Lecturer: Senior Lecturer, Department of Molecular Biology and Genetics, PhD, Smekenov I.T.
Subject: Genetic engineering

(Lecture 8)

Genetic engineering of plants. Regulatory elements of the plant genome

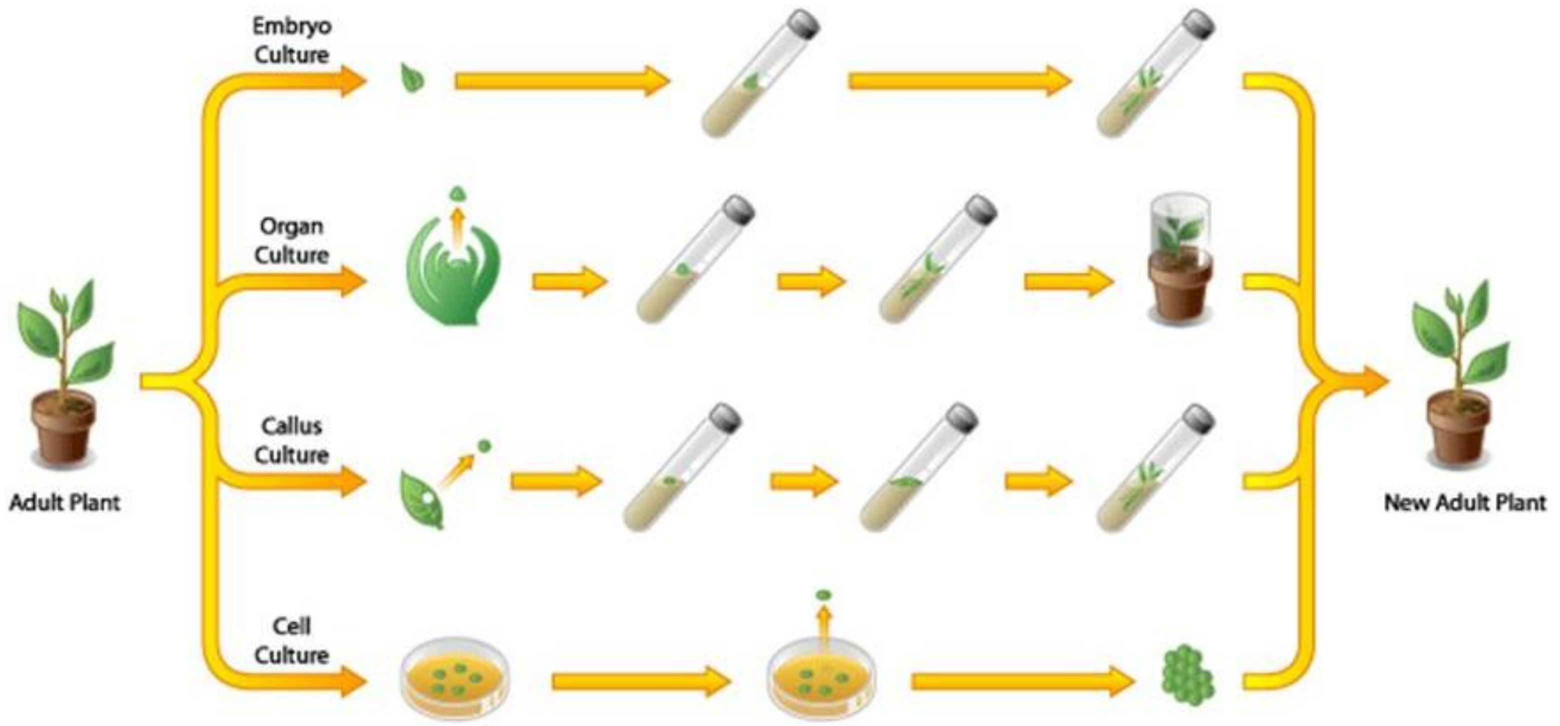
Lecture Goal:
To understand plant recombinant DNA technology using crown gall plasmids, focusing on the genetic engineering of plants and the regulatory elements of the plant genome

Tasks:

1. Describe the role of crown gall plasmids (Ti plasmids) in plant genetic engineering and their mechanism of action in transferring genes into plant cells.
2. Discuss the techniques used in the genetic engineering of plants, including the transformation methods and selection of transgenic plants.
3. Explain the regulatory elements of the plant genome that control gene expression, including promoters, enhancers, and terminators.

Keywords: *Plant recombinant DNA technology, crown gall plasmids, Ti plasmids, genetic engineering of plants, plant transformation, transgenic plants, regulatory elements, plant genome, promoters, enhancers, terminators*

Genetic engineering can be used to introduce specific traits into plants.



- It is possible due to the fact that plants are **totipotent**, enabling regeneration of a new plant from an isolated cell

Agricultural plants are one of the most frequently cited examples of genetically modified organisms (GMOs).

- Some benefits of genetic engineering in agriculture are:

- increased crop yields,
- reduced costs for food or drug production,
- reduced need for pesticides,
- enhanced nutrient composition and food quality,
- resistance to pests and disease,
- greater food security,
- medical benefits to the world's growing population.

Table 1: Examples of GMOs Resulting from Agricultural Biotechnology

Genetically Conferred Trait	Example Organism	Genetic Change
APPROVED COMMERCIAL PRODUCTS		
Herbicide tolerance	Soybean	Glyphosate herbicide (Roundup) tolerance conferred by expression of a glyphosate-tolerant form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) isolated from the soil bacterium *Agrobacterium tumefaciens*, strain CP4
Insect resistance	Corn	Resistance to insect pests, specifically the European corn borer, through expression of the insecticidal protein Cry1Ab from *Bacillus thuringiensis*
Altered fatty acid composition	Canola	High laurate levels achieved by inserting the gene for ACP thioesterase from the California bay tree *Umbellularia californica*
Virus resistance	Plum	Resistance to plum pox virus conferred by insertion of a coat protein (CP) gene from the virus

PRODUCTS STILL IN DEVELOPMENT		
Vitamin enrichment	Rice	Three genes for the manufacture of beta-carotene, a precursor to vitamin A, in the endosperm of the rice prevent its removal (from husks) during milling
Vaccines	Tobacco	Hepatitis B virus surface antigen (HBsAg) produced in transgenic tobacco induces immune response when injected into mice
Oral vaccines	Maize	Fusion protein (F) from Newcastle disease virus (NDV) expressed in corn seeds induces an immune response when fed to chickens
Faster maturation	Coho salmon	A type 1 growth hormone gene injected into fertilized fish eggs results in 6.2% retention of the vector at one year of age, as well as significantly increased growth rates

