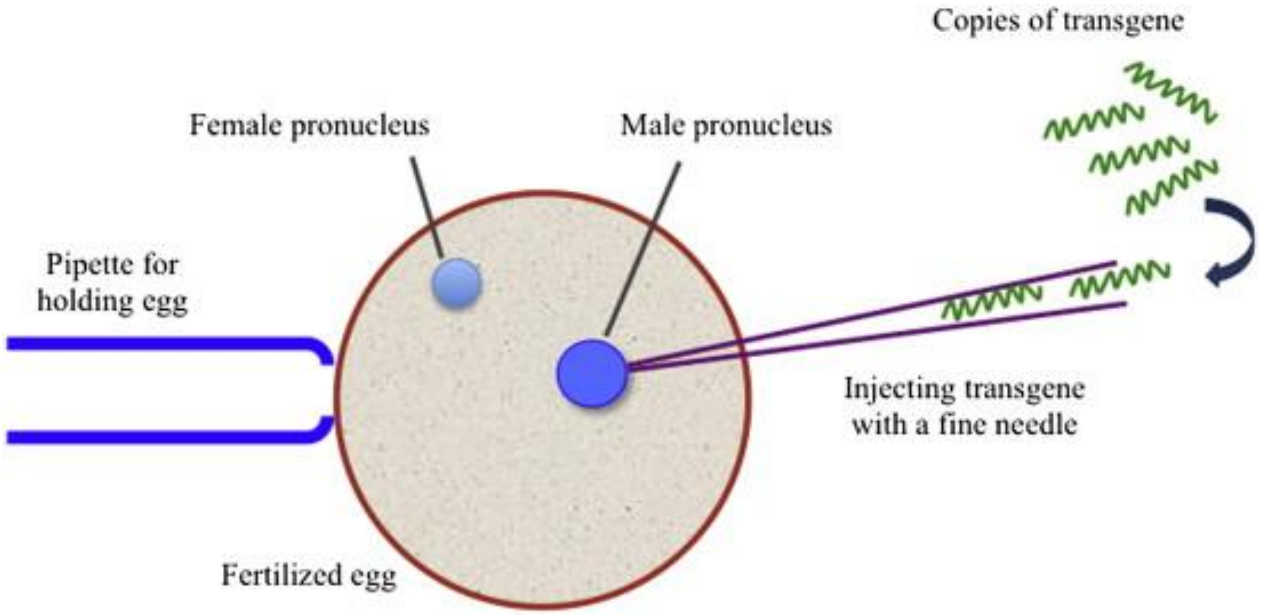


Particle bombardment :

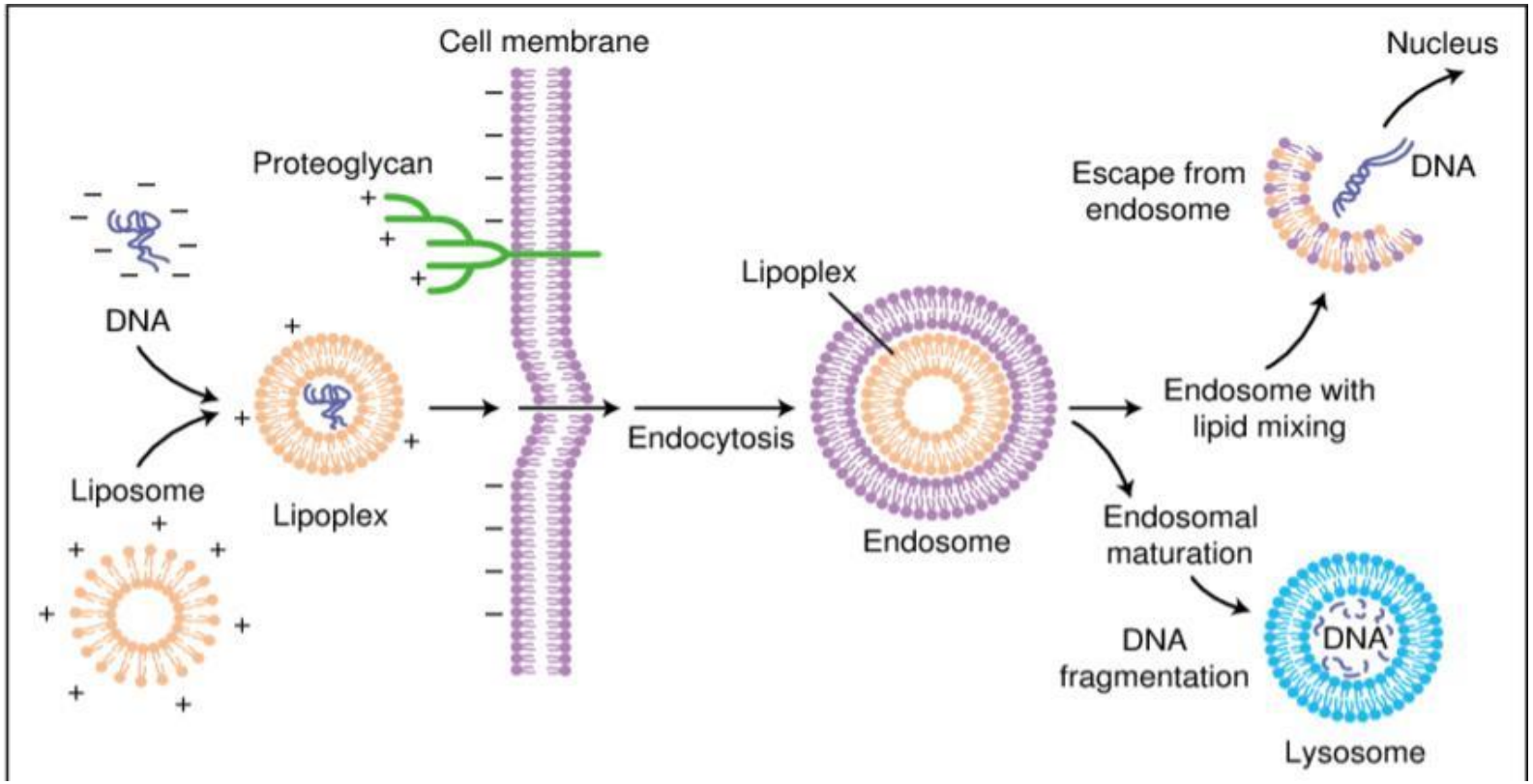
- It is also known as microprojectile bombardment, biolistics, gene gun, etc.
- Foreign DNA coated with high velocity **gold** or **tungsten** particles to deliver DNA into cells.



