MAJOR TECHNIQUES IN GENE MANIPULATION OF PLANTS

INTRODUCTION

- Plant genetic engineering has become one of the most important molecular tools in the modern molecular breeding of crops.
- Over the last decade, significant progress has been made in the development of new and efficient transformation methods in plants.
- Despite a variety of available DNA delivery methods, *Agrobacterium*- and biolistic-mediated transformation remain the two predominantly employed approaches.

- **Production of transgenic plants**

Isolate and clone gene of interest

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Add DNA segments to initiate or enhance gene expression

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Add selectable markers

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Introduce gene construct into plant cells (transformation)

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Select transformed cells or tissues

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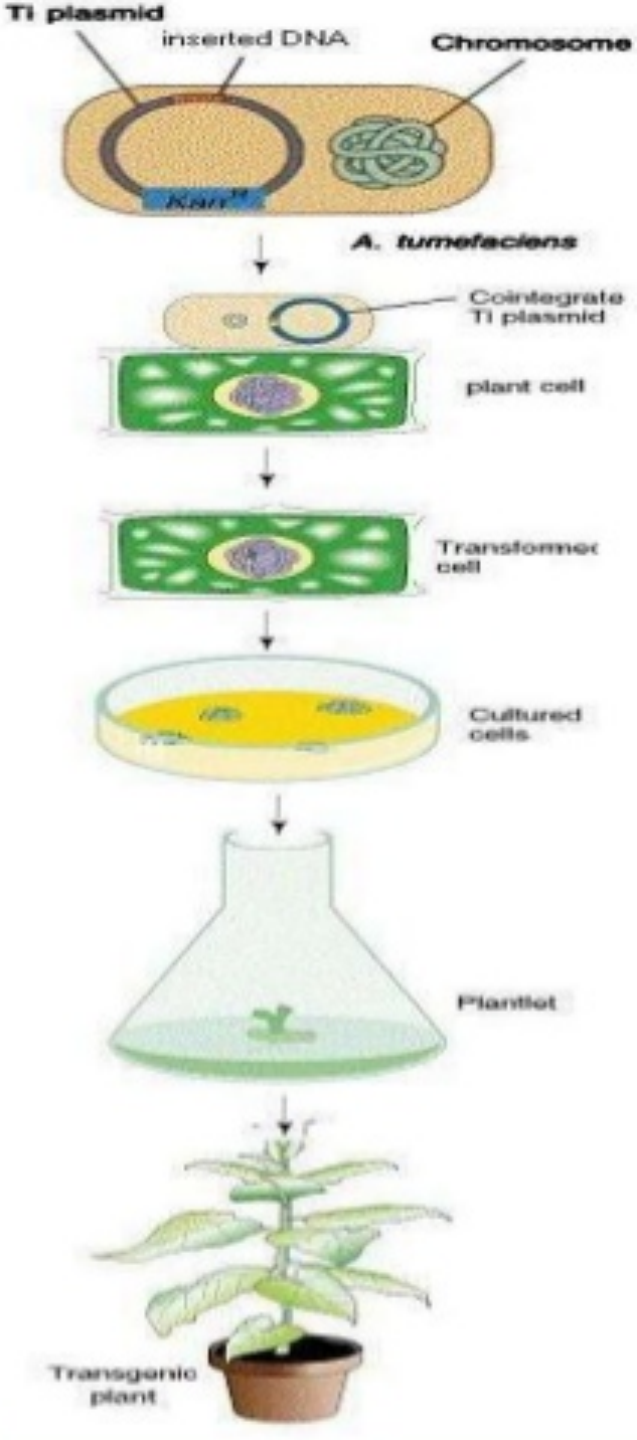
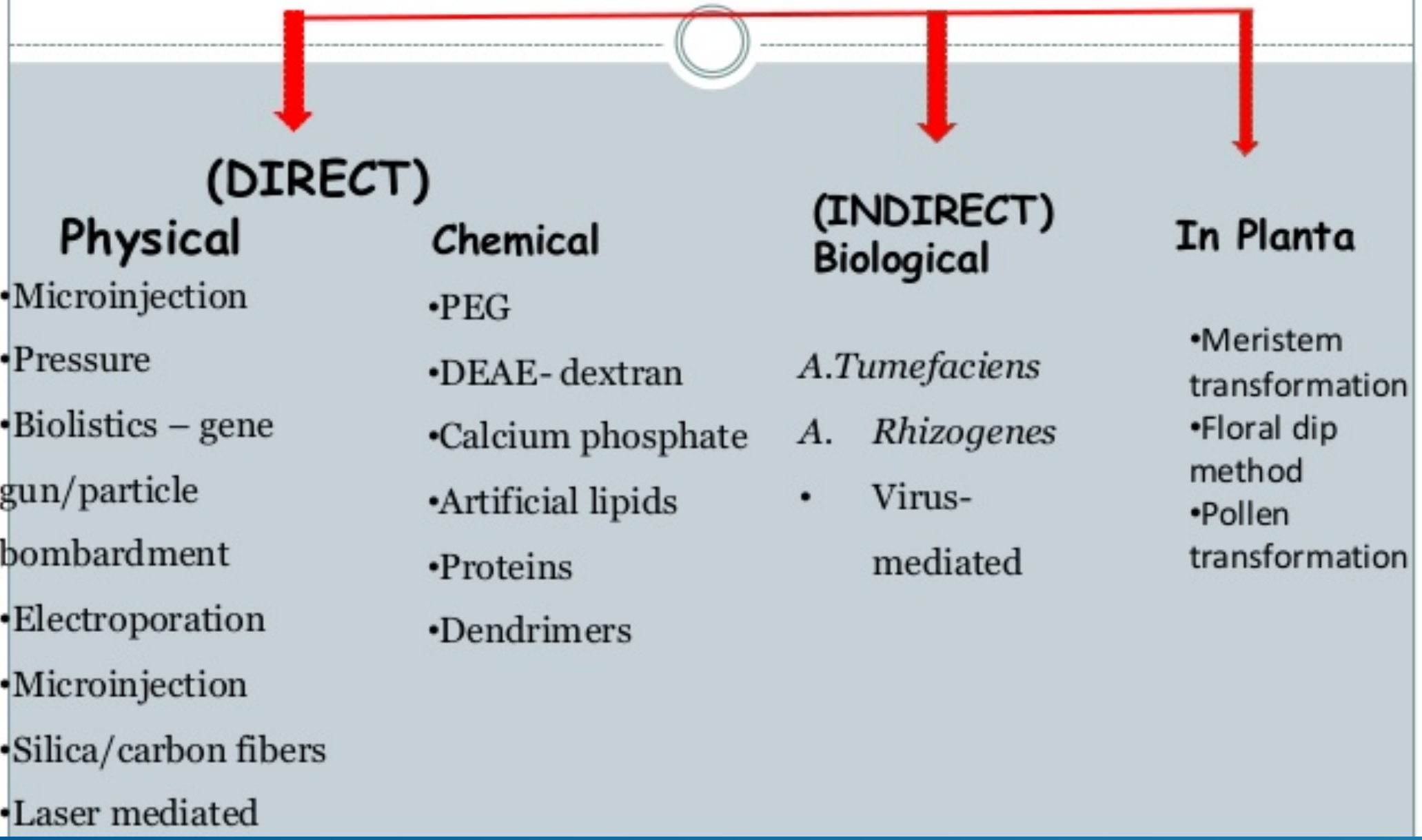
Regenerate whole plants



