

Part III

PLANT TRANSFORMATION

Le Bui Van
University of Science

Plant Biotechnology
Vietnam OpenCourseWare
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Transformation refers to the introduction of a gene of choice into the genome of an organism e.g. the generation of transgenic organisms.

Plant transformation: Integration of the DNA into a plant genome (nuclear or cytoplasmic).

Plant transformation consists of three steps:

1. Target gene (s)
2. Plant tissues
3. Transformation methods

3.1. TARGET GENES (Plasmid vectors)

Vectors

Vector is one of the most important elements in recombinant DNA (rDNA) technology and in gene cloning

A vector should have the following features:

- *contain a replicon that enables it to replicate in host cells*
- *it should have several marker genes*
- *it should have a unique cleavage site within one of the marker genes so that insertion of foreign DNA into the marker gene leads to its inactivation and identification of recombinant molecule*
- *for the expression on cloned DNA, the vector DNA should contain suitable control elements, such as promoters, terminators, and ribosome binding sites*

Plasmids

- Double-stranded, closed circular DNA molecules which exist in the cell as extrachromosomal units.
- Self-replicating, found in a variety of bacterial cells as accessory genetic units, advance growth to the bacterial host.
- 1-200 kb, dependent on host proteins for maintenance and replication functions.
- Frequently, plasmids contain some genes advantageous to the bacterial host, for example, antibiotics.
- By utilizing these antibiotic-resistance genes as dominant genetic markers in plasmid cloning vectors, it is possible to select for *E.coli* cells that have maintained high copy replication of plasmid DNA (e.g. pBR 322).

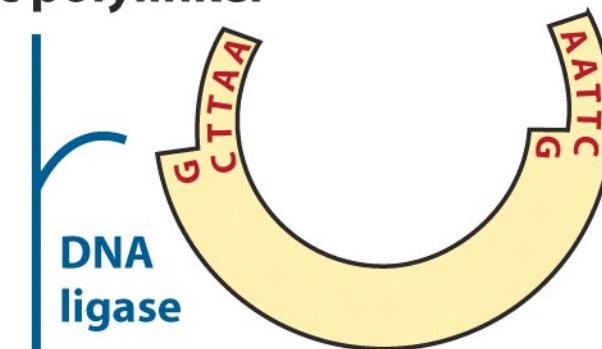
Plasmid vectors

1. It should contain an *origin of replication that operates in the* organism into which the cloned DNA is to be introduced.
2. Introduction of selectable markers.
3. Introduction of synthetic cloning sites termed *polylinkers, restriction site bank, or polycloning sites that are recognized by restriction enzymes.*
4. Incorporation of axillary sequences: visual identification of recombinant clones e.g. by histochemical tests, DNA sequencing, or expression of large amounts of foreign genes/proteins.

$PstI$ $HindIII$ $BamHI$ $SmaI$

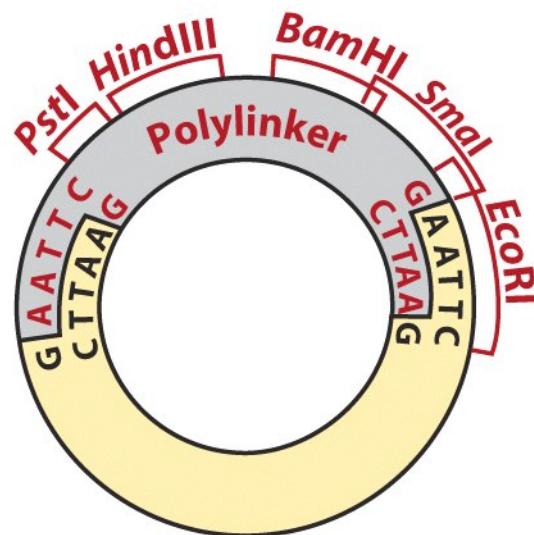
 AATTCCCTGCAGAAGCTTCCGGATCCCCGGG
 GGACGTCTTCGAAGGCCCTAGGGGCCCTTAA

Synthetic polylinker



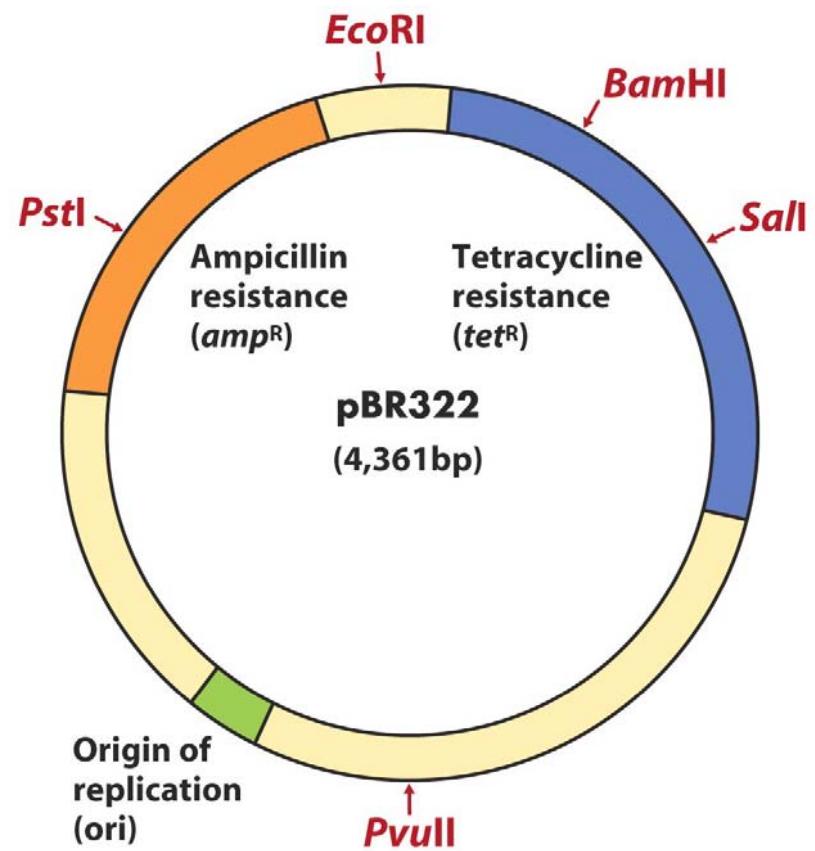
DNA ligase

Plasmid cloning vector
cleaved with *EcoRI*



Plasmid vectors: development

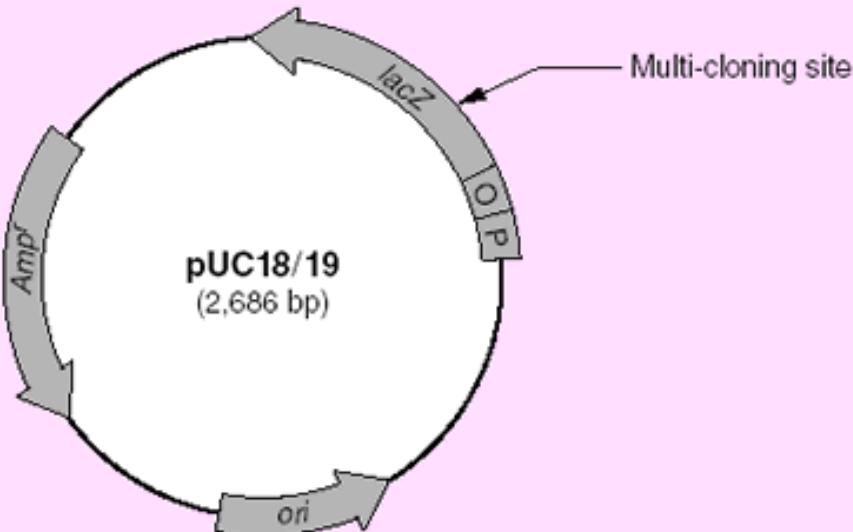
pBR 322 the most widely used cloning vector. Up to now cloning of a DNA fragment in any recognition sites for restriction enzymes has resulted in the inactivation of either one of the antibiotic resistance (ampicillin, tetracycline) markers.