- **Micro injection:**
- The most significant use of this is the introduction of DNA into the oocyte and the eggs of animals, either the transient expression analysis or to generate transgenic animals.



- **Liposome mediated gene transfer:**
- A method of gene transfer that uses liposomes, which are artificial lipid vesicles, to encapsulate and deliver genetic material (such as DNA or RNA) into cells.



CHEMICAL GENE MEDIATED TRANSFER

- **Polyethylene glycol mediated transformation**

It is used to introduce foreign DNA into cells by using polyethylene glycol (PEG) to facilitate the uptake of DNA through the cell membrane

- **Deae dextron mediated Transfer:**

The desirable DNA can be complexed with a high molecular weight polymer diethyl amino ethyl(DEAE)dextran and transferred.

Application

Transgenic plants have various applications -:

RESISTANCE TO BIOTIC STRESS

1. INSECT RESISTANCE
2. VIRUS RESISTANCE
3. FUNGAL AND BACTERIAL RESISTANCE

RESISTANCE TO ABIOTIC STRESS

1. HERBICIDE RESISTANCE
2. GLYPHOSATE RESISTANCE

IMPROVEMENT OF CROP YIELD & QUALITY

1. EXTENDED SELF LIFE OF FRUITS
2. IMPROVED NUTRITION
3. IMPROVED COLORATION

PRODUCTION OF LOW-COST PHARMACEUTICALS

1. EDIBLE VACCINES
2. ESSENTIAL PROTEINS

EXAMPLES OF TRANSGENIC PLANTS

Bt Cotton: Pest Resistant Crop

Bt cotton is genetically modified with the Bt gene.
Provides protection against bollworm, a major pest of cotton.

Bt Corn: Herbicide and Pest-Resistant Crop

- The most widely used GMO crop in the United States.
- **Herbicide Resistance**: Can withstand certain herbicides, making weed control easier.
- **Pest Resistance:** Produces a protein toxic to pests but safe for humans and animals.

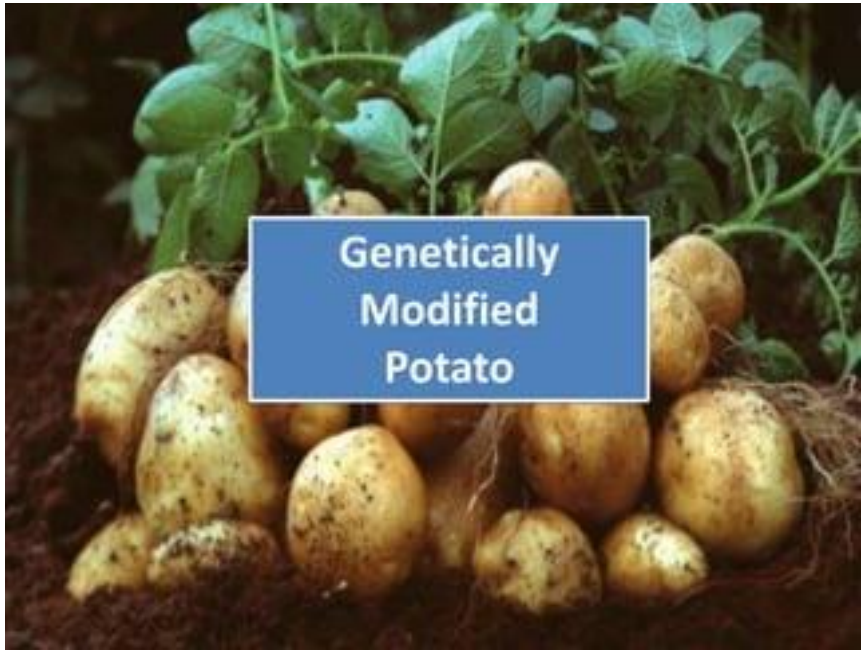
Golden Rice : Bio fortified crop

Engineered to produce beta-carotene, a precursor of vitamin A.

Beta-carotene is not naturally present in conventional rice varieties.

GMO Potatoes

- **Pest Resistance:** To protect plants from pests and reduce the need for pesticides.
- **Quality Improvement**: Preventing browning and bruising of tubers for better appearance and longer shelf life.



Flavr-savr Tomato

- This is Produced by antisense technology.
- The polygalactouronase gene, which is responsible for fruit decay is silenced.