Plant Transformation Methods



Techniques for plant genetic transformation

- Indirect method- *Agrobacterium* mediated gene transfer
- Direct methods-
  - Particle bombardment (biolistics)
  - Microprojectile gun method
  - Electroporation
  - Silicon carbide fibres
  - Polyethylene glycol (PEG)/protoplast fusion
  - Liposome mediated gene transfer

Transformation vector requirements

- Origin of replication
- Bacterial selectable marker
- Gene constructs of interest
- T-DNA borders and other *Agrobacterium* genes if using *Agrobacterium*
- Compatible with helper plasmid if using *Agrobacterium*

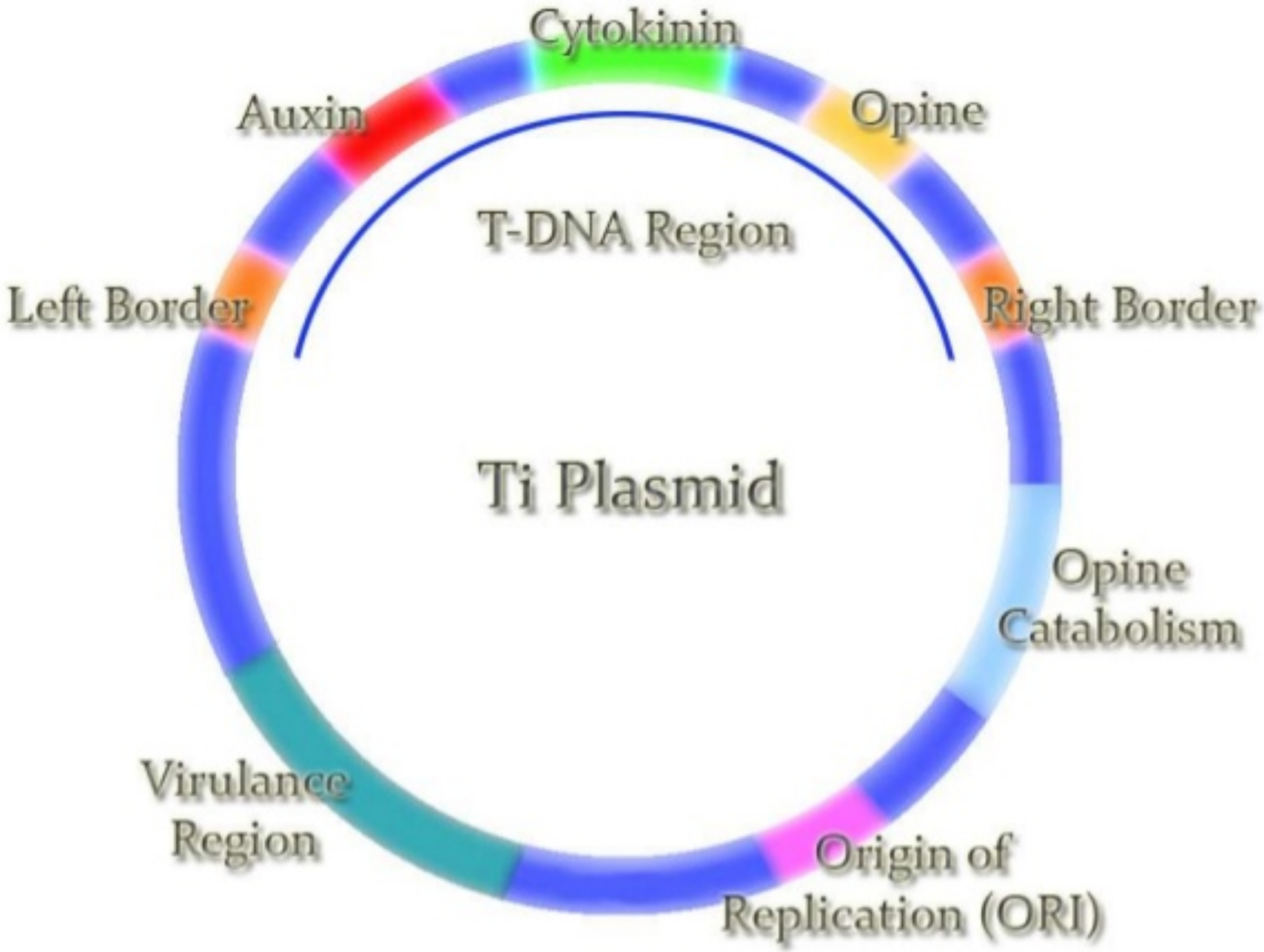
Agrobacterium mediated gene transfer

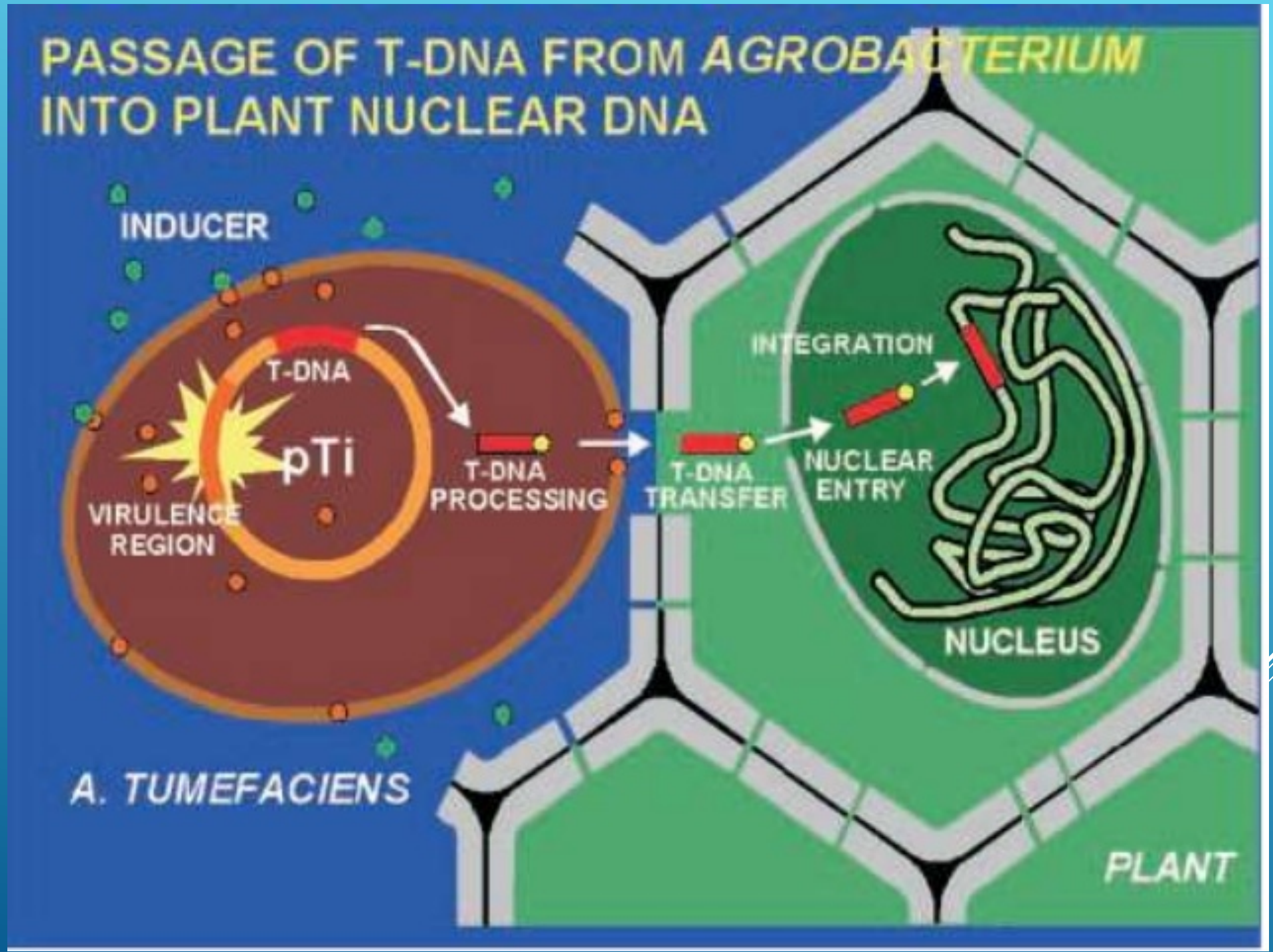
Agrobacterium-

- Soil borne, gram negative, rod shaped, motile found in rhizosphere
- Causative agents of "Crown gall" disease of dicoltyledones
- Have ability transfer bacterial genes to plant genome
- Attracted to wound site via chemotaxis in response to chemicals (sugar and Phenolic molecules: acetosyringone) released from damaged plant cells
- Contains Ti plasmid which can transfer its T-DNA region into genome of host plants