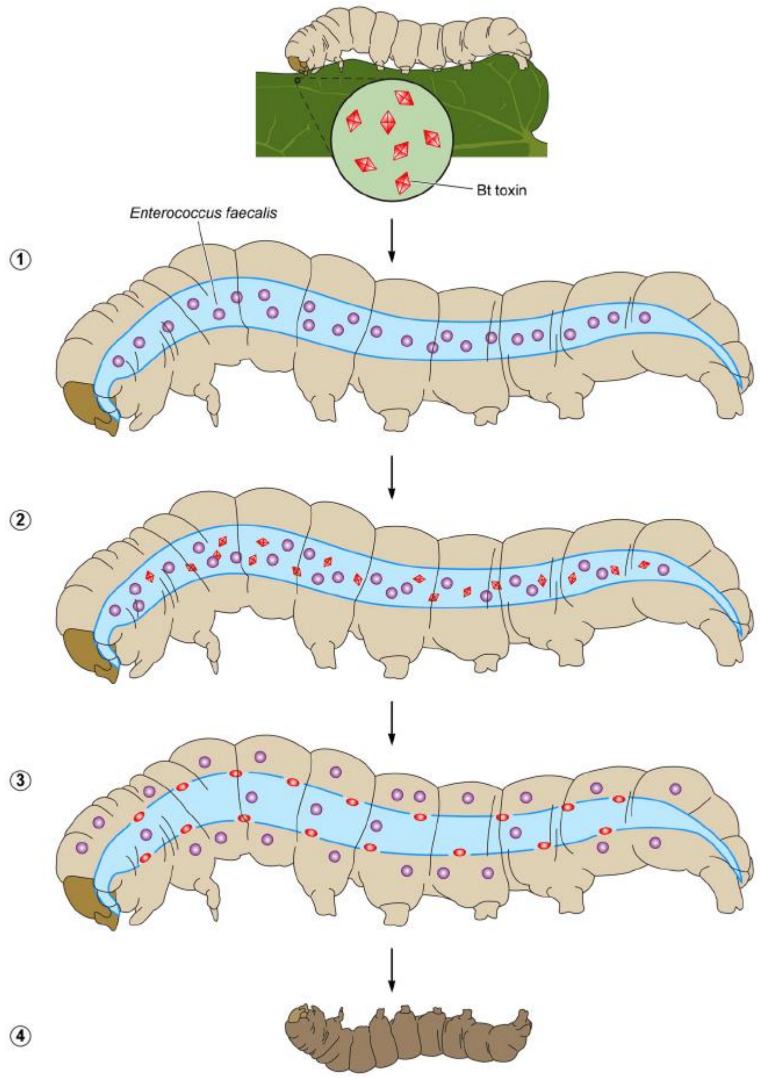
Trait I. Bt (*Bacillus thuringiensis*) toxin

- **What does it do?** It kills caterpillars (in most cases) that eat it (or genetically engineered plants that contain it). Other insects, including pollinators, are unaffected.

- **How does it work?** Bacterial genes that result in production of a protein harmful to insect cells are inserted into genes of the plant. The plant cells now contain the toxic protein and caterpillars that feed on the plant will be killed.

- **Which crops have Bt toxin genes?** Corn, cotton, and eggplant (Bangladesh) (not all seed/plants are genetically modified).

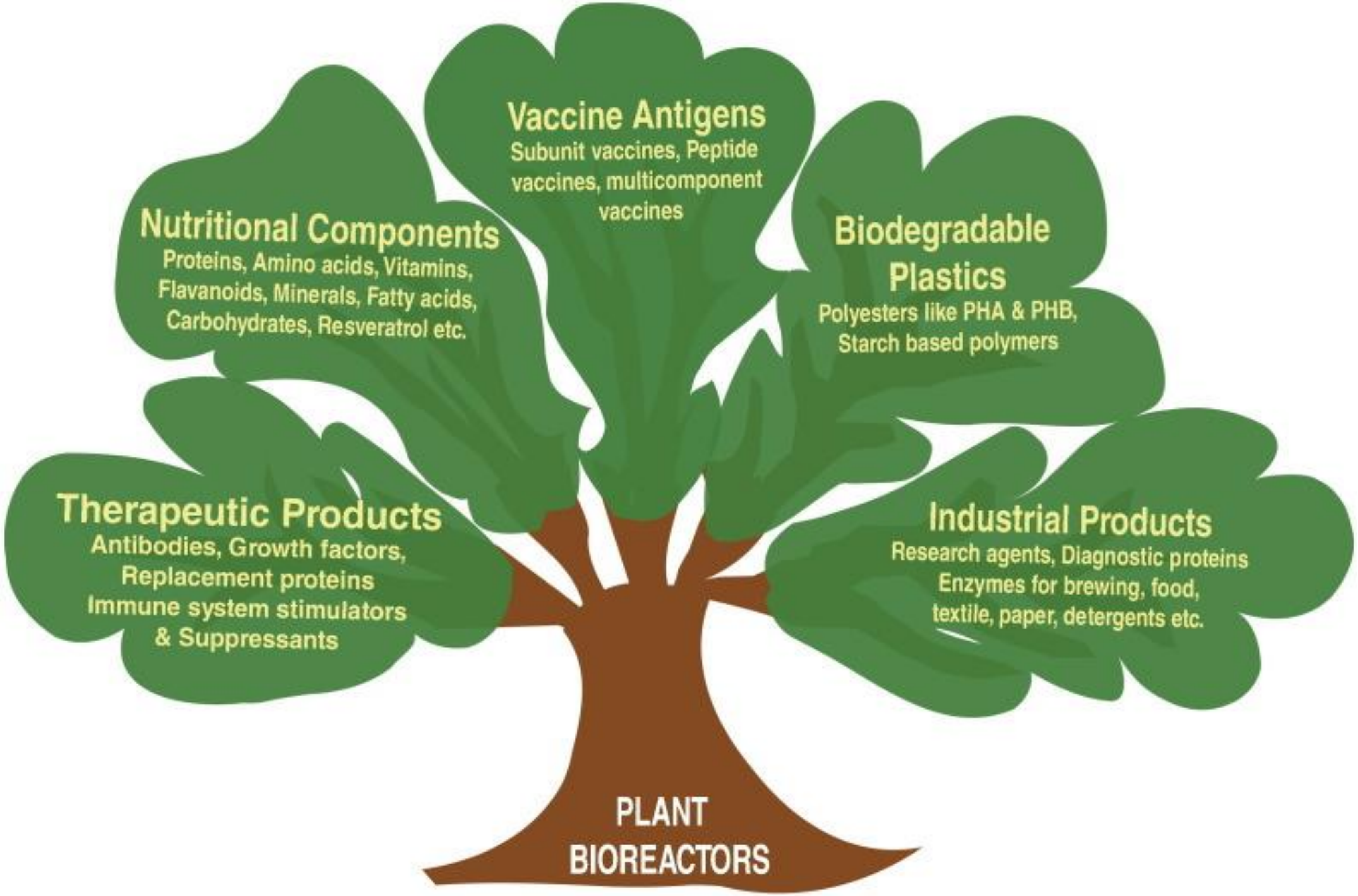
- **Why was this trait introduced?** Some of the most damaging pests of these crops, typically requiring regular applications of insecticides, are caterpillars. By making the plant toxic to the pest, chemical insecticide applications can be reduced. This can reduce harmful effects of pesticides on non-target organisms, handlers and the environment while reducing costs.



Trait II. Resistance to papaya ringspot virus (PRSV).

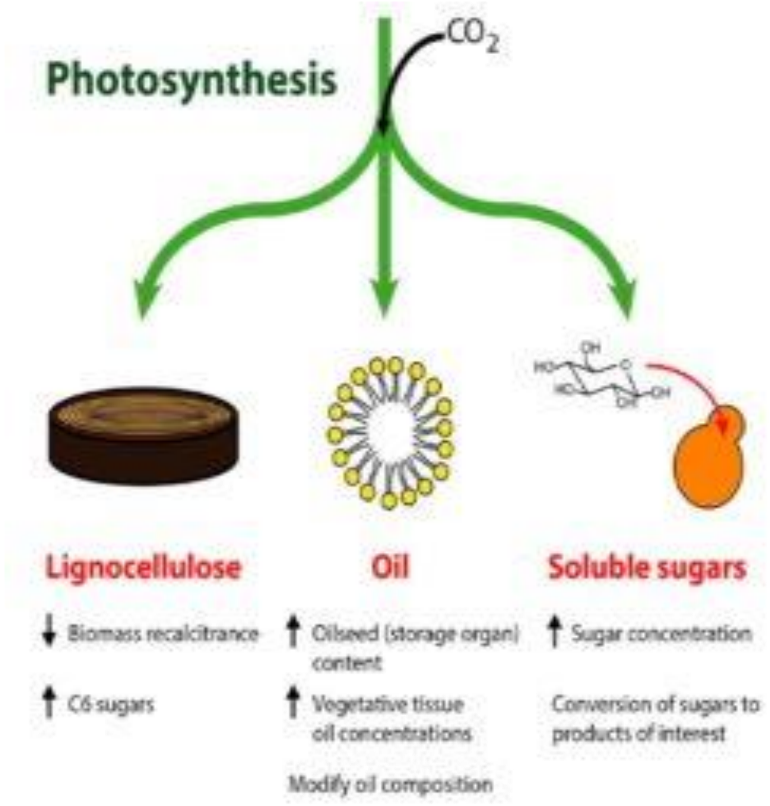
- **What does it do?** *Transgenic (genetically engineered) papaya is resistant to PRSV.*
- **How does it work?** *Genes from part of the virus itself have been incorporated into the papaya genome to achieve resistance.*
- **Which crops have PRSV resistance?** *Rainbow papaya*.
- **Why was this trait introduced?** *No other preventive or curative options were available to protect papaya in Hawaii from this disease. It is credited with saving the papaya industry in Hawaii.*