Disadvantages:

Damage to human health

- Allergies
- horizontal transfer and antibiotic resistance
- eating foreign DNA
- changed nutrient levels

Damage to the natural environment

- crop-to-weed gene flow
- leakage of GM proteins into soil
- reductions in pesticide spraying

Disruption of current practices of farming and food production in developed countries

- crop-to-crop gene flow

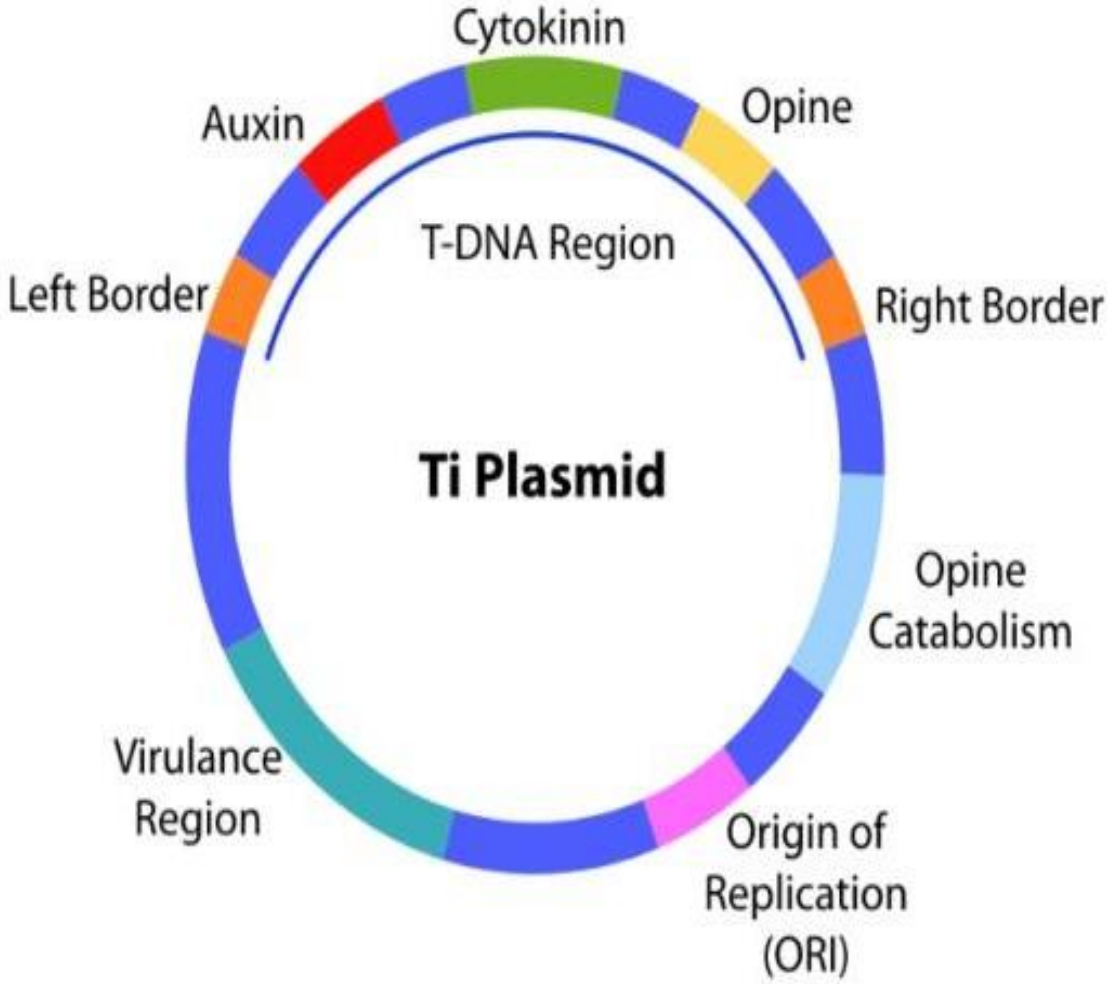
- **Disruption of traditional practices and economies in less developed countries**
- **Lack of research on consequences of transgenic crops**

AGAROBACTERIUM MEDIATED GENE TRANSFER

Agarobacterium tumifaciens

Agrobacterium tumefaciens (synonym Agrobacterium radiobacter) is a rod shaped, Gram negative soil bacterium, the causal agent of **crown gall disease** in over 140 species of dicots.