Ti-plasmid features

- Two strains of Ti-plasmid:
  - -Octopine strains- contains two T-DNA region: T_L (14 kb) and T_R (7 kb)
  - -Nopaline strains- contain one T-DNA region(20 kb)
- Size is about 200 kb
- Has a central role in Crown-gall formation
- Contains one or more T-DNA region that is integrated into the genome of host plants
- Contain a *vir* region ~ 40 kb at least 8~11 *vir* genes
- Has origin of replication
- Contains a region enabling conjugative transfer
- Has genes for the catabolism of opines

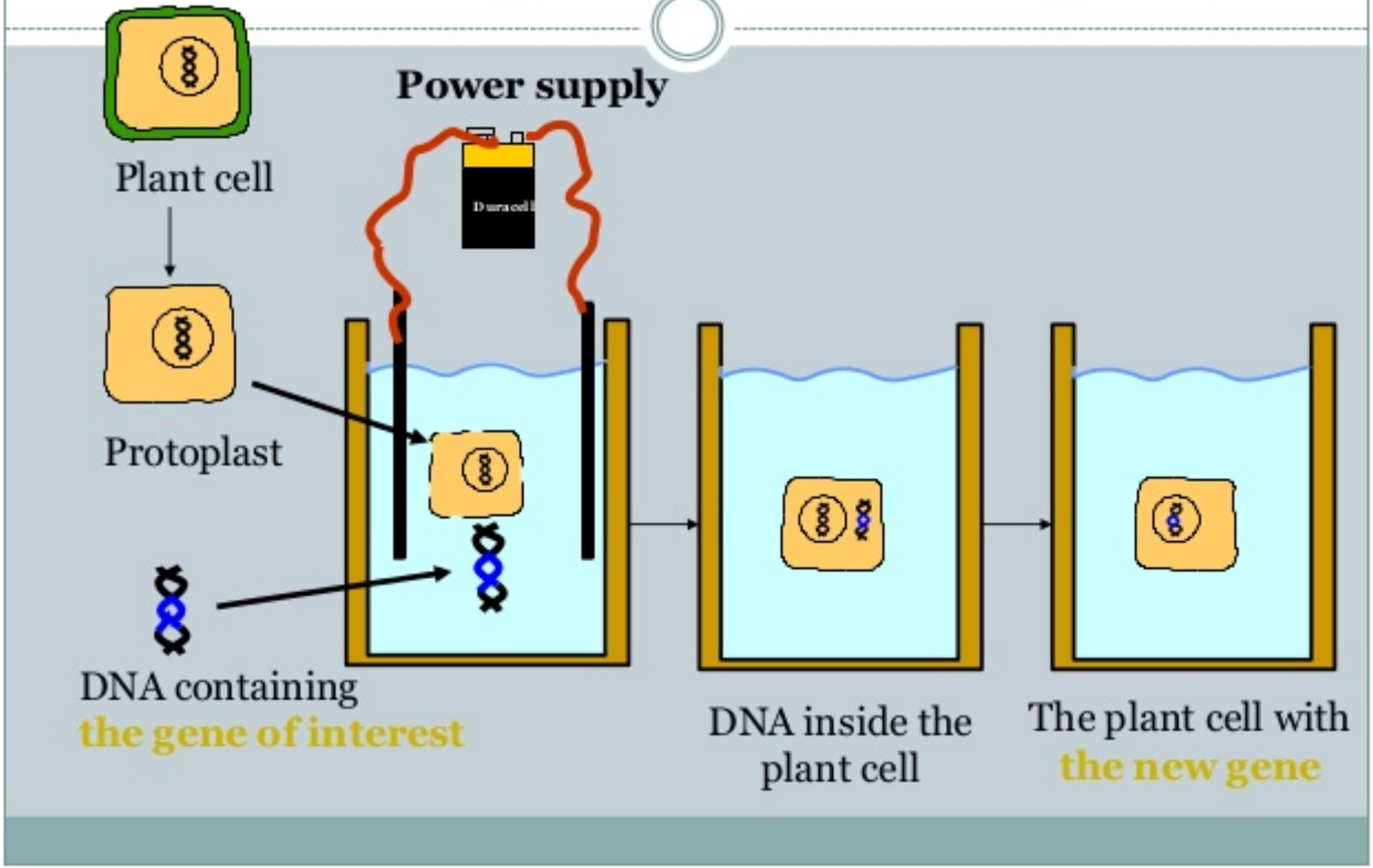

Cytokinin
Auxin
Opine
T-DNA Region
Left Border
Right Border
Ti Plasmid
Opine
Catabolism
Virulance
Region
Origin of
Replication (ORI)


PASSAGE OF T-DNA FROM AGROBACTERIUM
INTO PLANT NUCLEAR DNA
INDUCER
T-DNA
pTi
VIRULENCE
REGION
T-DNA
PROCESSING
T-DNA
TRANSFER
INTEGRATION
NUCLEAR
ENTRY
NUCLEUS
A. TUMEFACIENS
PLANT

Electroporation technique.