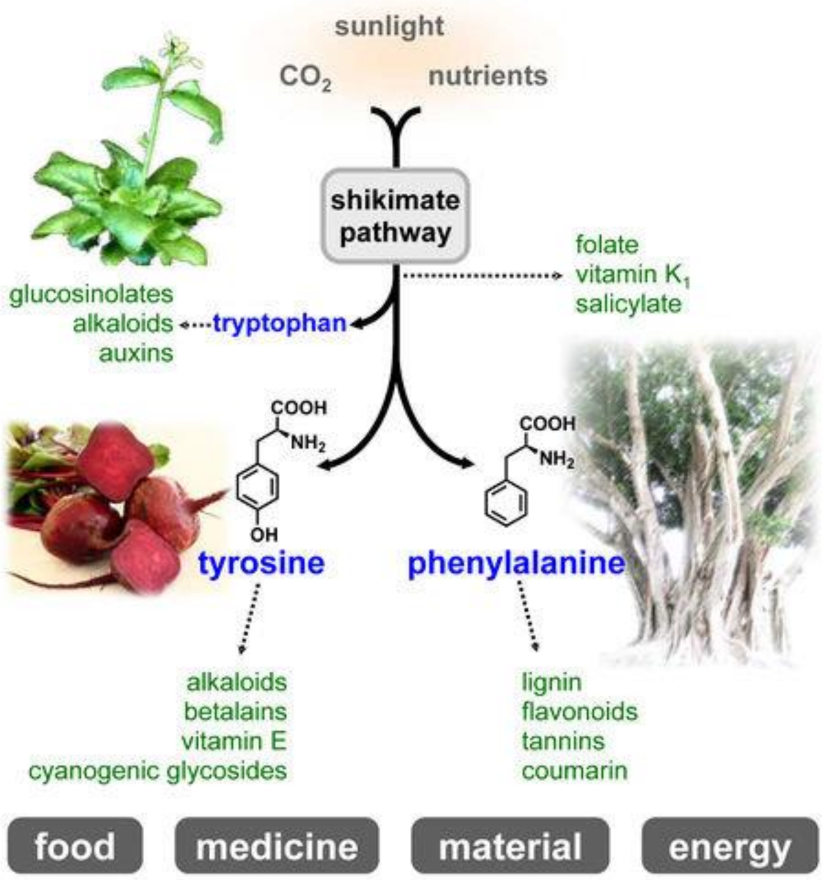
- **Biopharming -** the creation of plants capable of synthesizing medicinal proteins
- **Model plants** to study the action of target genes



Metabolic engineering

Metabolic engineering is the design of plants with targeted changes in the metabolic conversion of substrates into target products.



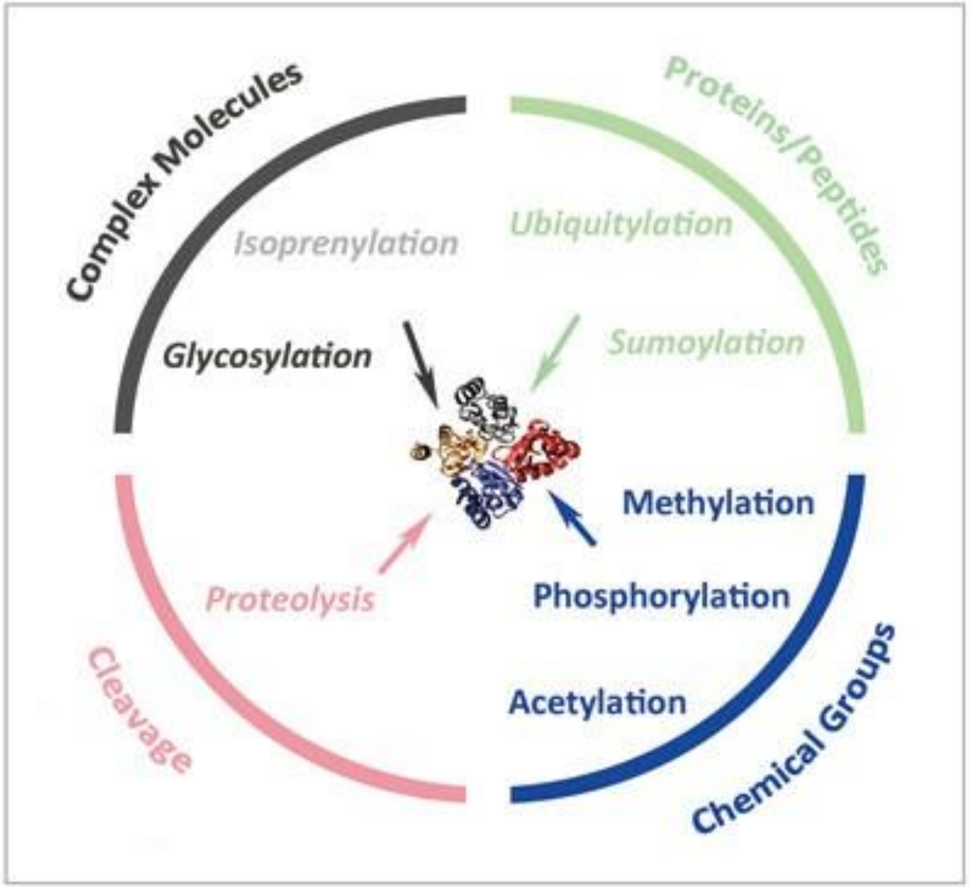


Plants as the basis for eukaryotic gene expression

1) Plants - eukaryotes, genetics and physiology are well studied, genomes of plants of different classes are sequenced;
2) Plants can be obtained in laboratory, greenhouse and field conditions
3) Genetic engineering technologies developed;
4) In plant cells, post-translational modifications are carried out adequately to other eukaryotes, in contrast to bacteria;
5) Many plants are recognized as safe and are used for human consumption. Therefore, the procedure for the approval of medicines is simplified;
6) Binary vectors are used to work with plants: the main manipulations are carried out in bacteria, and the final structures are transferred to plants;

Post-translational modifications

- ✓ **Amino acid modifications**: phosphorylation, acetylation, acylation, carboxylation, etc.;
- ✓ **Glycosylation**: proteins acquire stability, attachment of the sugar residue to serine / threonine / asparagine;
- ✓ **Proteolytic cleavage** of precursor proteins;
- ✓ The formation of **disulfide bonds** is catalyzed by the enzyme protein disulfide isomerase, the incorrectly folded protein is unstable and inactive;



Promoters

For plants, constitutive and inducible are used as strong promoters:

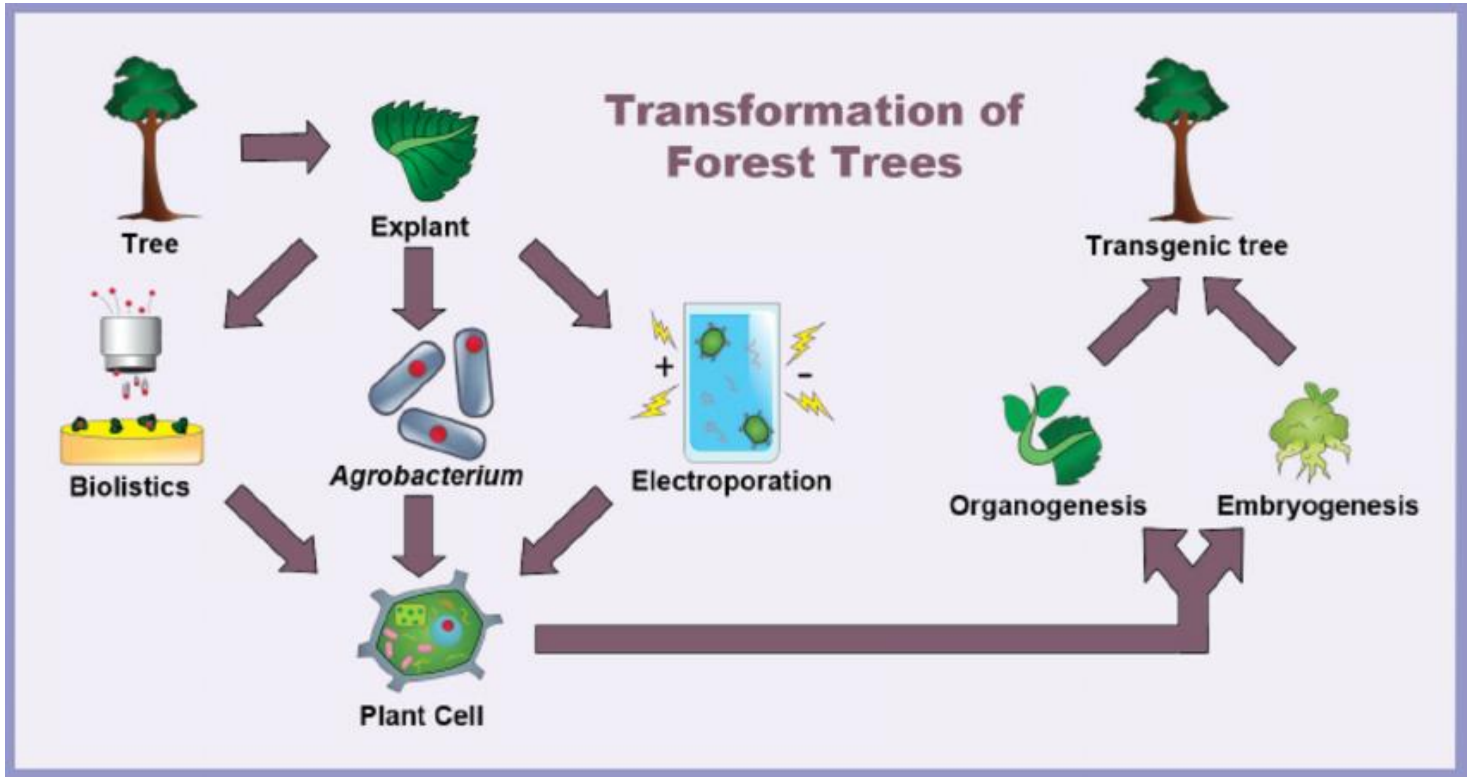
Constitutive promoters
<u>For dicotyledonous plants</u>:
• 35S promoter of cauliflower mosaic virus (CaMV);
• nos-promoter of agrobacterium nopaline synthase gene.
<u>For monocotyledonous plants</u>:
• the promoter of the corn alcohol dehydrogenase gene (Adh);
• rice actin gene promoter (Act).

Inducible promoters
Activated under certain conditions:
• temperature
• lighting
• concentration of phytohormones
• mechanical stress – injury
• treatment of plants with elicitors

If the induction of the expression of the target gene occurs when the plants are cut, then the gene product accumulates in the harvested biomass: until the harvesting stage, transgenic plants can be grown as normal

STAGES OF OBTAINING TRANSGENIC PLANTS

- **Stage 1** - selection of the target gene and the method of its cloning;
- **Stage 2** - selection of the genotype of the recipient plant;
- **Stage 3** - creation of a construct containing the target gene;
- **Stage 4** - transfer of the vector molecule into the genome of the recipient plant• 5 stage - selection of transgenic plants;