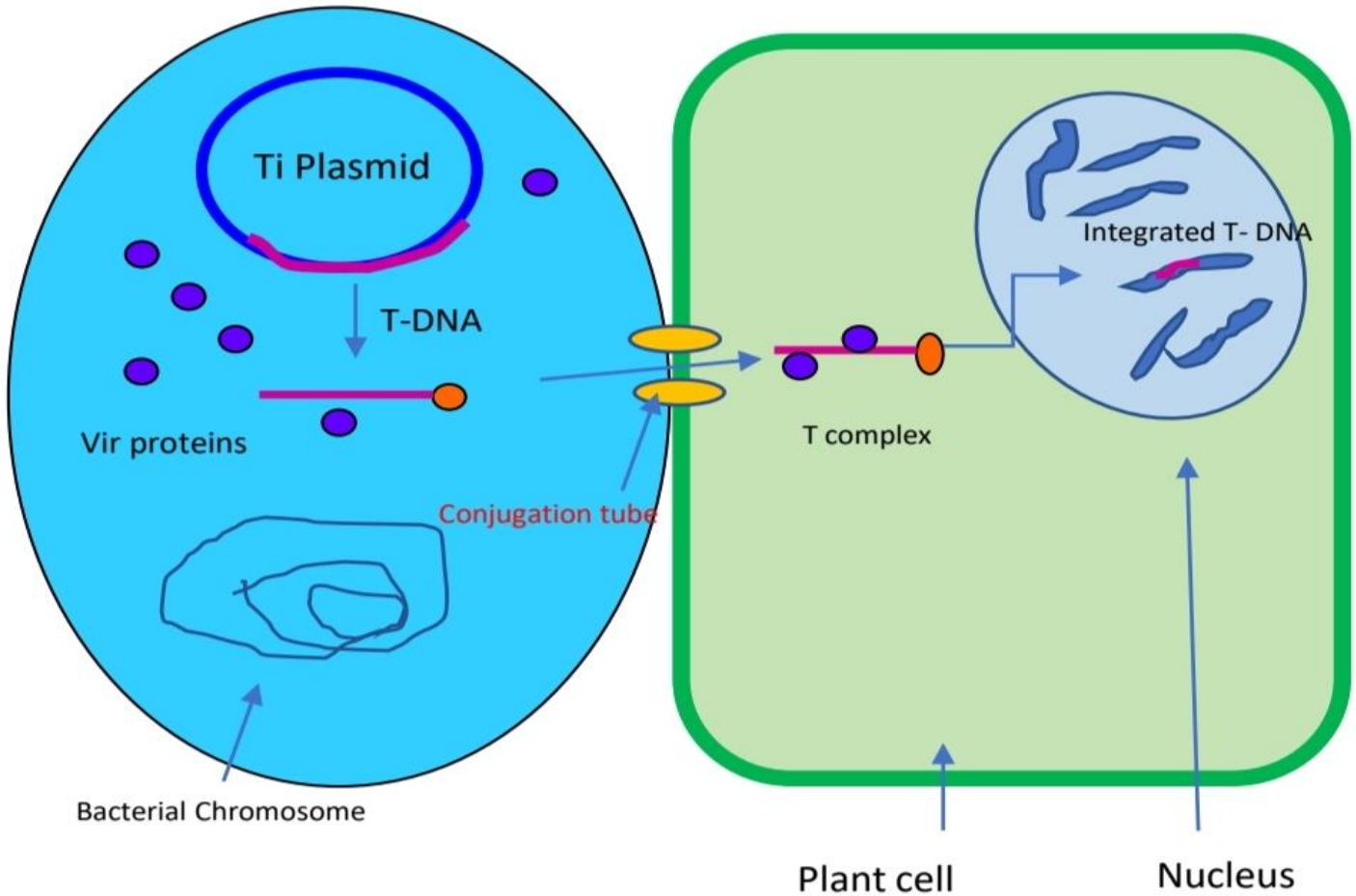
A. tumefaciens harbors a plasmid called Ti plasmid, is capable of transferring a particular DNA segment (T DNA) of the tumour-inducing (Ti) plasmid into the nucleus of infected cells where it is subsequently stable integrated into the host genome and transcribed, causing the crown gall disease



- T-DNA contains eight or so genes that are expressed in the plant cell and are responsible for the cancerous properties of the transformed cells. These genes also direct synthesis of unusual compounds, called opines, that the bacteria use as nutrients.
- Genes in the virulence region are grouped into the operons virA,B,C,D,E,F,G, which code for the enzymes responsible for mediating transduction of T-DNA to plant cells

The T-DNA contains two types of genes:

- the **tumor inducing genes,**
- encoding for enzymes involved in the synthesis of auxins and cytokinins which are responsible for tumour formation;
- and the **Opine synthase genes that is genes encoding for the synthesis of opines,**
- a product resulted from condensation between amino acids and sugars, which are produced and excreted by the crown gall cells and consume by A. tumefaciens as carbon and nitrogen sources.


Ti Plasmid
T-DNA
Vir proteins
Conjugation tube
Bacterial Chromosome
T complex
Integrated T- DNA
Plant cell
Nucleus

Overall Mechanism:

The process of gene transfer from Agrobacterium tumefaciens to plant cells requires these essential steps:

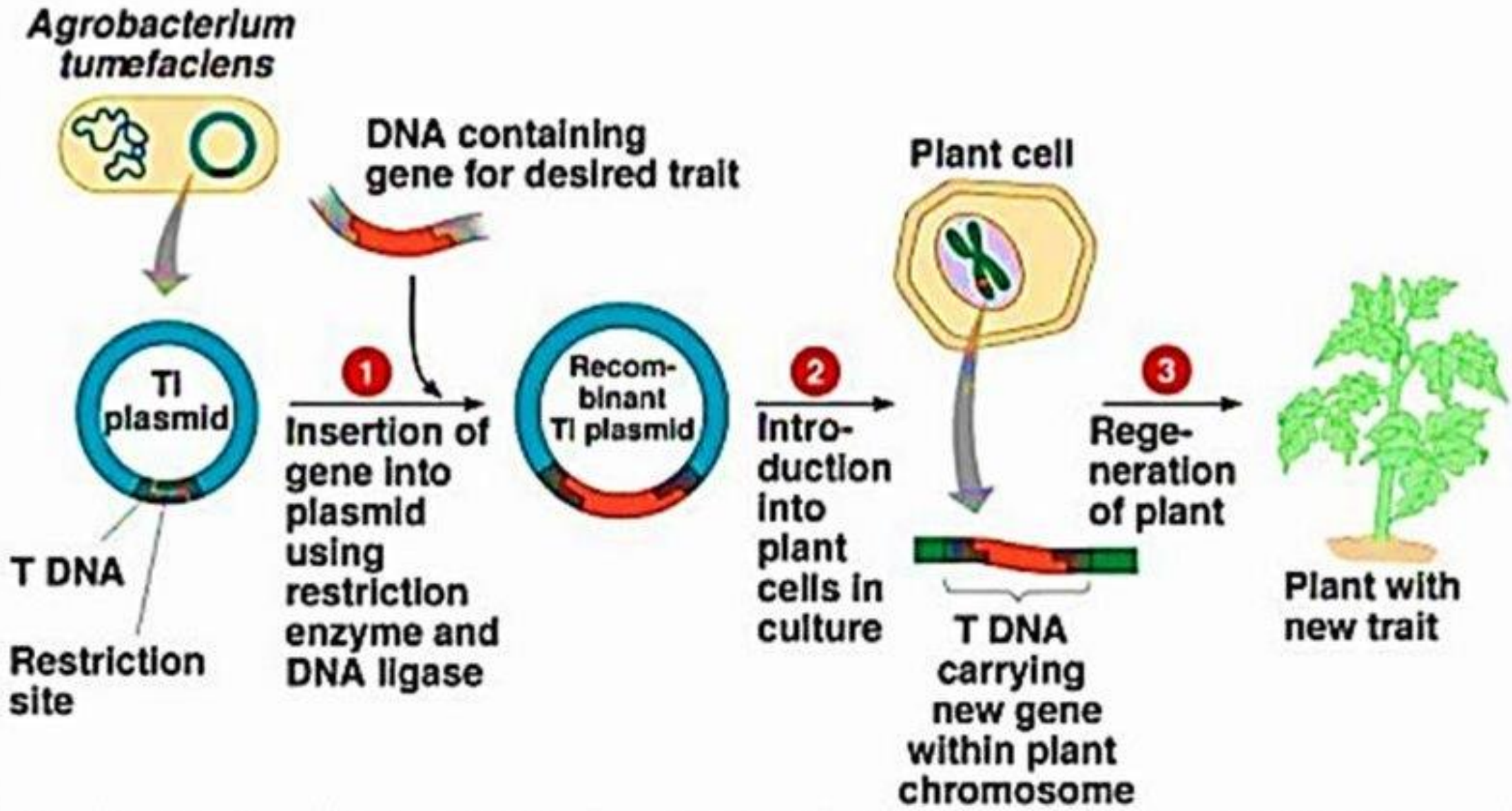
(1) bacterial colonisation

(2) induction of bacterial virulence system,

(3) Generation of T-DNA transfer complex

(4) T-DNA transfer and

(5) integration of T-DNA into plant genome


Agrobacterium tumefaciens
DNA containing gene for desired trait
Plant cell
Ti plasmid
1
Recom-binant Ti plasmid
2
3
Rege-neration of plant
Plant with new trait
T DNA
Restriction site
Insertion of gene into plasmid using restriction enzyme and DNA ligase
Intro-duction into plant cells in culture
T DNA carrying new gene within plant chromosome

- The tumour formation is a transformation process of plant cells resulted from transfer and integration of T-DNA and the subsequent expression of T-DNA genes.
- Any foreign DNA placed between the T-DNA borders can be transferred to plant cell, no matter where it comes from.

PLANT TISSUE CULTURE

INTRODUCTION:

- Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition.
- Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation.

METHODS OF PLANT TISSUE CULTURE:

- **Plant tissue culture includes two major methods.**
- **(A) Type of in vitro growth-callus and suspension cultures.**
- **(B) Type of explant- single cell culture, shoot and root cultures, somatic embryo culture, meristem culture, anther culture and haploid production, protoplast culture and somatic hybridisation, embryo culture, ovule culture, ovary.**

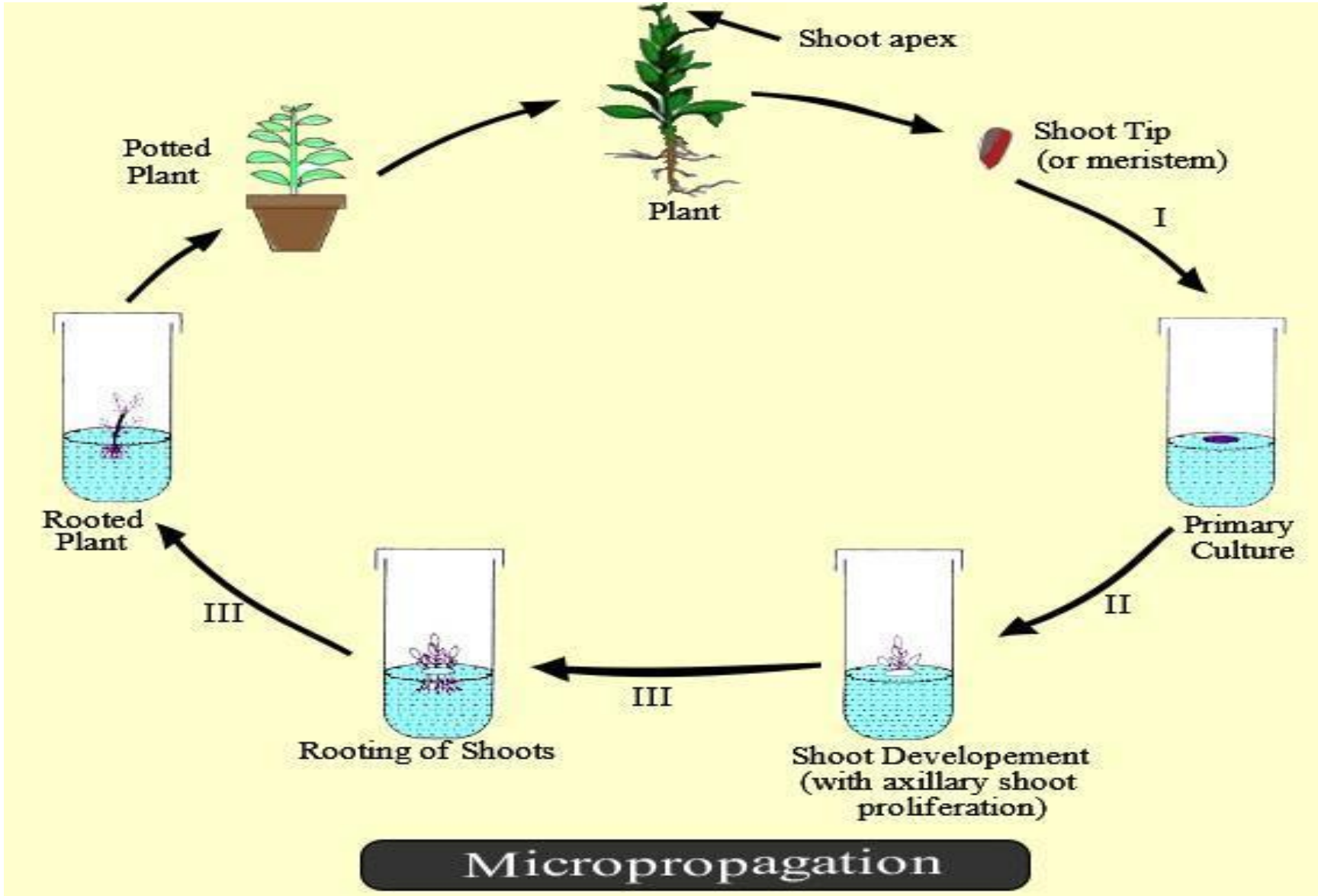
STERILIZATION TECHNIQUES:

- **Sterilization is achieved by one of the following approaches:**
- **(i) dry heat treatment**
- **(ii) flame sterilization**
- **(iii) autoclaving**
- **(iv) filter sterilization**
- **(v) wiping with 70% ethanol**
- **(vi) surface sterilization**

Types of Plant Tissue Culture:

MICROPROPAGATION:

- Micropropagation is also called micro cloning or clonal propagation, is the vegetative propagation of plants by tissue culture techniques, using cells, tissues, organs, etc.
- Method of plant propagation using extremely small pieces of plant tissue taken from a carefully chosen and prepared mother plant, and growing these under laboratory conditions to produce new plants.


Shoot apex
Plant
Shoot Tip
(or meristem)
I
Primary
Culture
II
Shoot Developement
(with axillary shoot
proliferation)
III
Rooting of Shoots
III
Rooted
Plant
Potted
Plant
Micropropagation

ADVANTAGES

- **Rapid multiplication of plants within a short period and on small space.**
- **Plants are obtained under controlled conditions, independent of seasons.**
- **Production of virus free plants like potato, sugarcane, banana and apple for horticulture and agriculture.**
- **DISADVANTAGES:**
- **High startup costs**
- **Not all plants can be micropropagated**
- **Risk of contamination**
- **Genetic changes in plant**
- **Need for skilled workers**

PROTOPLAST ISOLATION

- Protoplasts can be isolated from a range of plant tissues: leaves, stems, roots, flowers, anthers and even pollen.
- The isolation and culture media used vary with the species and with the tissue from which the protoplasts were isolated.
- Protoplasts in labs are isolated mainly using two techniques:
- Mechanical Method
- Enzymatic Method

- Mechanical Method: It is done by cutting plasmolyzed tissue with knife and releasing the protoplast by deplasmolysis. In this method the protoplast released are very few in number.
- Enzymatic Method: A concentrated solution of cellulase enzyme is taken and the leaves are dipped in the solution. This isolates the protoplast by degrading the cell wall.