A typical plasmid vector contains a polylinker which can recognize several different restriction enzymes, an ampicillin resistance gene (*ampr*) for selective amplification, and a replication origin (ORI) for proliferation in the host cell.

Plasmid vectors: pUC18/19

- *lacZ* gene encoding β -galactosidase, which cleaves lactose into glucose and
- galactose → identification by the X-gal assay (blue/white colonies)

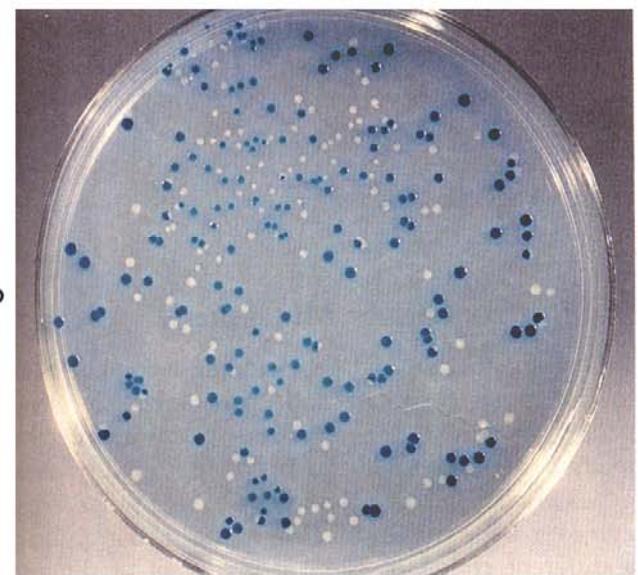
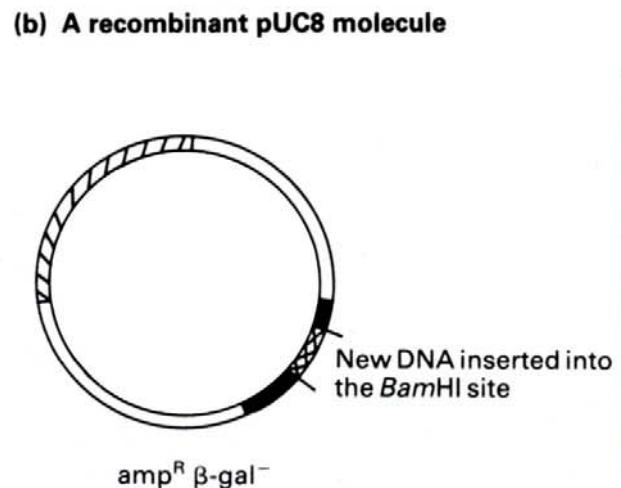
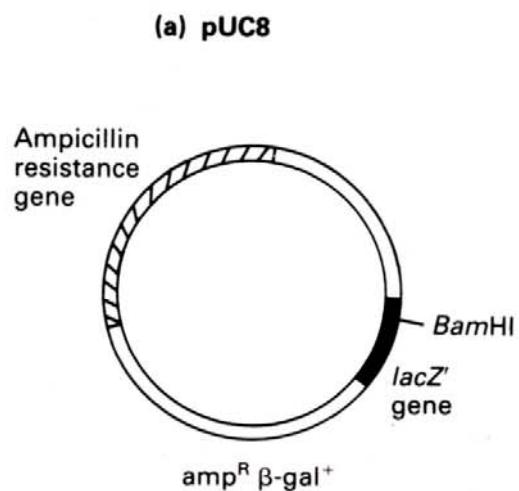


pUC18

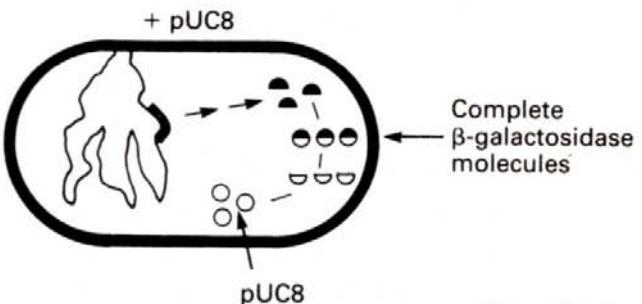
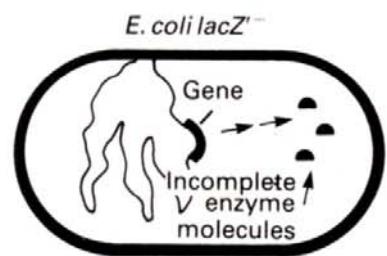
455 → *lacZ*
 5'-GAATT CGAGCT CGGT ACCCGGGG AT CCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGC-3'
 EcoR I Sac I Kpn I Sma I Xba I BamH I Xba I Pst I Sph I Hind III
 Sal I Acc I Hinc II Sse8387 I

pUC19

452 → *lacZ*
 5'-GCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATT C-3'
 Hind III Sph I Pst I Sal I Acc I Hinc II Xba I BamH I Sma I Xma I Kpn I Sac I EcoR I
 Sse8387 I

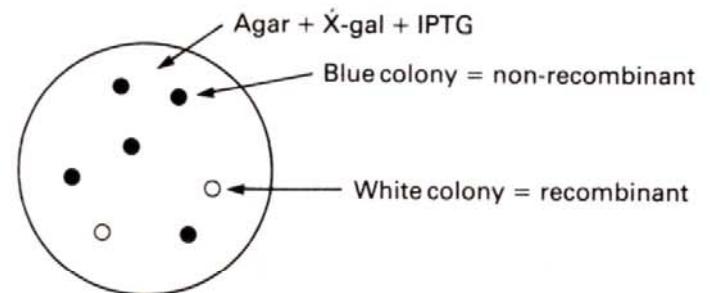


(a) The role of the *lacZ'* gene



- β -galactosidase fragment coded by bacterial gene
- β -galactosidase fragment coded by plasmid gene
- Complete β -galactosidase molecule

(b) Screening for pUC8 recombinants



Blue colonies = β -galactosidase synthesized
X-gal \rightarrow blue product

White colonies = β -galactosidase not
synthesized
X-gal \rightarrow no blue product

Ti plasmids

- Most cloning vectors for plants are based on the Ti plasmid, which is not a natural plant plasmid but belongs to a soil bacterium.