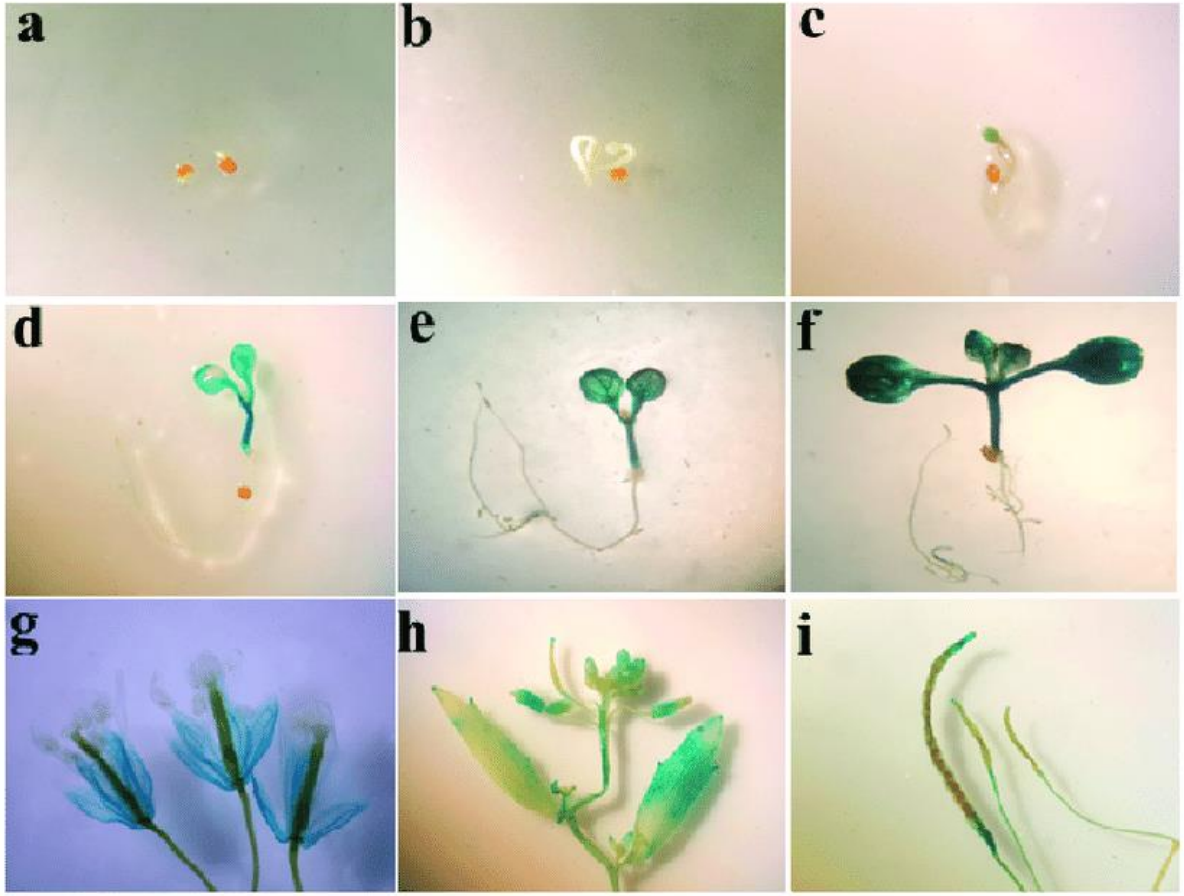


Inclusion of reporter genes:
Serve for the identification of transgenic plants

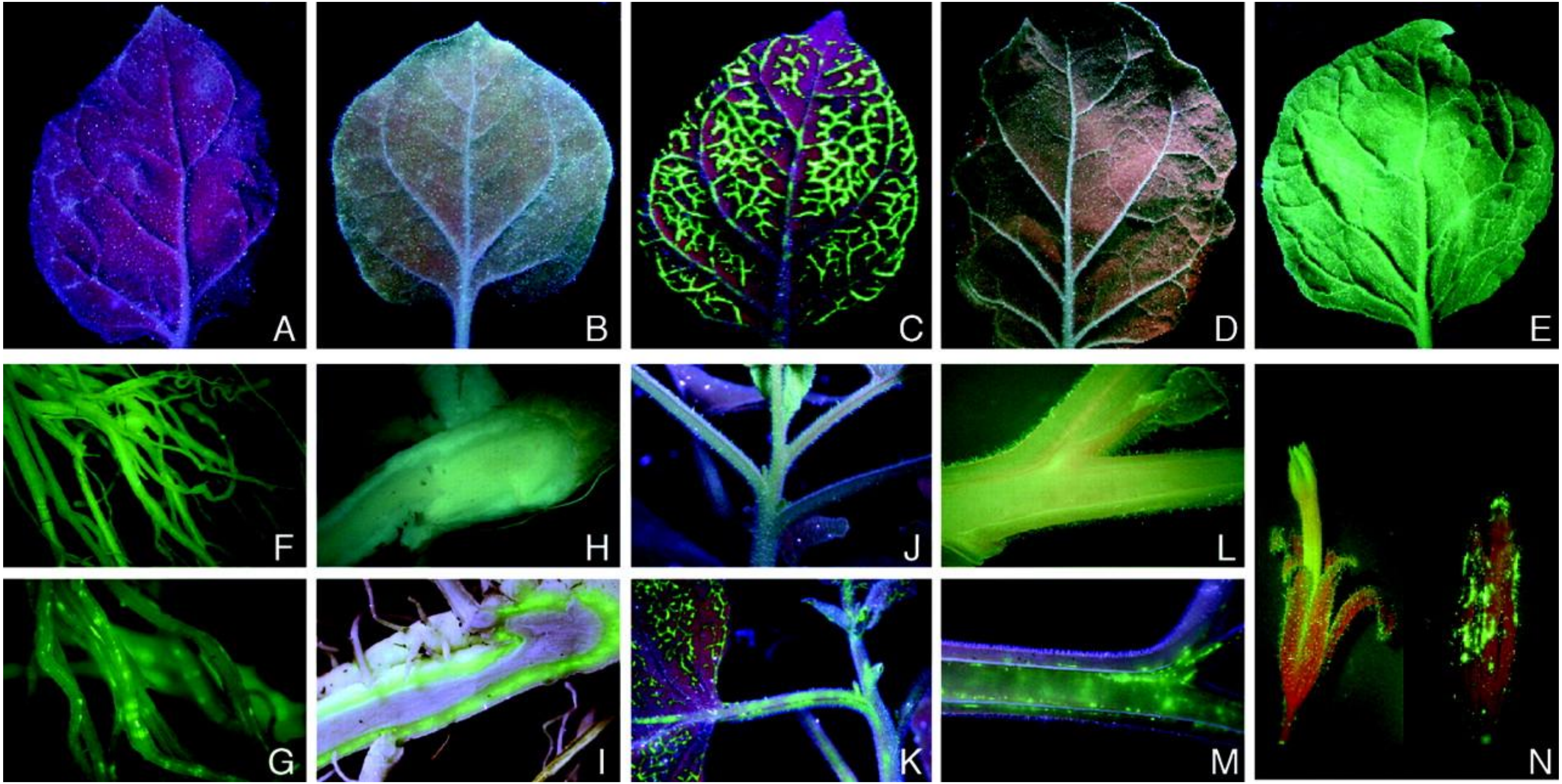
Replacing marker genes with reporter genes eliminates the risk to the environment and human health

Reporter genes encode proteins that are neutral for cells and are easy to test for in tissues

Reporter genes:
β-glucuronidase (GUS) - hydrolyzes glucuronides, tissues are colored blue when using a substrate
green fluorescent protein (GFP) - able to fluoresce when exposed to UV
luciferase (LUC) - causes the clones to glow



GFP expression in transgenic plants infected with TYLCSV or TYLCV.
Tomato yellow leaf curl Sardinia virus /



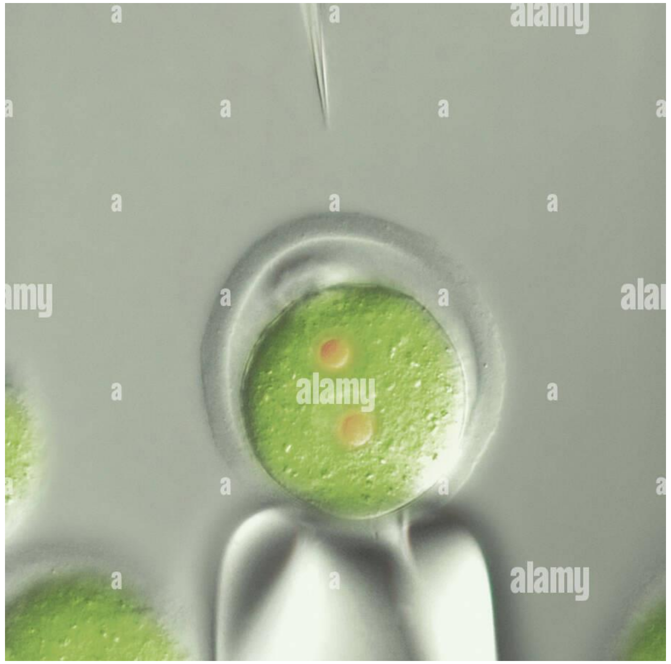
Methods for delivering foreign DNA to plants