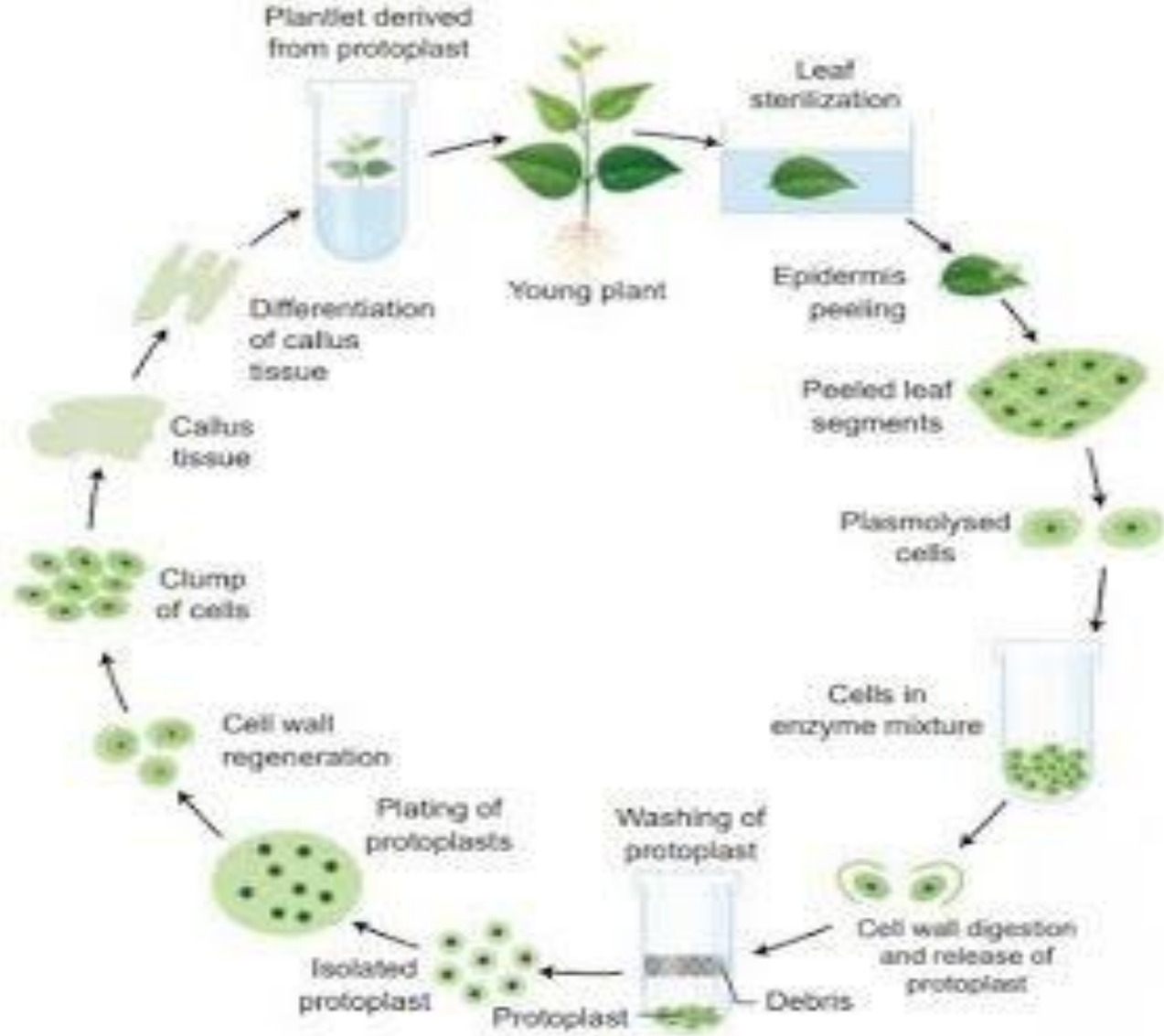
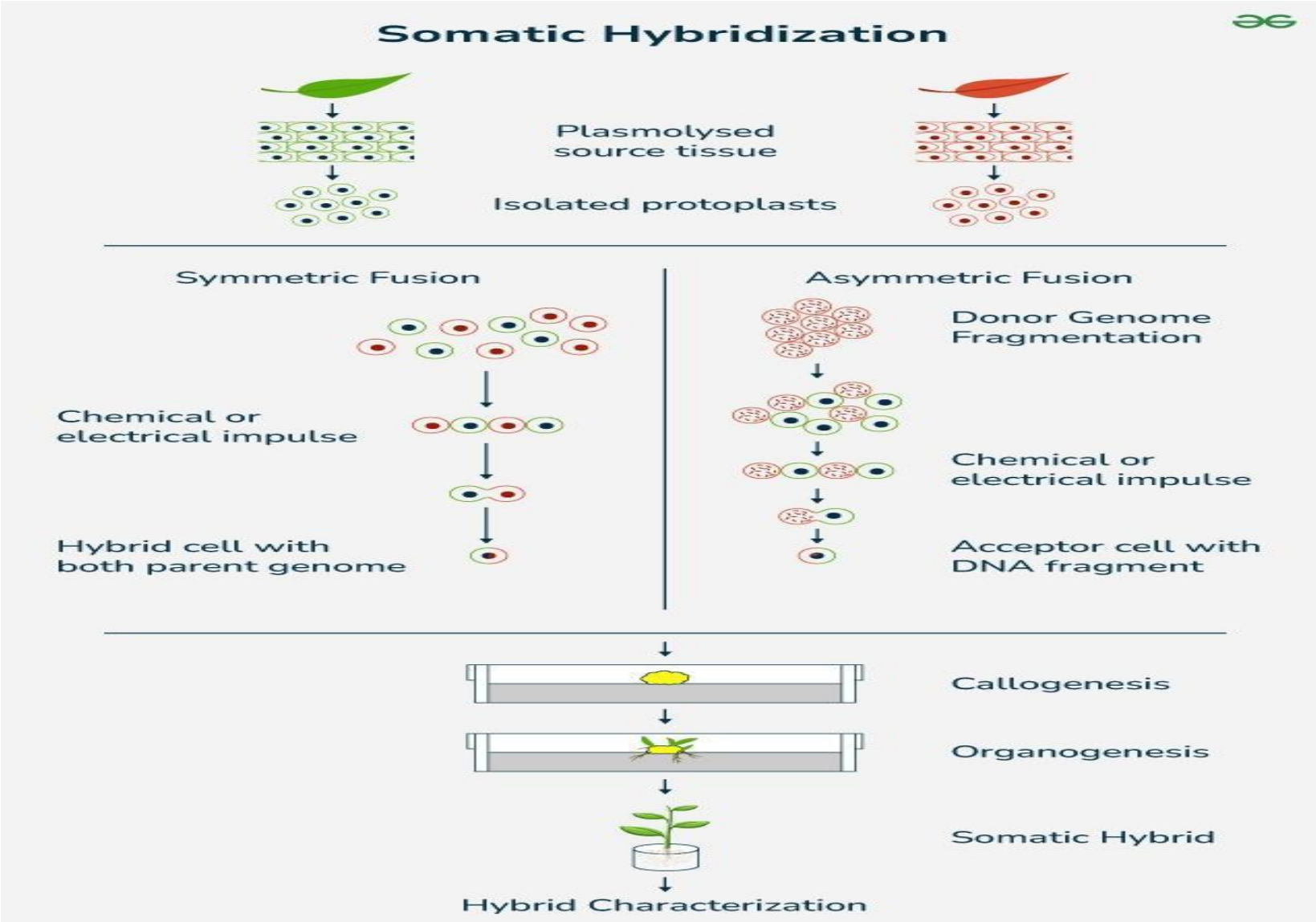