Agrobacterium tumefaciens

- Invades plant tissues, causing a cancerous growth called a crown gall.
- During the infection, a part of the Ti plasmid (T-DNA) is integrated into the plant chromosomal DNA.

vir genes

25 bp repeats

T DNA

Ti plasmid

Target gene(s) and gene cloning

In order to clone a DNA sequence that codes for a required gene product, the gene has to be removed from the organism and cloned in a vector molecule

- Libraries of such cloned fragments are made.
- A gene library is a random collection of cloned fragments in a suitable vector that ideally includes all the genetic information of that species.
- Two ways to make libraries:
 - complementary (cDNA) libraries
 - genomic libraries

cDNA libraries and cloning

- ✓ For many species, a complete genome library contains such a vast number of clones that the identification of the desired gene is more facilitated.
- ✓ cDNA libraries are used when it is known that the gene of interest is expressed in a particular tissue or cell type.

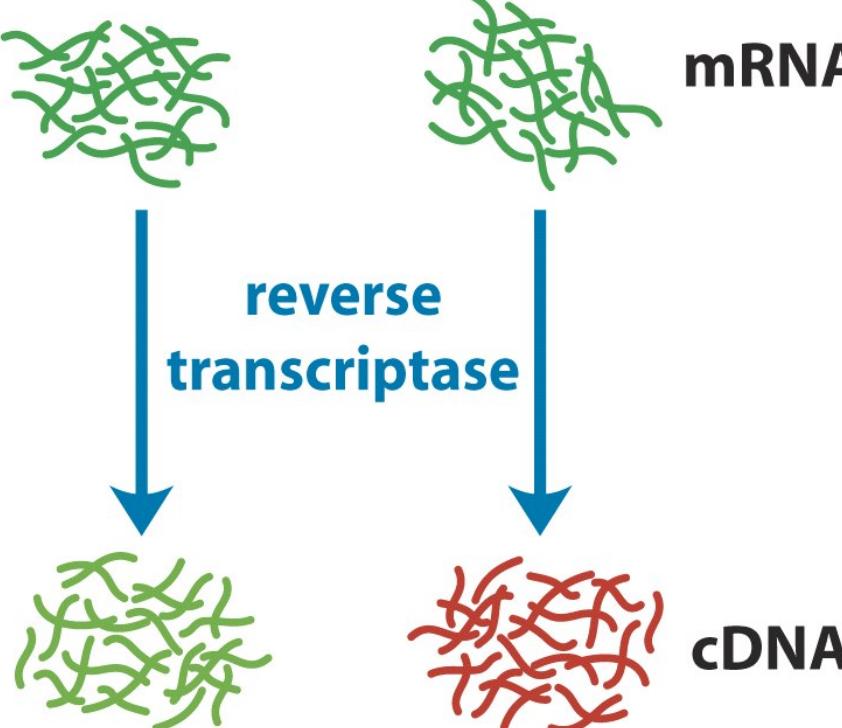
①

Isolate mRNAs from cells at two stages of development; each mRNA sample represents all the genes expressed in the cells at that stage.



②

Convert mRNAs to cDNAs by reverse transcriptase, using fluorescently labeled deoxyribonucleotide triphosphates.



Genomic cloning

- Genomic libraries and clones are necessary in addition to cDNA clones because cDNA clones are generated from processed mRNA which lack introns and other sequences surrounding the gene.
- If genes are to be modified and returned to plants, genomic sequences will be more useful.
- The genome of plants is remarkably complex, and a particular fragment of interest comprises only a small fraction of the whole genome.
- Genomic library must contain at least one copy of every sequence of DNA in the genome.

Expression vectors

Any cloned vector after insertion can be *in vivo* transcribed into RNA, and in many cases, even translated into proteins.

Typical plant expression vectors contain the following features:

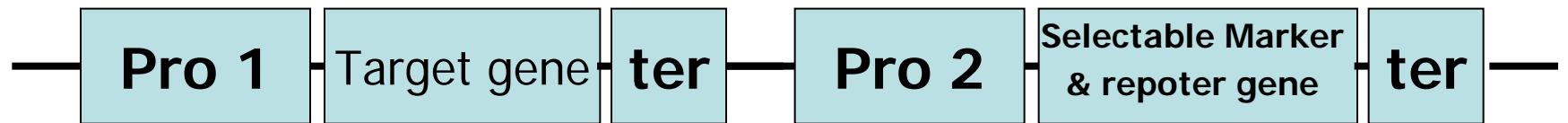
- *E. coli* origin of replication so that the plasmid can be manipulated in *E. coli*.
- A bacterial antibiotic resistance gene, typically ampicillin. This allows for the selection of bacteria that contain the plasmid.
- A plant promoter, often Cauliflower Mosaic Virus (CaMV) 35S promoter.
- A plant selectable marker & reporter genes. This can be a gene whose expression is easy to observe (GUS gene), or an antibiotic resistance gene (often kanamycin).

Design transgenic construct

One cassette



Two cassettes



Promoters

Type of promoter	Name	Comment
Constitutive	35S	Cauliflower Mosaic Virus (CaMV) 35S promoter is a strong promoter for plant tissue
	Ubi	Maize ubiquitin promoter strong promotor for monocot
Specific	Oleosin, Vicilin, glutelin Patatin RuBisCo	Expression is found in the seed Expression in tube specific Expression in green tissue
Inducible	Ethanol	Activation of a synthetic promoter

Some transgenes are known to impact plant growth or yield, so it is prudent to turn them on only when needed.

- *Inducing male sterility for hybrid seed production without the need for a restorer line;*
- *Turning on disease resistance genes only when the pathogen appears, to counter pathogen adaptation;*
- *Delaying expression of transgenes when the new protein interferes with early plant growth.*

Selectable Markers & Reporter Genes

- **Selectable Markers**
 - Antibiotics
 - Herbicides
- **Reporter Genes**

code for a gene product that has an easily detectable phenotype.

 - GUS** (β -glucuronidase gene)
 - LUC** (luciferase gene)
 - GFP** (Green Fluorescent Protein)

