# Roles of Plant Tissue Culture in Plant Genetic Engineering

# What are Transgenic Plants?

- Plants that contain a source gene inserted from a species outside of the target plant species.
- The gene may 1) have a similar function to a gene that is already present but with a different DNA sequence or 2) not already present in that species.

  - Herbicide-tolerant plants : allowing the effective control of weed species in a field without damage to the crop
  - Disease-resistant plants :
  - Chill-tolerant plants :
  - Salt tolerance plant :
  - Metal tolerance :

# Why Transgenic Plants?

Sexual cross barriers were a problem for moving genes between species.

Now these barriers are not a problem for mixing gene pools. Animal, fungus, and bacteria genes can now become plant genes.

# Plant Transformation

* Application of plant genetic manipulation to agronomic traits (**herbicide, pest, disease resistance**) as first-generation plant biotechnology
* More challenging and diverse advances : stress resistance, crop yield and quality and molecular pharming
* Improve crop plant characteristics
* Depends on the stable introduction of transgene(s) into the genome of the plant
* Transformation methods :
  - non-direct gene transfer (dicotyl) : *Agrobacterium*
  - direct gene transfer (monocotyl) : particle bombardment (biolistics)
* Transgenic Plant

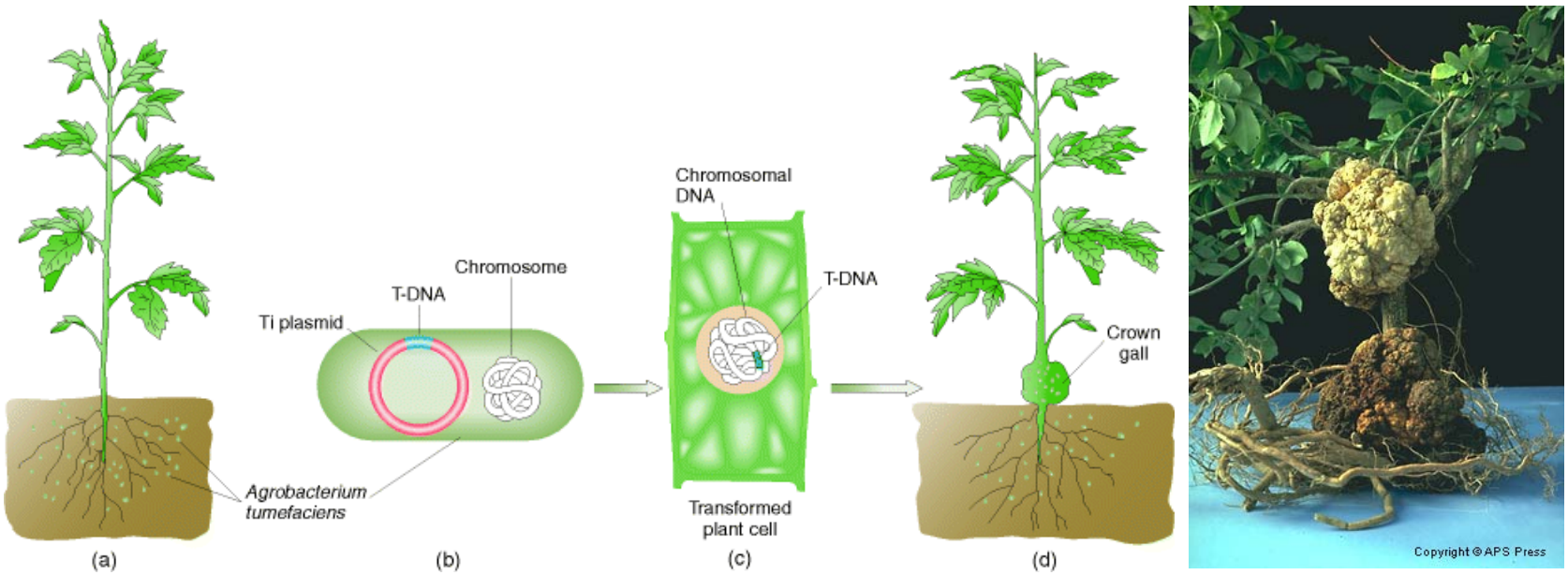
# Three Steps of Genetic Engineering

1. Isolating a genetic segment (DNA), and constructing a DNA recombinant DNA molecule (includes promoter).
2. Finding a ***vector*** that moves the DNA into the cell.
3. Placing the vector in the plant cell. The plant cell must have the potential to regenerate into a whole plant. It needs to be sexually fertile in order to pass the gene on to subsequent generations.