Figure 5.8: Protoplast Culture

SOMATIC HYBRIDS

- Somatic hybridization is a technique of fusing protoplasts from different plant species to create hybrid plants .For example Banana breeding: Employed to combine traits for disease resistance and improved fruit quality from different banana varieties.
- Somatic hybridization involves three processes. They are as follows:
- Protoplast fusion
- Hybrid Cell Selection
- Identifying hybrid plants


Somatic Hybridization
Plasmolysed source tissue
Isolated protoplasts
Symmetric Fusion
Chemical or electrical impulse
Hybrid cell with both parent genome
Asymmetric Fusion
Donor Genome Fragmentation
Chemical or electrical impulse
Acceptor cell with DNA fragment
Callogenesis
Organogenesis
Somatic Hybrid
Hybrid Characterization

APPLICATION

- Crop Improvement: Somatic hybridization is used to develop crop varieties with desirable traits such as disease resistance, stress tolerance, and improved yield.
- Biotechnological Research: Somatic hybridization is instrumental in studying gene expression, functional genomics, and understanding the mechanisms of plant development and stress responses.
- Disease Resistance: Disease-resistance genes have been able to spread from one plant to many others due to somatic hybridization.

- **Advantages of Somatic Hybridization:**
- Somatic hybridization can be performed on young, immature plants.
- It is now simple to research cytoplasmic genes and how they work.
- **Limitations of Somatic Hybridization:**
- The created plants are not always healthy and fruitful.
- There are only a few selection criteria.

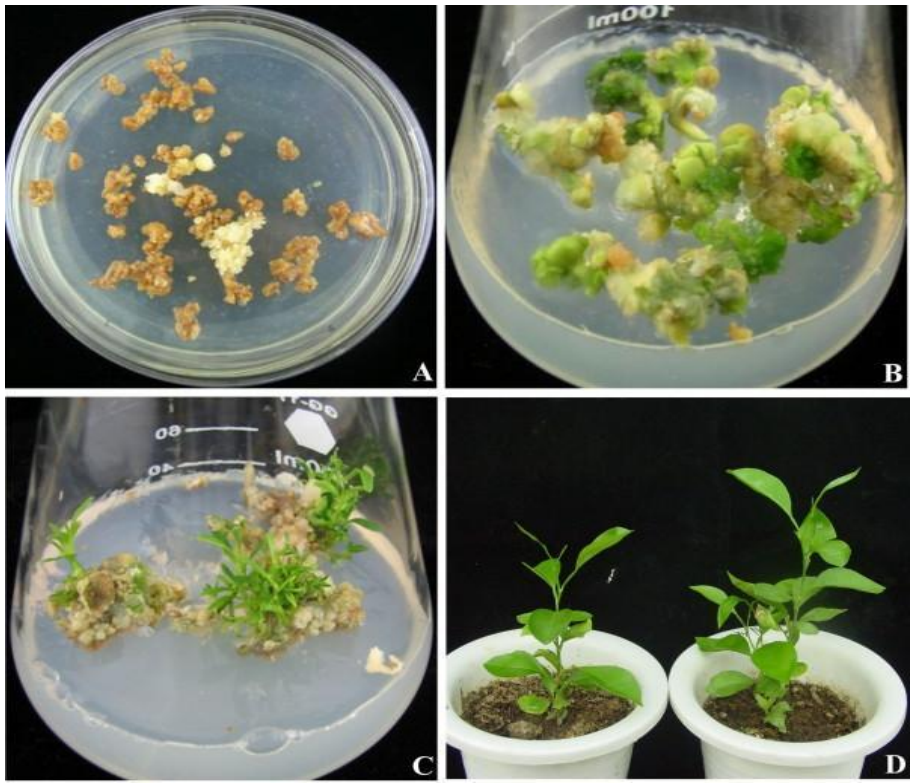
IDENTIFICATION OF TRANSFORMED CELLS INTO CALLUS

- Identifying transformed cells within a callus involves several steps and techniques to ensure that only the cells that have successfully incorporated the transgene are selected and regenerated into whole plants.
- Observation of Morphological Changes: Transformed cells exhibit distinct morphological changes. Look for unorganized, undifferentiated mass of cells, typically forming a lump or callus.
- Under a microscope, the callus cells can be observed for changes in cell structure.

- Use Selective Media: Grow cells on a medium with antibiotics or other selective agents. Only transformed cells will survive and form callus .
- Perform Molecular Tests- PCR: Check for specific DNA sequences from the transformation.
- Reporter Genes: Use markers like GFP (green fluorescent protein) to see if the cells glow under UV light.
- These methods help confirm which cells have successfully transformed into callus.

REGENERATION OF TRANSGENIC PLANTS

- Regeneration of transgenic plants means growing a whole new plant from a single cell that has been genetically modified (transformed) in a laboratory. This process involves:
- Taking a cell from a plant and changing its DNA (genetic material).
- Helping the modified cell grow into a small mass of cells (callus).
- Inducing the callus to form shoots and roots.
- Transferring the shoots with roots to soil, where they grow into a mature plant.



FACTORS

- Genotype: Plant species and variety.
- Medium composition: Nutrients, hormones, and pH.
- Temperature and light: Environmental conditions.
- Hormone balance: Auxins, cytokinins, and gibberellins.
- Time and duration: Length of regeneration process.

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Genetics- analysis of genes and genome by Daniel L. Harti