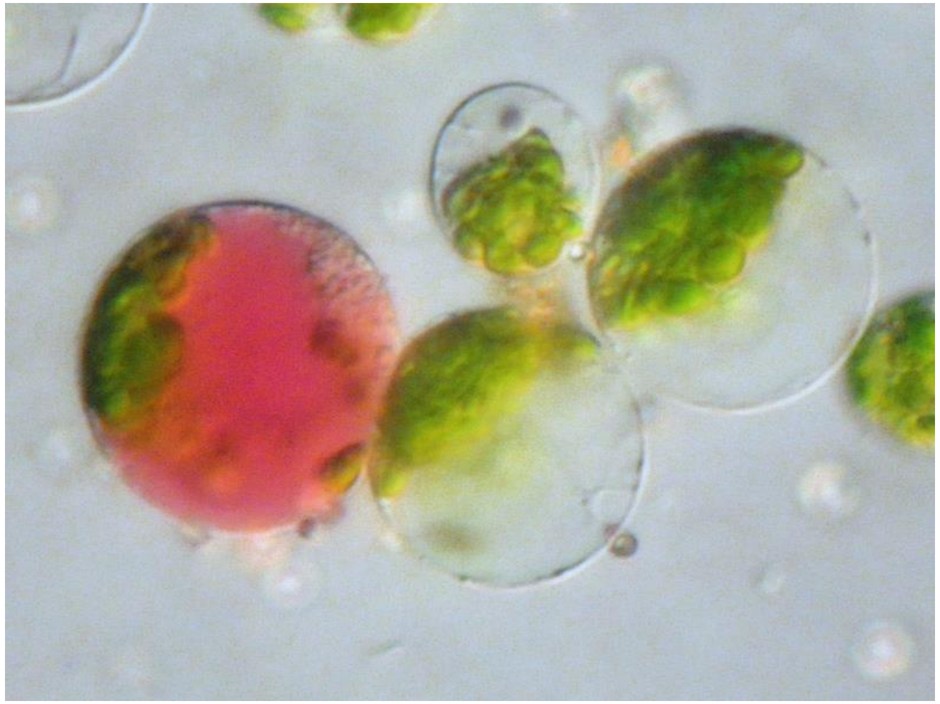
<u>Vector (indirect) delivery:</u>

- ✓ Agrobacterial transformation
- ✓ Viral delivery

<u>Drop shipping:</u>

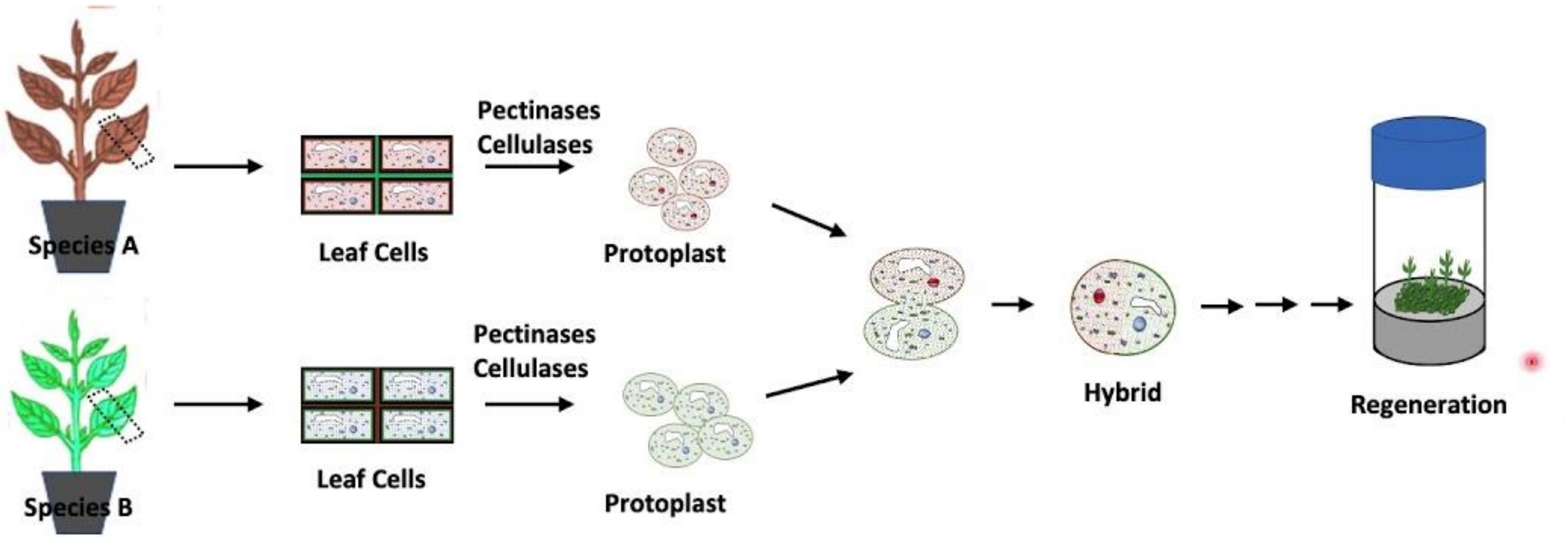
- ✓ Bioballistics
- ✓ Somatic hybridization method
- ✓ Microinjection
- ✓ Electroporation