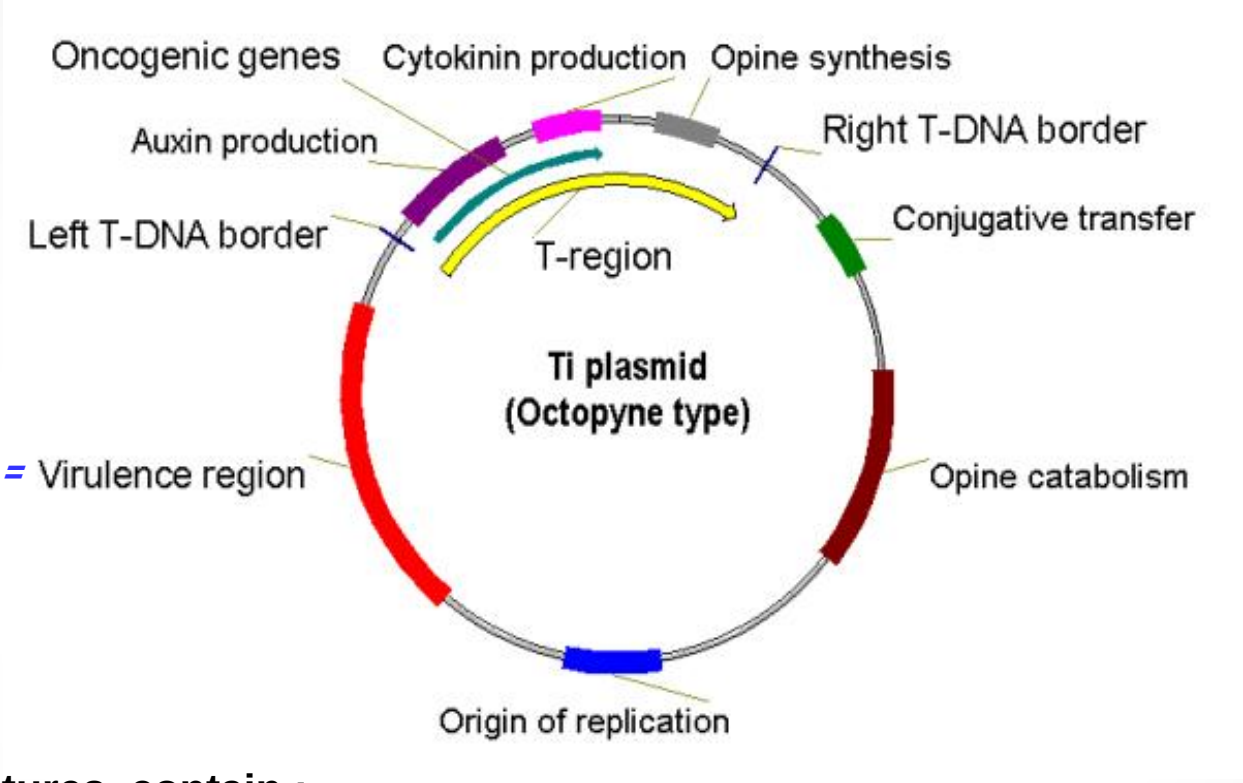
Hopkins, 1999

# *Agrobacterium tumefaciens*

- Gram-negative, rod-shaped, motile bacterium
- Found in rhizosphere where it normally survives on nutrients released from plant roots
- If a plant is wounded or damaged → *A. tumefaciens* : is attracted to the wound site via chemotaxis in response to chemicals (sugars and phenolic molecules) released from the damaged plant cells → infect the wound site → cause disease symptoms → **crown-gall disease**
- Crown-gall formation depend on the presence of **Ti (tumor-inducing) plasmid** in *A. tumefaciens*
- **T-DNA regions:** a part of Ti plasmid which is transferred from the bacterium into the plant cell → integrated into the genome of the host plant