- Is the process where by electrical impulses of high field strength are used to reversibly permeabalize cell membrane to facilitates uptake of large molecules, including DNA.
- It has been used for long time for transient and integrative transformation of protoplasts.
- 1 to 1.5 k V. so uses low capacitance hence short decay time .

Electroporation Technique



Biolistic/Particle bombardment

- High velocity micro projectile were utilize to deliver nucleic acids into loving cells.
- **Advantages :**

➢ Transformation of organized tissue

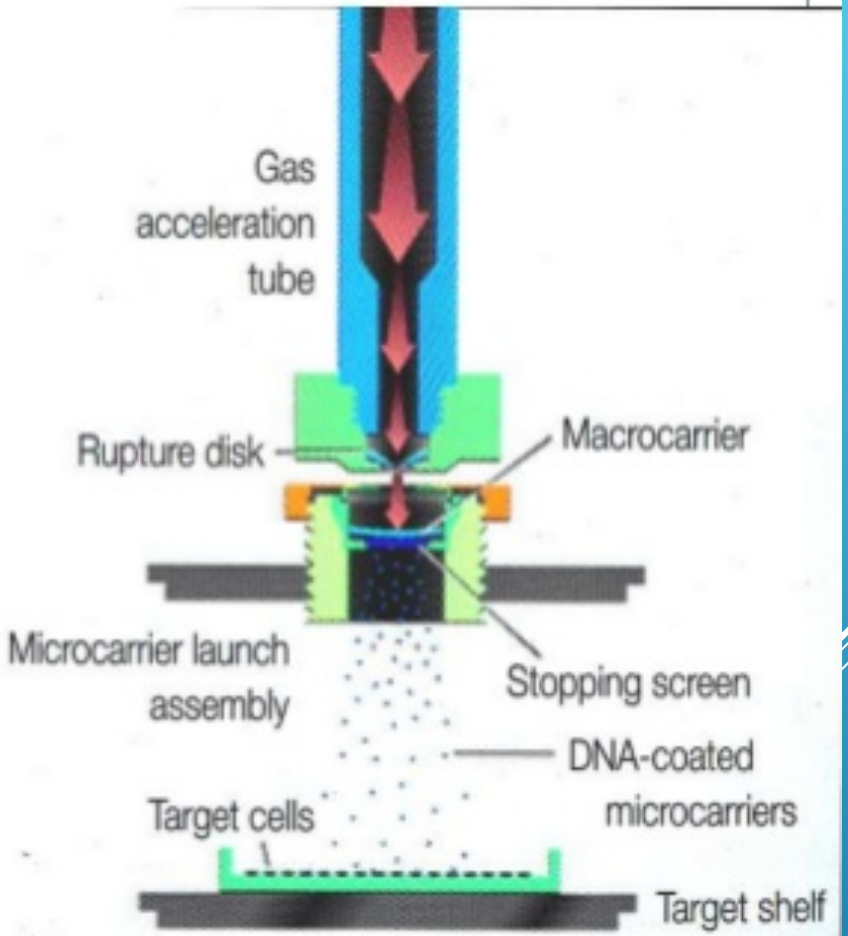
➢ Universal delivery system

➢ Transformation of recalcitrant spp

➢ Study of basic plant development processes.