Selectable Markers

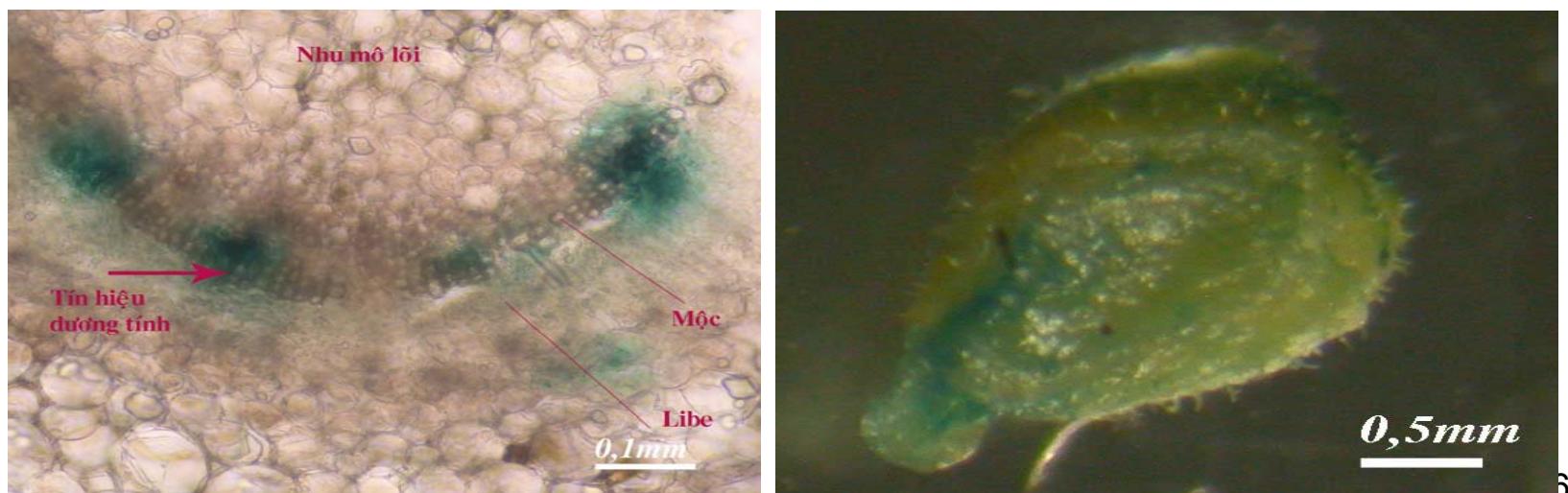
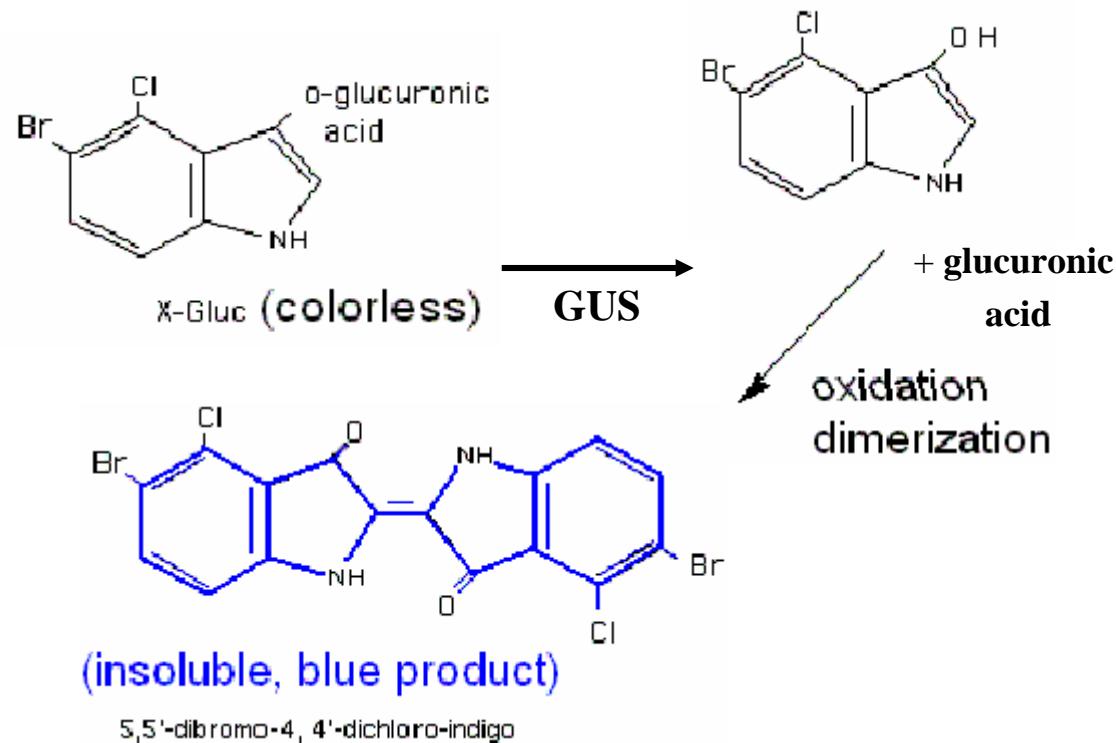
Selective Agent	Mode of Action	Resistance gene/ enzyme
Antibiotics		
Kanamycin	Binds to 30S ribosomal subunit to inhibit translation	<i>nptII</i> /neomycin phosphotransferase
Geneticin (G418)		
Hygromycin	Interacts w/EF2 to inhibit peptide chain elongation	<i>hpt</i> /hygromycin phosphotransferase
Herbicide		
Phosphinothricin (PPT, bialaphos Ignite)	Glutamine synthase inhibitor	<i>bar</i> /phosphinothricin acetyl transferase

Reporter genes

Substrate	Name	Source
Visual		
GUS (β -glucuronidase) blue	E.coli	5-bromo-4-chloro 3-indoyl-1-glucuronide (X-gluc)
LUC (Luciferase)	Firefly	luciferin & ATP
GFP (Green Flourescent Protein)	Jelly fish	none

β -Glucuronidse (GUS) gene

GUS or β -glucuronidase is an E.coli K12 enzyme encoded by the gusA gene (or uidA). Histochemical GUS assay uses the substrate X-Gluc (5-bromo-4-chloro-3-indolyl- β -D-glucuronide) which is incubated with whole mounts or sectioned specimens (organs/tissues). GUS cleaves colorless X-Gluc to form a blue product, insoluble in water and ethanol. Oxidative dimerization is required for formation of the insoluble blue pigment



Luciferase (*luc*) gene

*The North American firefly releases green light during the oxidation of its chemical substrate, luciferin. The glow is widely used as an assay for *luc* expression, which acts as a "reporter" for the activity of any regulatory elements that control its expression. Luciferase is particularly useful as a reporter-low-light cameras can detect bioluminescence, in real time and with high sensitivity, in living cells and organisms*



Source: www.campus.queens.edu 27

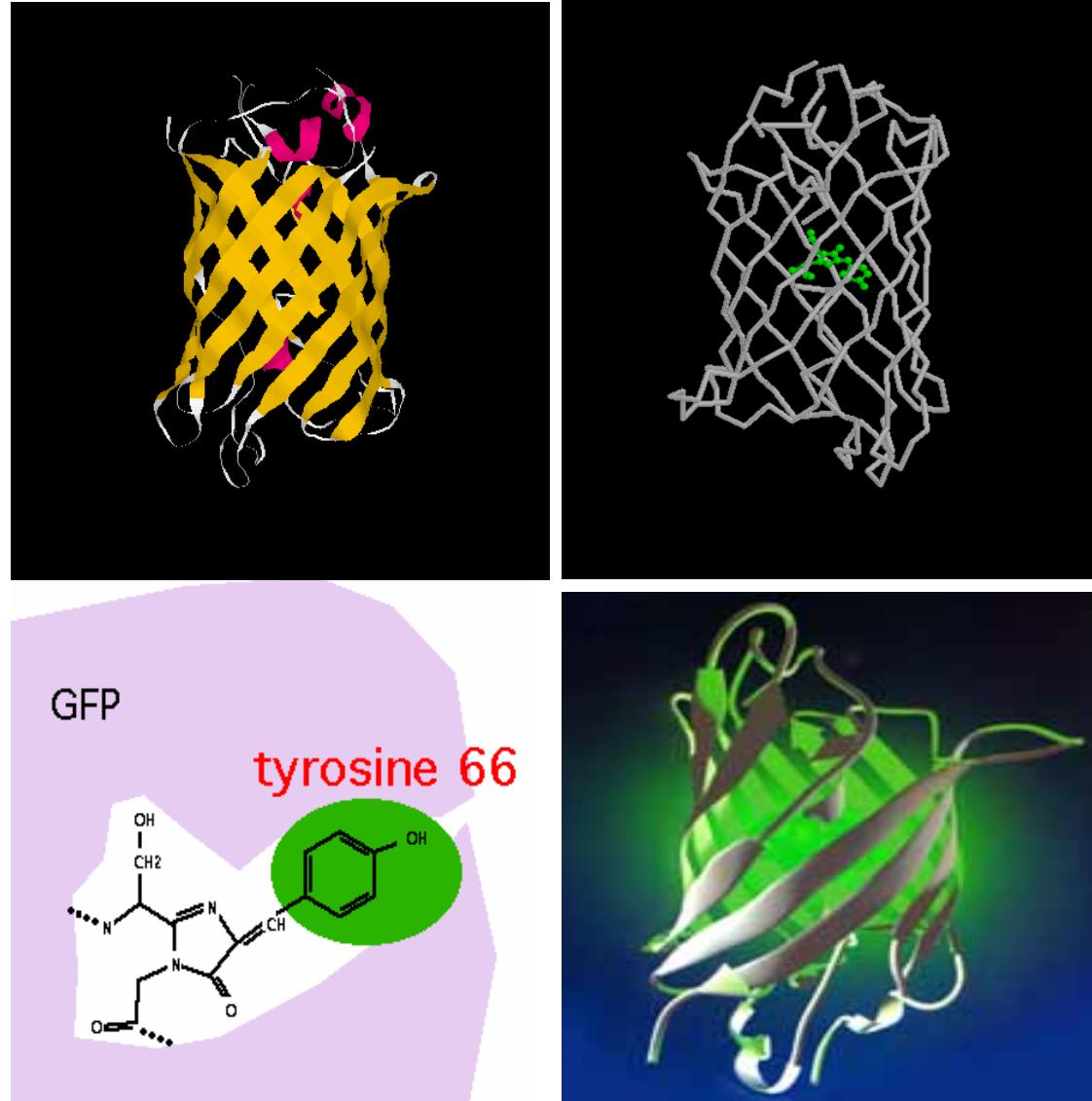
Green fluorescent protein (GFP)