# Ti Plasmid - Agrobacterium




**Ti Plasmid features, contain :**

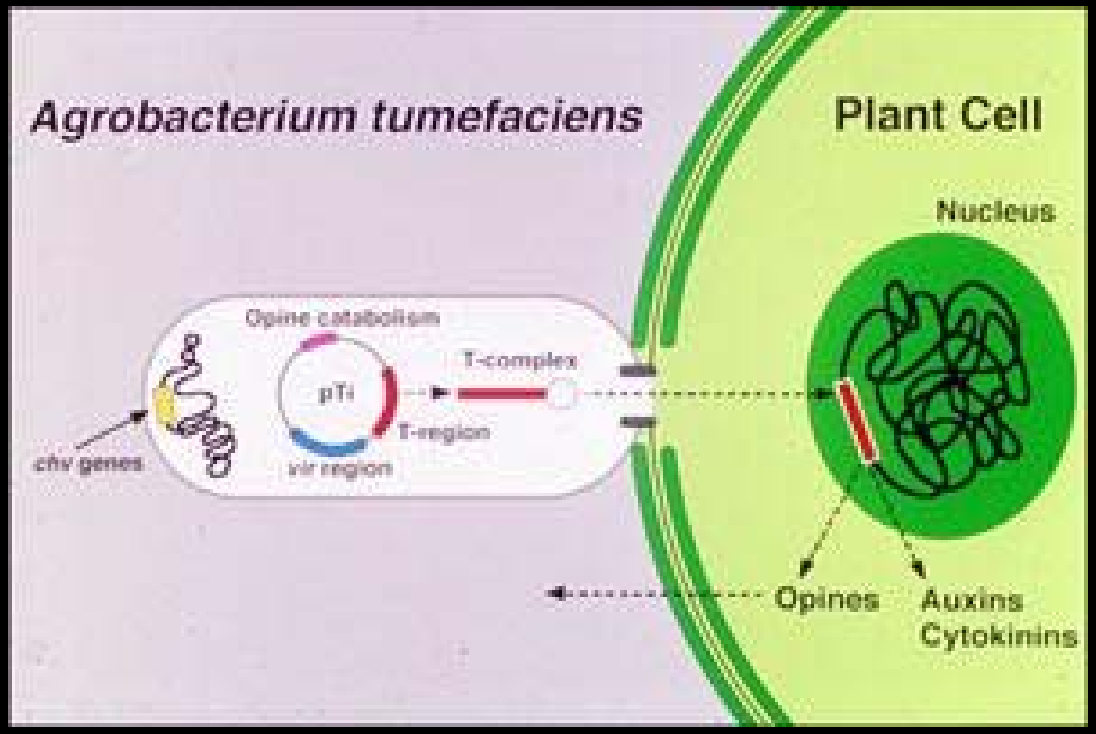
1. **one (or more) T-DNA regions that carries gene encode proteins involved in hormone (cytokinin and auxin) biosynthesis and biosynthesis of novel plant metabolites : 'agropines' and 'opines'**
2. **a *vir* region : Genes directing T-DNA transfer into plant house (at least 9 *vir*-gene operons)**
3. **an origin of replication**
4. **a region enabling conjugative transfer**
5. **genes for the catabolism of opines**