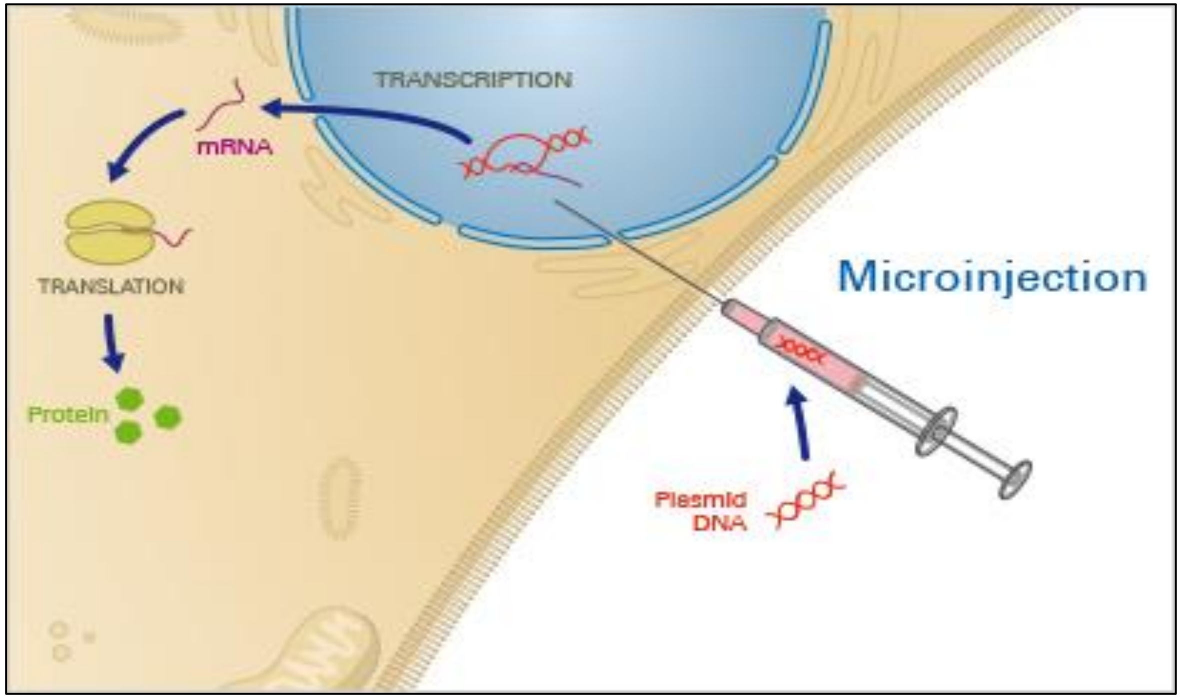


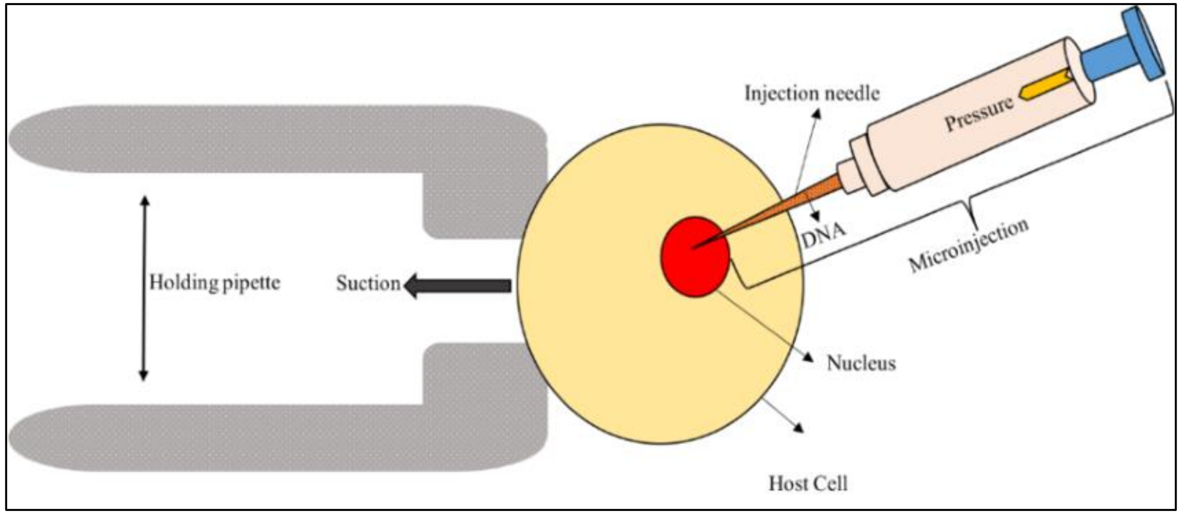
Somatic hybridization method

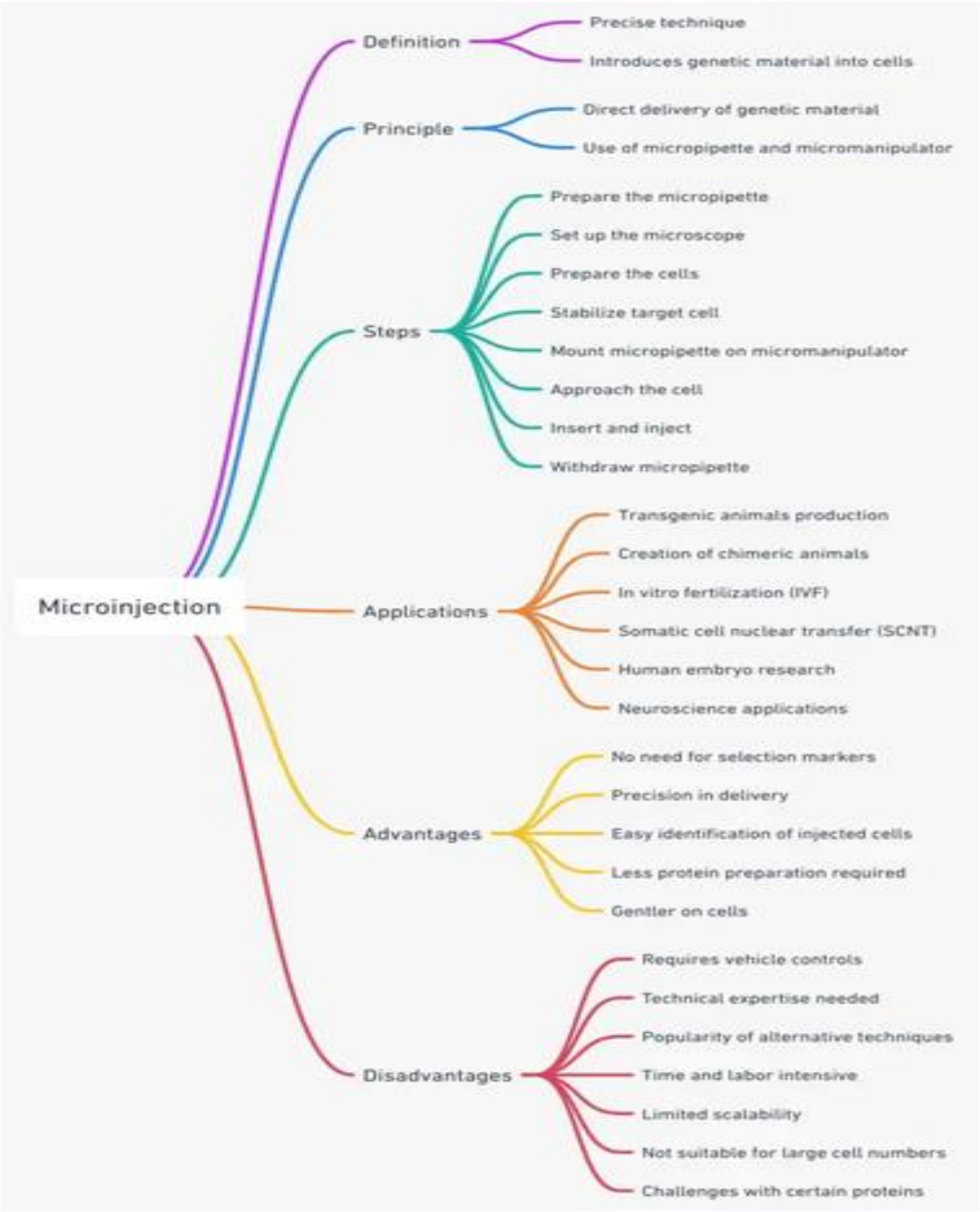
The process of fusion of protoplasts isolated from somatic cells of two different plant species/cultivars to regenerate hybrid plants is called as Somatic hybridization.