*Protein identified from luminescent jellyfish *Aequorea victoria*. GFP has now been produced in a number of heterologous cell types and there appears to be little requirement for specific additional factors for post-translational modification of the protein, which may be autocatalytic or require ubiquitous factors. There are many structural variants now available commercially (e.g. red fluorescent protein). GFP provides a "window" onto the mechanisms that regulate the activity of specific genes, in specific, living cells.*

Structurally, GFP represents a new motif which has been named the "B-can". The "can" consists of 11 B-strands and a central α -helix. Fluorescence is mediated by a fluorophore located at the center of the protein. The fluorophore is derived from the tripeptide -Ser⁶⁵-Tyr⁶⁶-Gly⁶⁷- Formation of the fluorophore occurs in an autocatalytic manner.

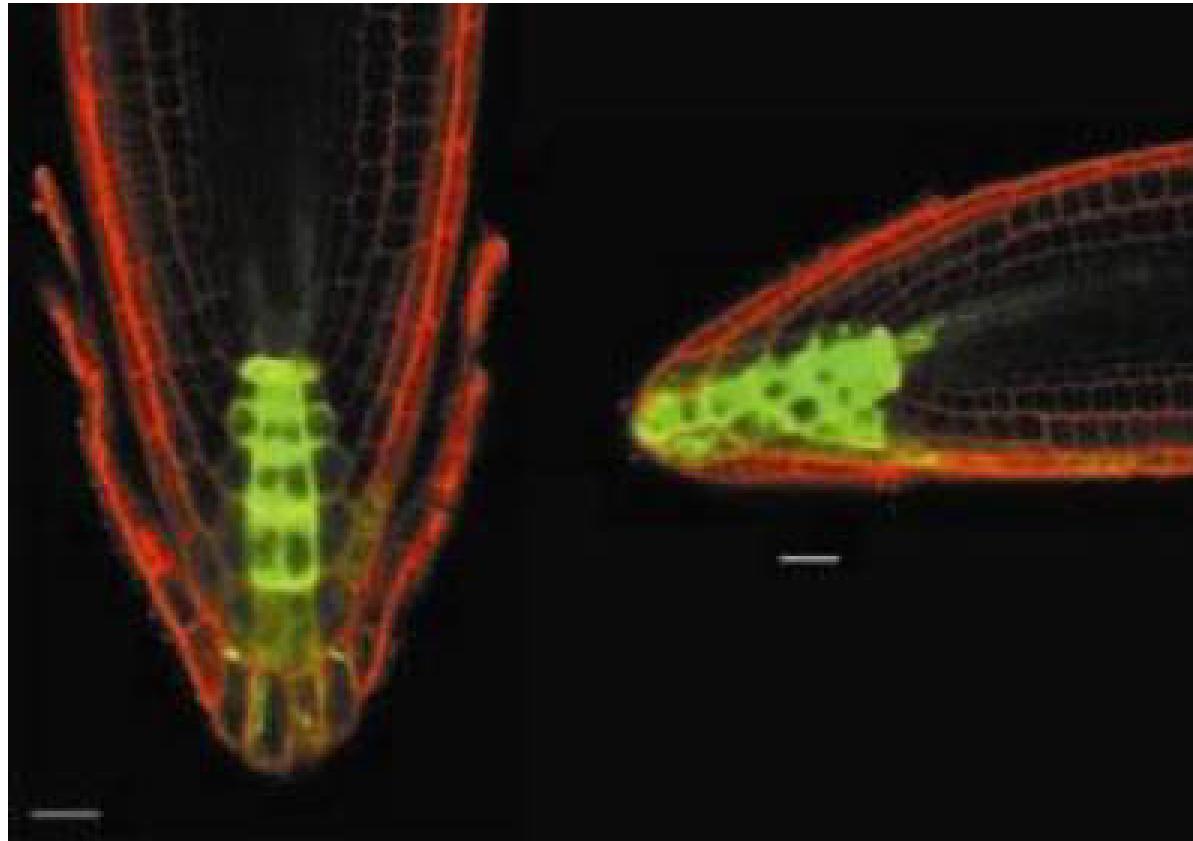
Green fluorescent protein (GFP)

- History of invent.
- Characteristic of structure and expression.