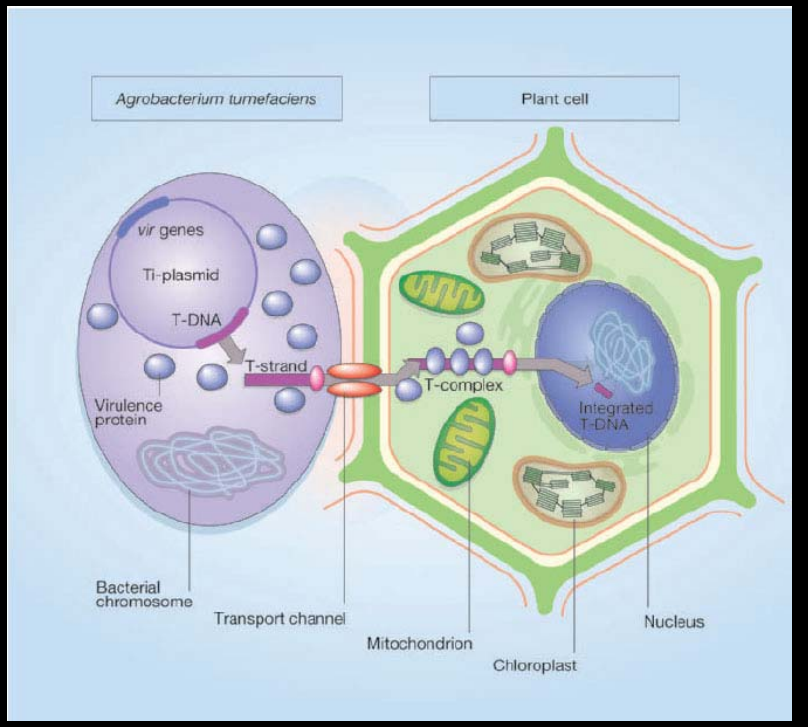
# T-DNA TRANSFER AND INTEGRATION

Signal recognition by *Agrobacterium*

Attachment to Plant cells

Generation of T-DNA transfer complex



# In vitro process of T-DNA transfer and integration

| | | |
|---|---|---|
| 1 | Identify a suitable explant | |
| 2 | Co-cultivate with the *Agrobacterium* | Signal recognition by agrobacterium |
| | | Attachment to plant cells |
| | | vir Gene induction |
| | | T-strand production |
| | | Transfer of T-DNA out of the bacterial cell |
| | | Transfer of T-DNA and Vir proteins into the plant cell and nuclear localization |
| 3 | Kill the *Agrobacterium* with a suitable antibiotic | |
| 4 | Select for transformed plant cells | |
| 5 | Regenerative whole plants | |

# Antibiotic Resistant Genes

* **Most common is neomycin phosphotransferase type II (NPT II)**
* **Confers resistance to kanamycin**
* **Cells contain NPT II will grow in media containing kanamycin - others will die**

* The neo (neomycin-resistance) gene of transposon Tn5 encodes the enzyme neomycin phosphotransferase II (**npt II**) (EC 2.7.1.95), which confers resistance to various aminoglycoside antibiotics, including kanamycin and G418. The gene is widely used as a selectable marker in the transformation of organisms as diverse as bacteria, yeast, plants, and animals.