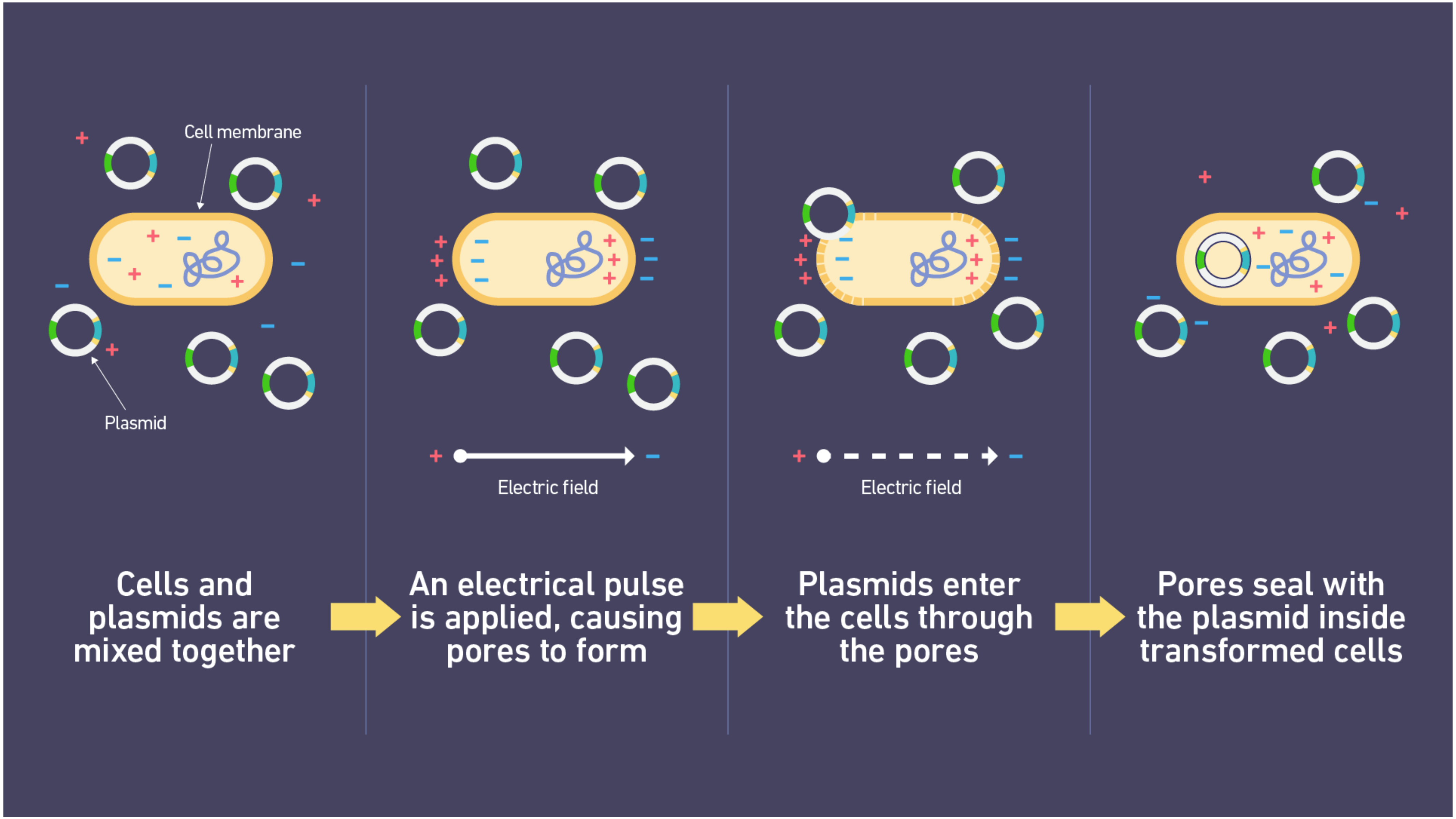
Microinjection

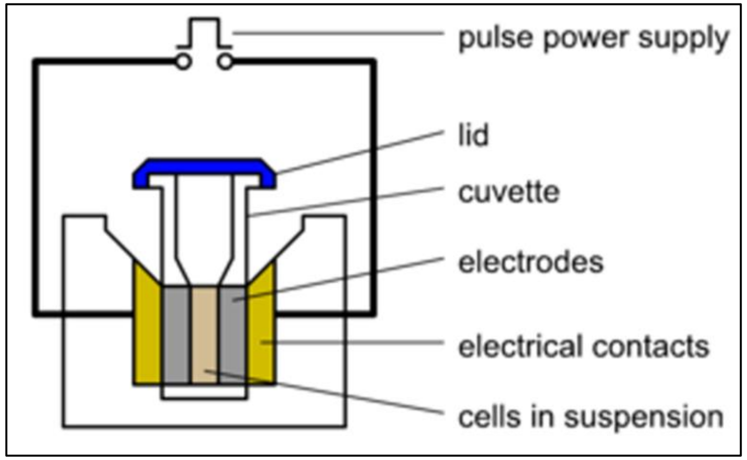


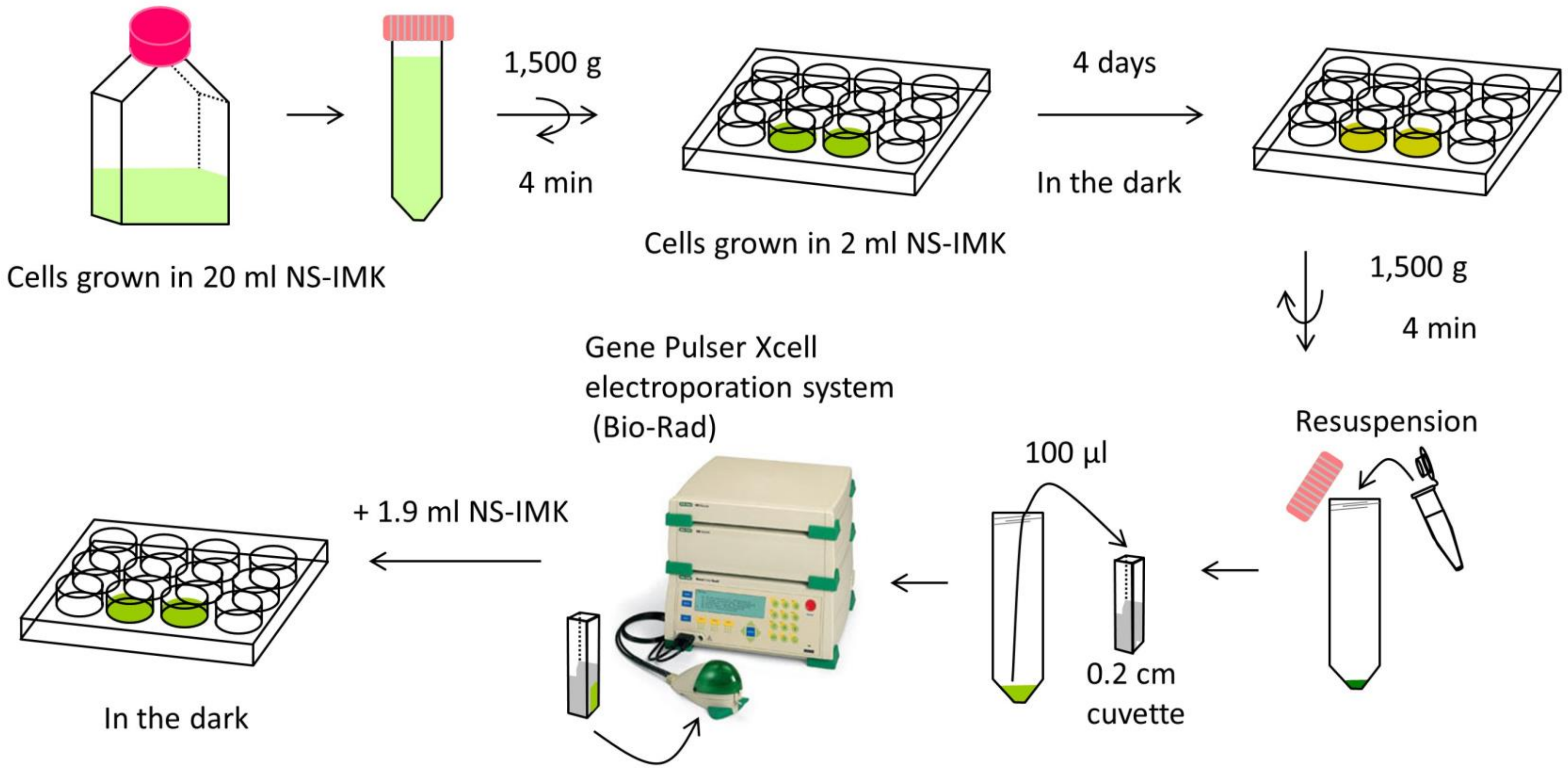


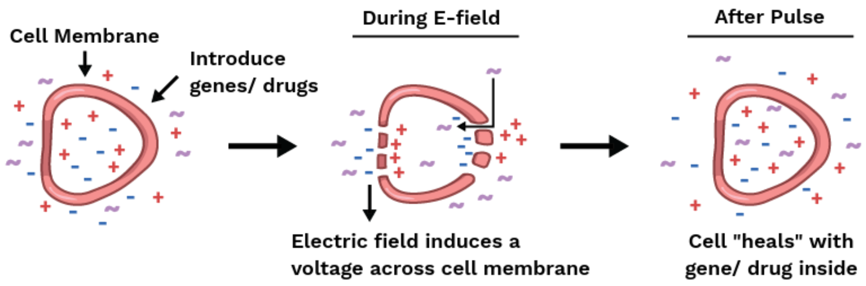


Electroporation




pulse power supply
lid
cuvette
electrodes
electrical contacts
cells in suspension


Cells grown in 20 ml NS-IMK
1,500 g
4 min
Cells grown in 2 ml NS-IMK
4 days
In the dark
1,500 g
4 min
Resuspension
100 µl
0.2 cm
cuvette
Gene Pulser Xcell
electroporation system
(Bio-Rad)
+ 1.9 ml NS-IMK
In the dark


Cell Membrane
Introduce
genes/ drugs
During E-field
Electric field induces a
voltage across cell membrane
After Pulse
Cell "heals" with
gene/ drug inside