Source:
www.tsienlab.ucsd.edu

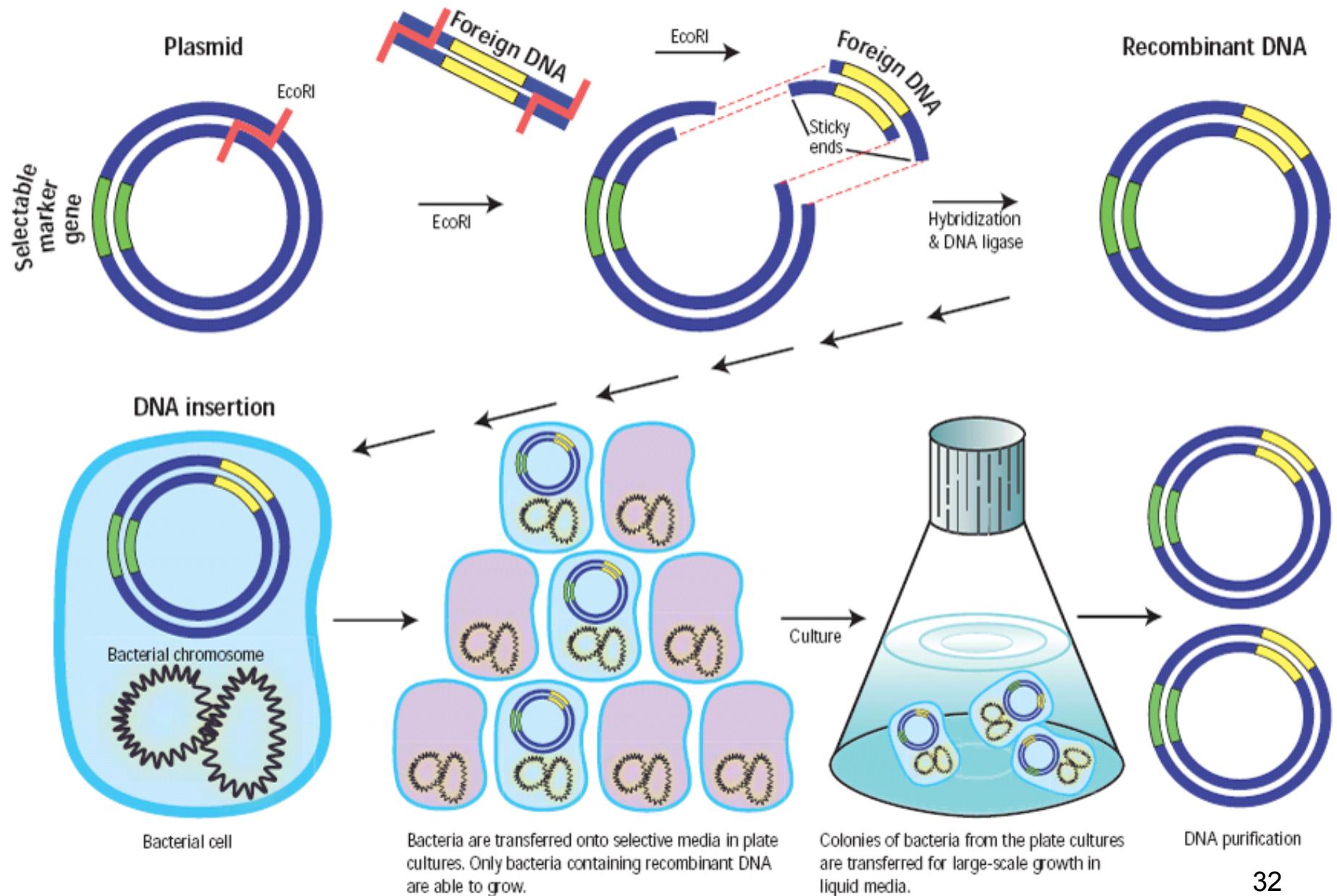
Expression of GFP in specific, living cells



GFP expression in *Arabidopsis* root tips under the control of an auxin-responsive DR5 promoter

(from ‘Plant Physiology On-line’ – Sinauer 2004, and Ottenschlager *et al* 2002) 30

*After construction of the vector using standard lab techniques (restriction digests, ligation) the vectors are typically introduced in *E. coli* and then propagated. The plasmid is then harvested and is either used for direct transformation (e.g. Biolistic) or is introduced in *A. tumefaciens*.*



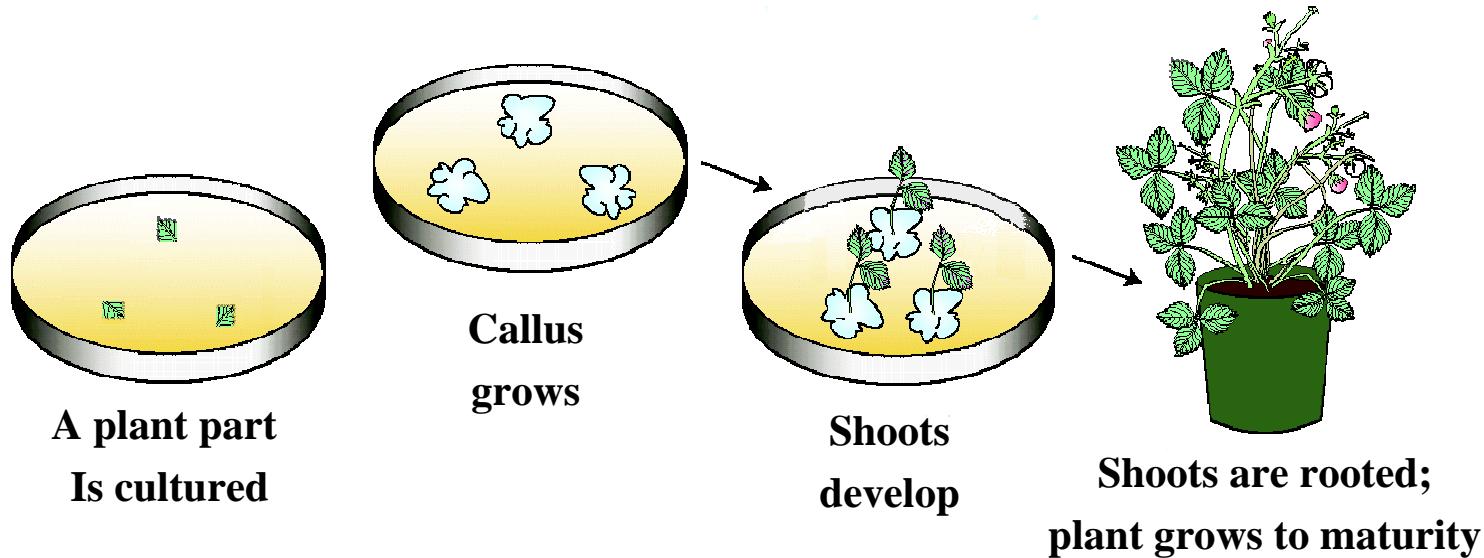
3.2. PLANT TISSUES (Cloning in plants)

PLANT TISSUE CULTURE FOR TRANSFORMATION

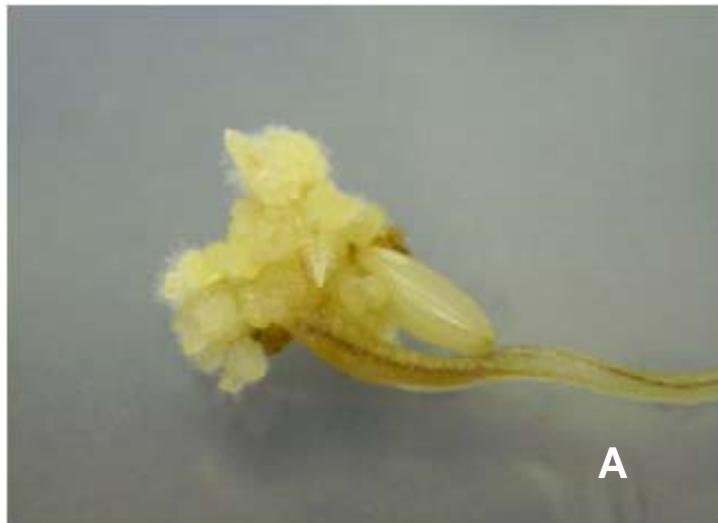
- Morphogenetically competent cells *In planta* transformation.

Morphogenetic Processes That Lead to Plant Regeneration (shoot formation from Organogenesis, Somatic embryogenesis or callus growth on explants).