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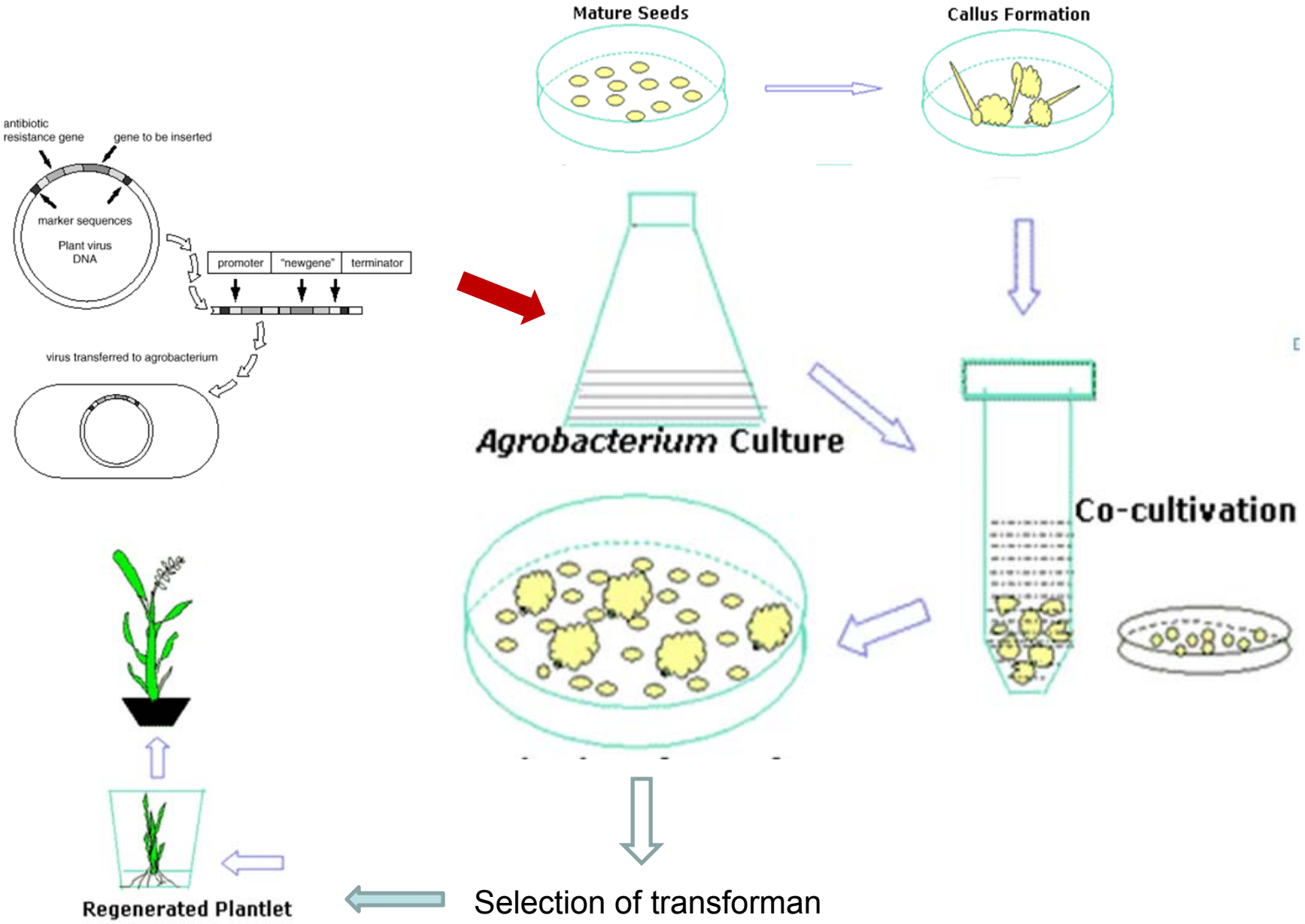