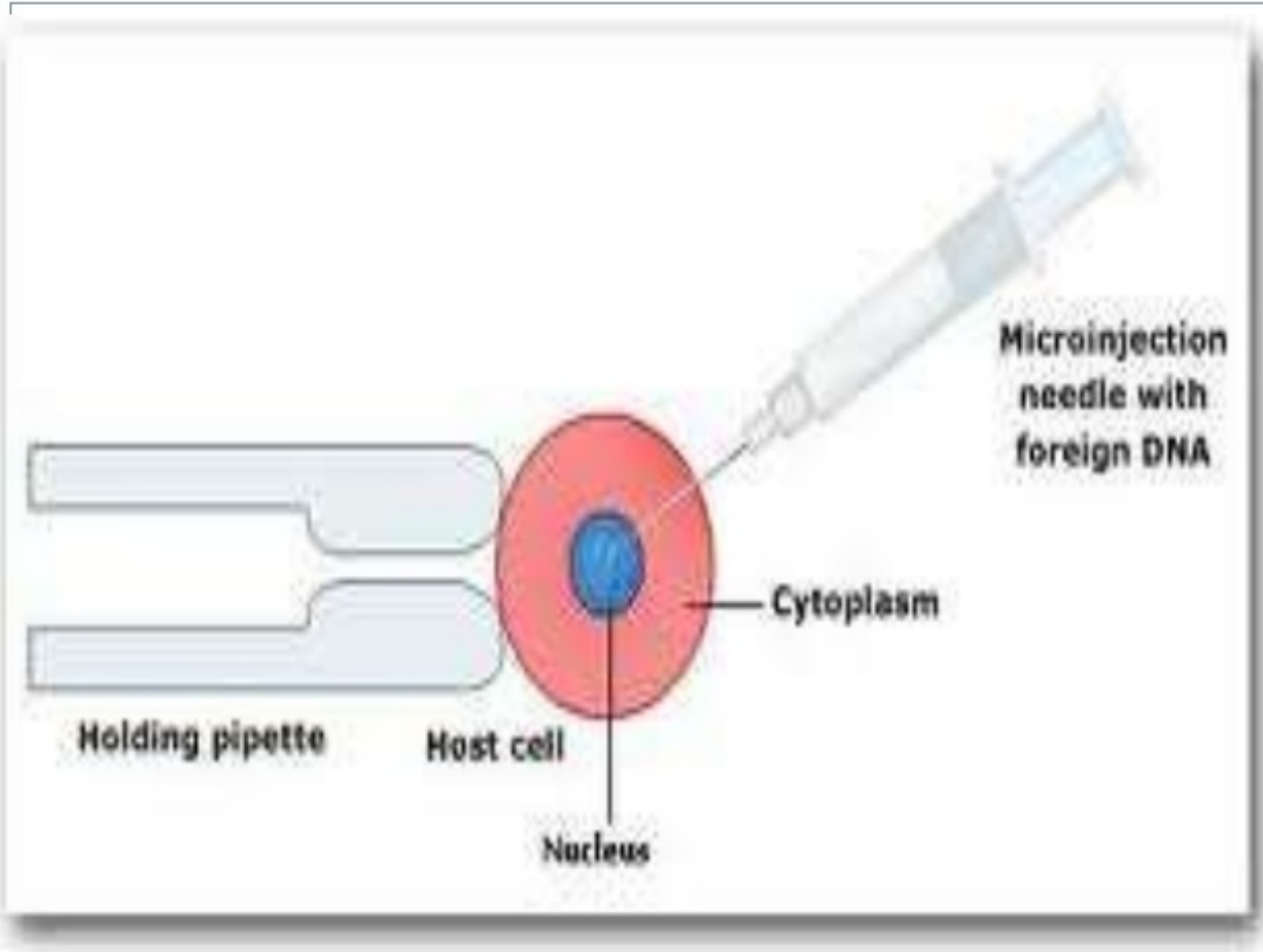
Particle Gun

The Helium Gas Gun – Circa 2000



Microinjection

- Under a microscope, a cell is manipulated to a blunt capillary. Gentle suction holds the cell in place.
- With a micromanipulator, a very fine tipped pipet is inserted into the cytoplasm or nucleus.
- DNA or RNA is injected directly into the nucleus or cytoplasm.
- Microinjection has been successfully used with large frog eggs, cultured mammalian cells, mammalian embryos, and plant protoplasts and tissues


Microinjection needle with foreign DNA
Cytoplasm
Holding pipette
Host cell
Nucleus

Thank you