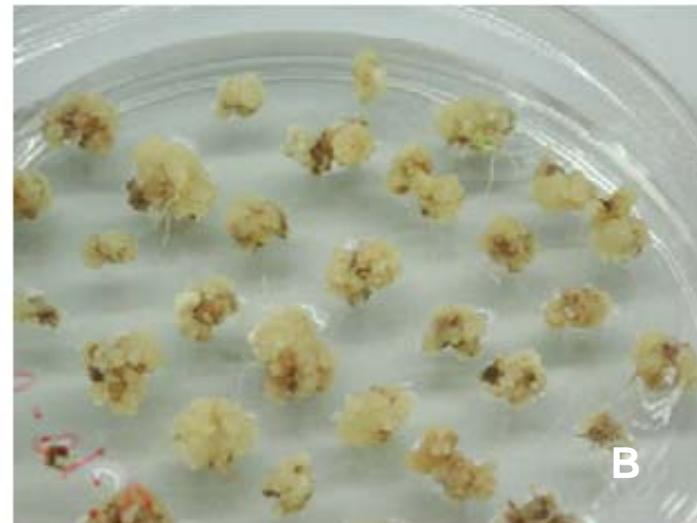
i.e. Produce callus → Transform callus → Stimulate shooting by cytokinin addition in rice. - Induction embryo for transformation in cassava.



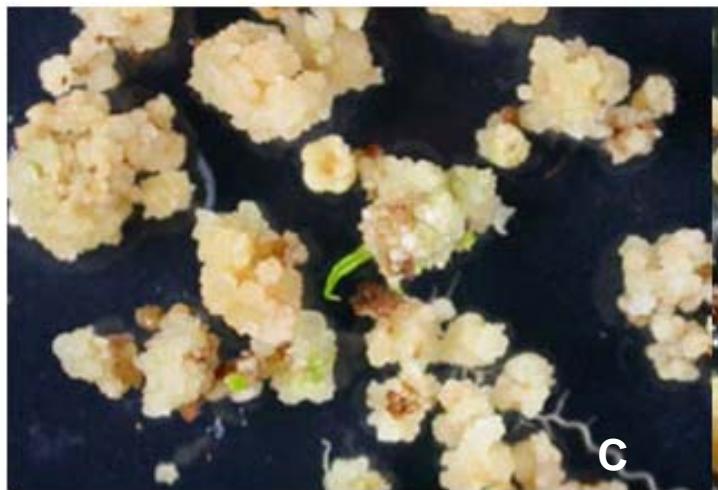
Produces callus for transformation in rice