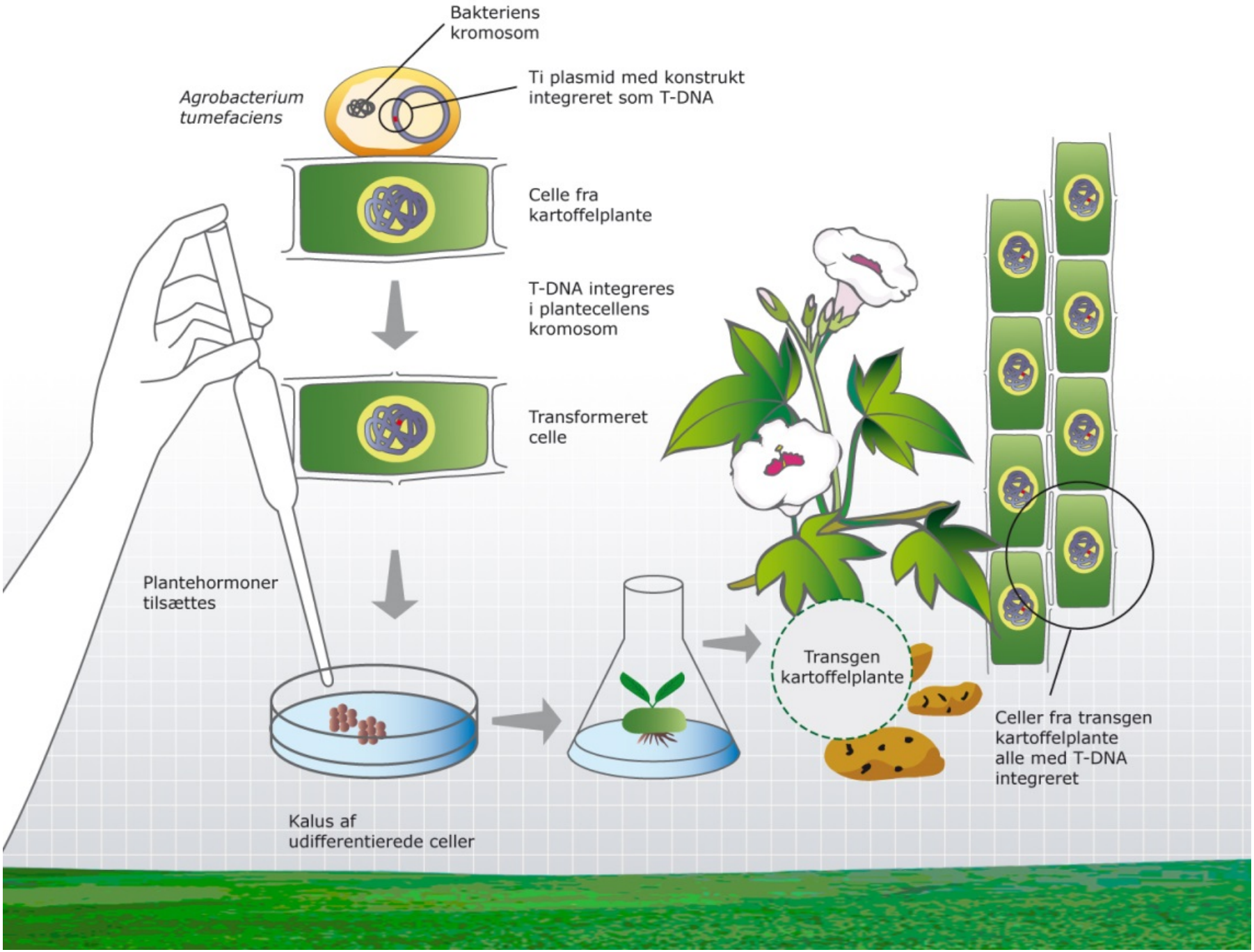
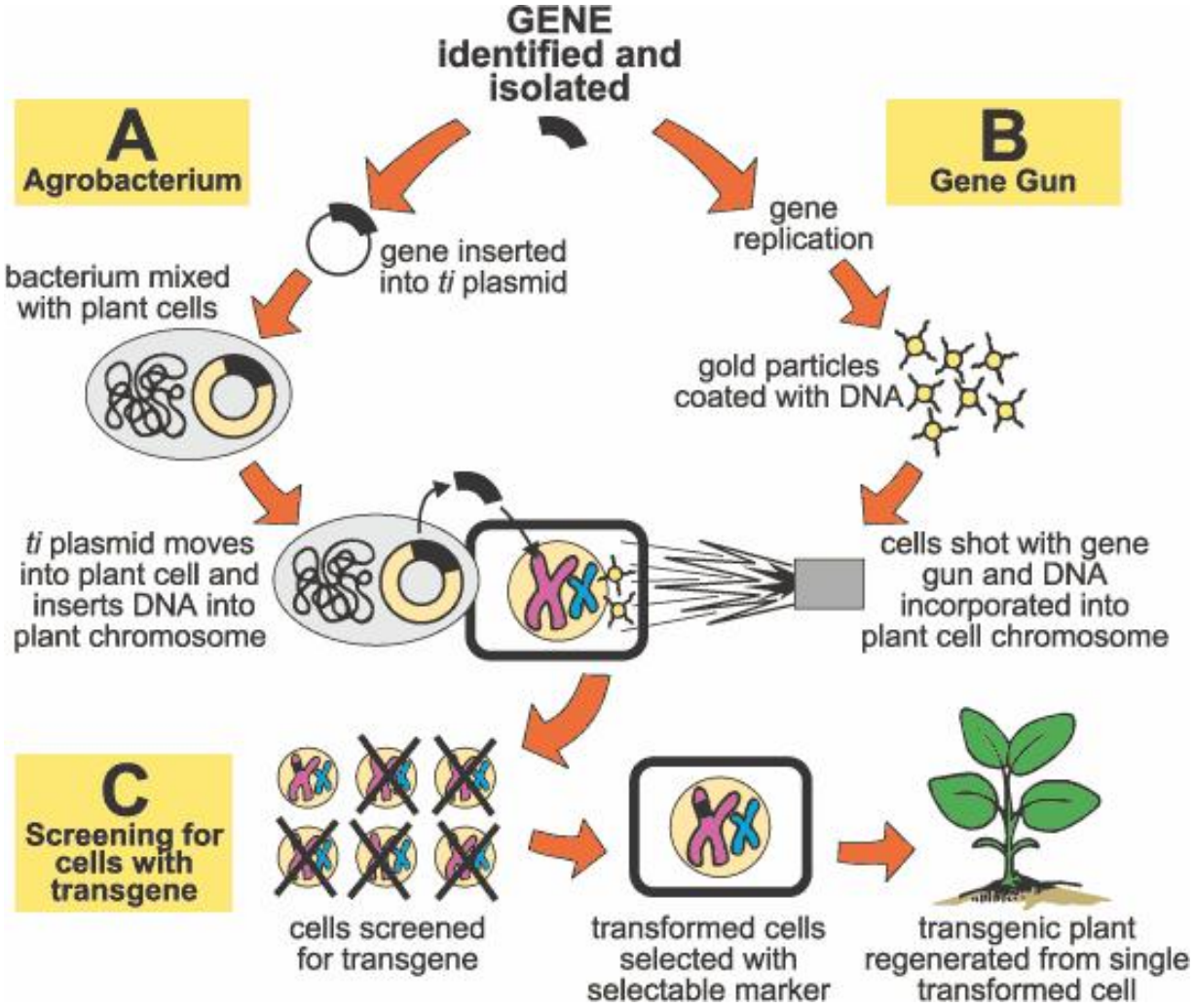
# Plant Transformation - *Agrobacterium*




Bakteriens kromosom
Ti plasmid med konstrukt integreret som T-DNA
Agrobacterium tumefaciens
Celle fra kartoffelplante
T-DNA integreres i plantecellens kromosom
Transformeret celle
Plantehormoner tilsættes
Kalus af udifferentierede celler
Transgen kartoffelplante
Celler fra transgen kartoffelplante alle med T-DNA integreret




Biolistic, free DNA or “gene gun”

⇩

DNA fragments coated onto metals

⇩

“Shot” into cells with high pressure helium gun

⇩

Some enter nucleus and incorporate into host DNA

⇩

Antibiotic resistant gene also inserted for selection

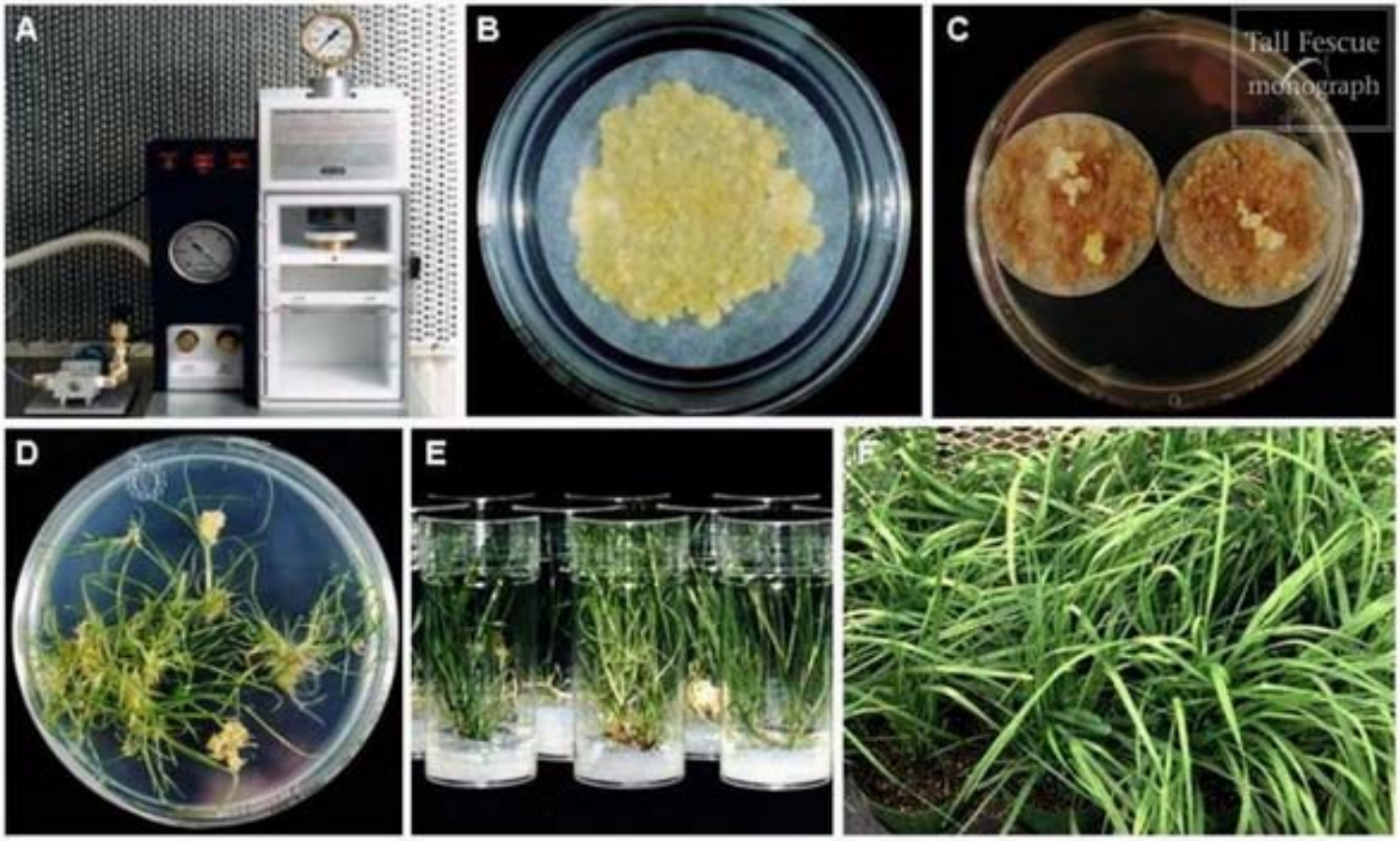
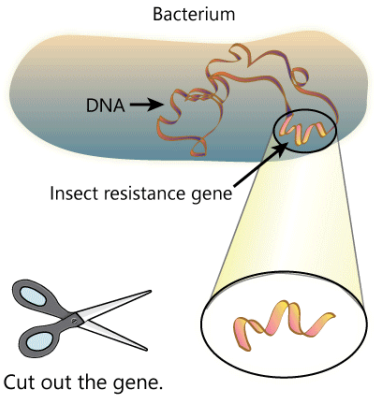


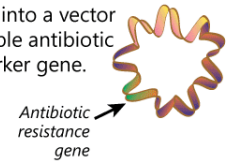

Fig. 22-1. Transgenic tall fescue plants obtained by biolistic transformation. (A) PDS/1000 biolistic device used for biolistic transformation. (B) Embryogenic cells of tall fescue plated on filter paper for microprojectile bombardment. (C) Hygromycin resistant calluses obtained after bombardment and hygromycin selection. (D, E) Transgenic shoots and plantlets regenerated from the hygromycin resistant calluses. (F) Transgenic tall fescue plants growing in the greenhouse.

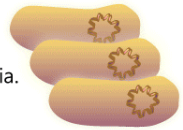
# Creation of an Insect Resistant Tomato Plant




1. Cut out the gene.

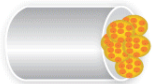
2. Insert gene into a vector with a selectable antibiotic resistance marker gene.



3. Copy vector in bacteria.

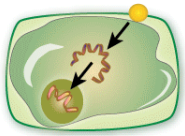
4. Coat tungsten or gold particles with DNA vectors.



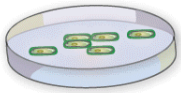
5. Load vector-coated particles onto teflon bullet.

6. Load bullet into gene gun.


7. Shooting the gene gun releases the particles at a high velocity penetrating the plant cells.

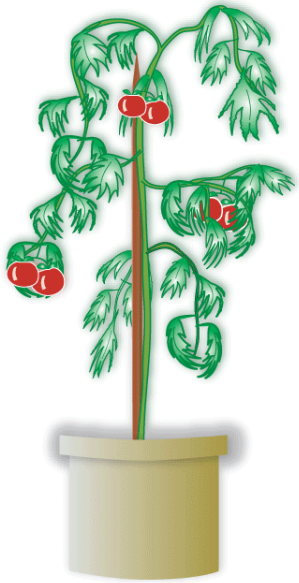


8. The vector enters the cell. The genes are incorporated into the plant genome.



9. The cells are plated on a selective antibiotic media. Only cells that have incorporated the vector will grow.

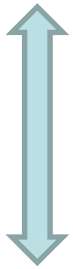
10. These cells are transferred to medium containing plant growth factors.



**Insect resistant tomato plant**

# Gene Gun - Problems

- Not very precise - no idea where new DNA will be located in host genome
- No guarantee the new DNA will be expressed



# Plant Transformation methods

- *Agrobacterium tumefaciens*
- The T-DNA is then used to carry other genes into the plant
- More precise than gene gun
- Also uses antibiotic resistant marker genes for selection