A



B

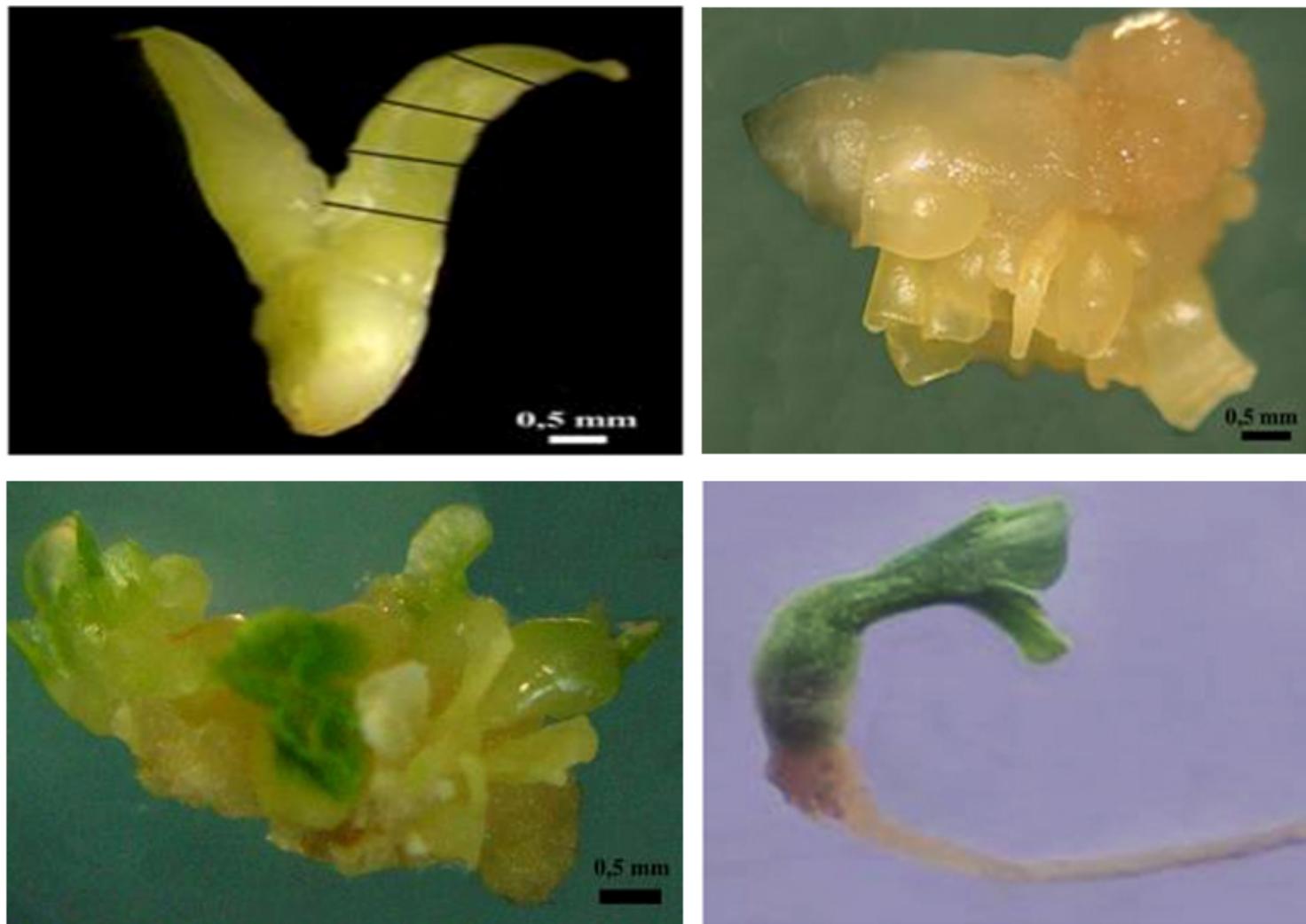


C



D

Induction of embryo culture for transformation in cassava



3.3. TRANSFORMATION METHODS (DNA delivery)

Methods of delivery DNA into the cell

3.3.1. Direct methods

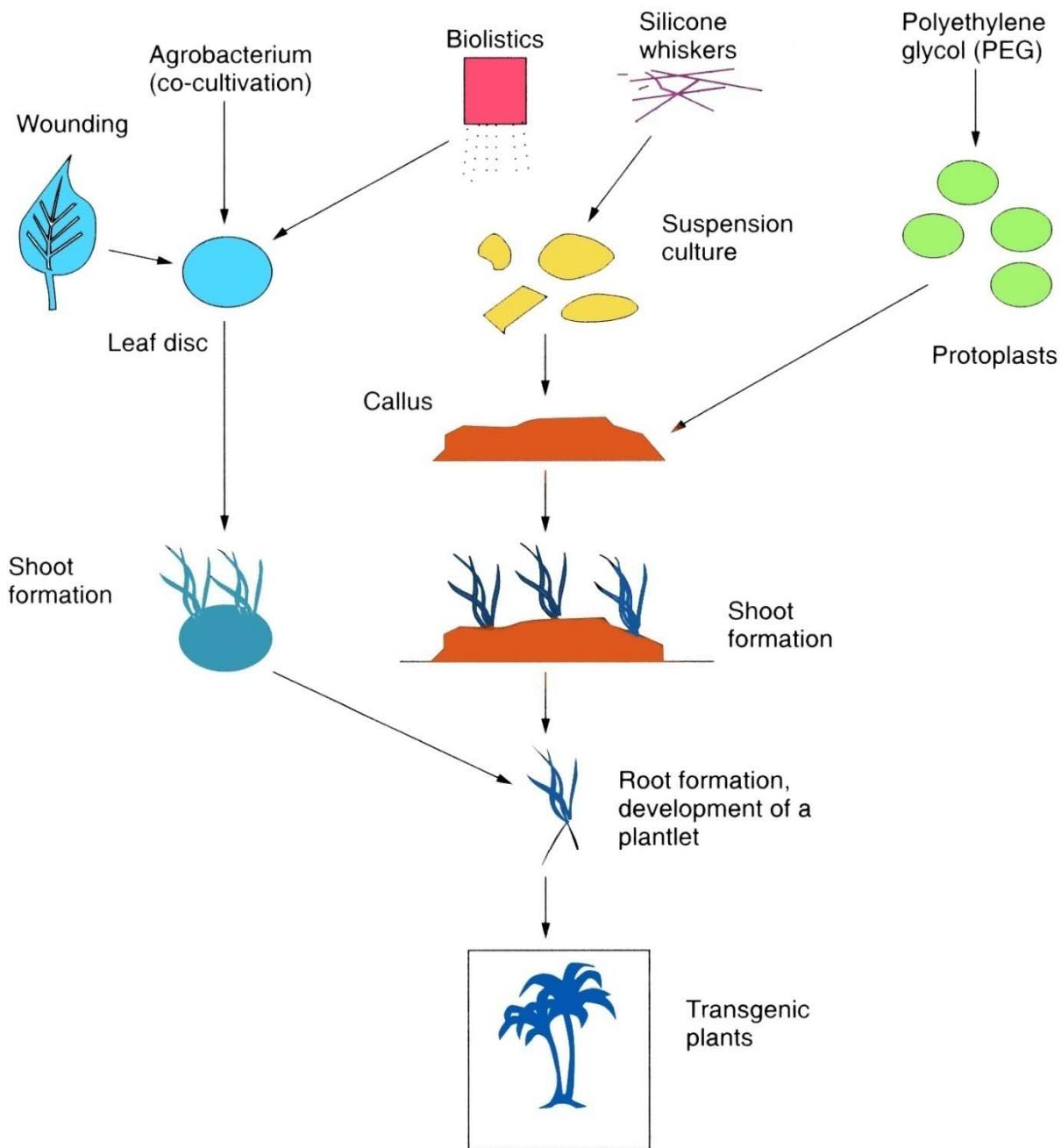
- microprojectile bombardment (biolistic)**
- *protoplast polyethyleneglycol (PEG) method*
- *protoplast electroporation*
- *silicone whiskers*

3.3.2. Indirect methods

Agrobacterium -mediated transformation:

- *Agrobacterium tumefaciens*
- *Agrobacterium rhizogenes (Hairy Root)*

Agrobiotechnology



3.3.1. Direct methods

*The most common technique for direct transformation is **microprojectile** or **particle bombardment**. This technique is based on the use of a “particle gun” or “gene gun”. The expression vector with target gene (s) is precipitated onto tungsten or gold particles which are then shot into the plant tissue. In most cases we will see only transient expression (i.e. the DNA does not integrate into the genome but is transcribed until it degrades). In a small percentage of the cells the DNA will integrate and we will see stable expression. The main drawback of this technique is that often multiple copies of the transgene insert. It is also necessary to have an *in vitro* regeneration system in place.*

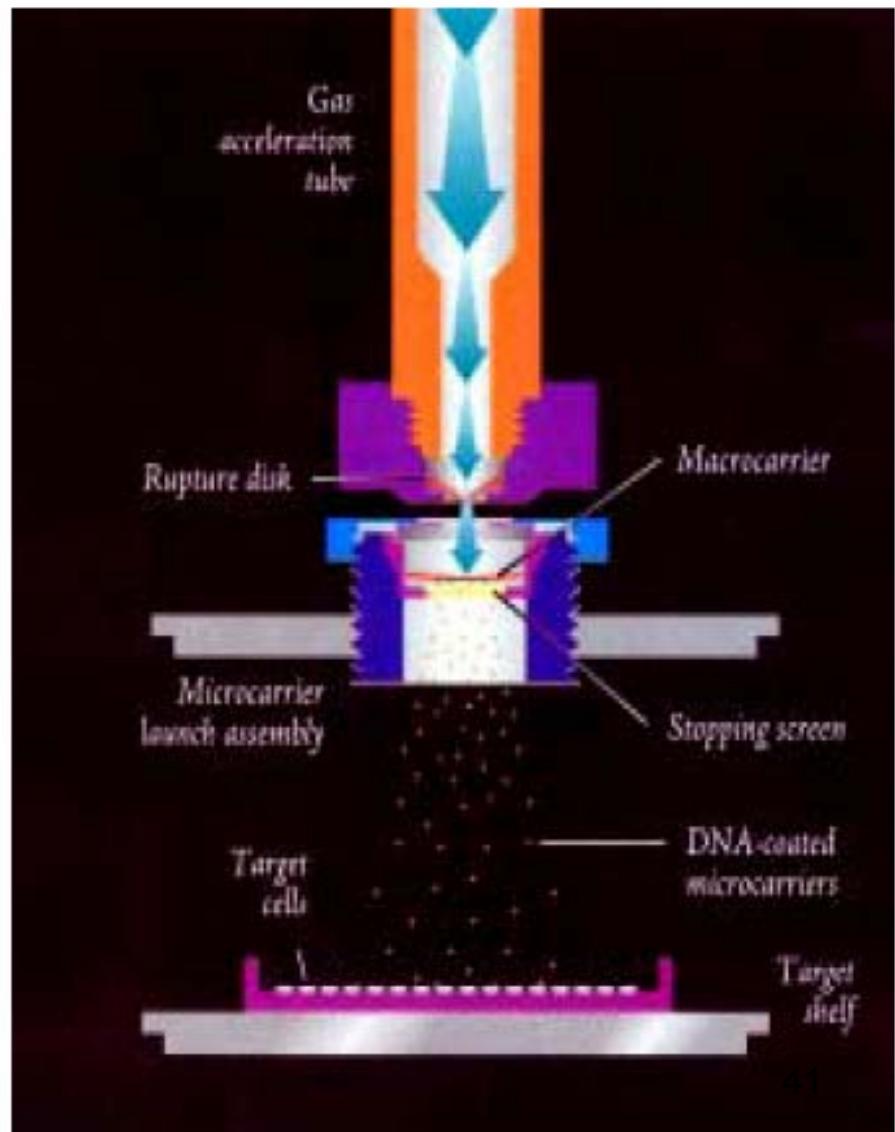
Particle bombardment

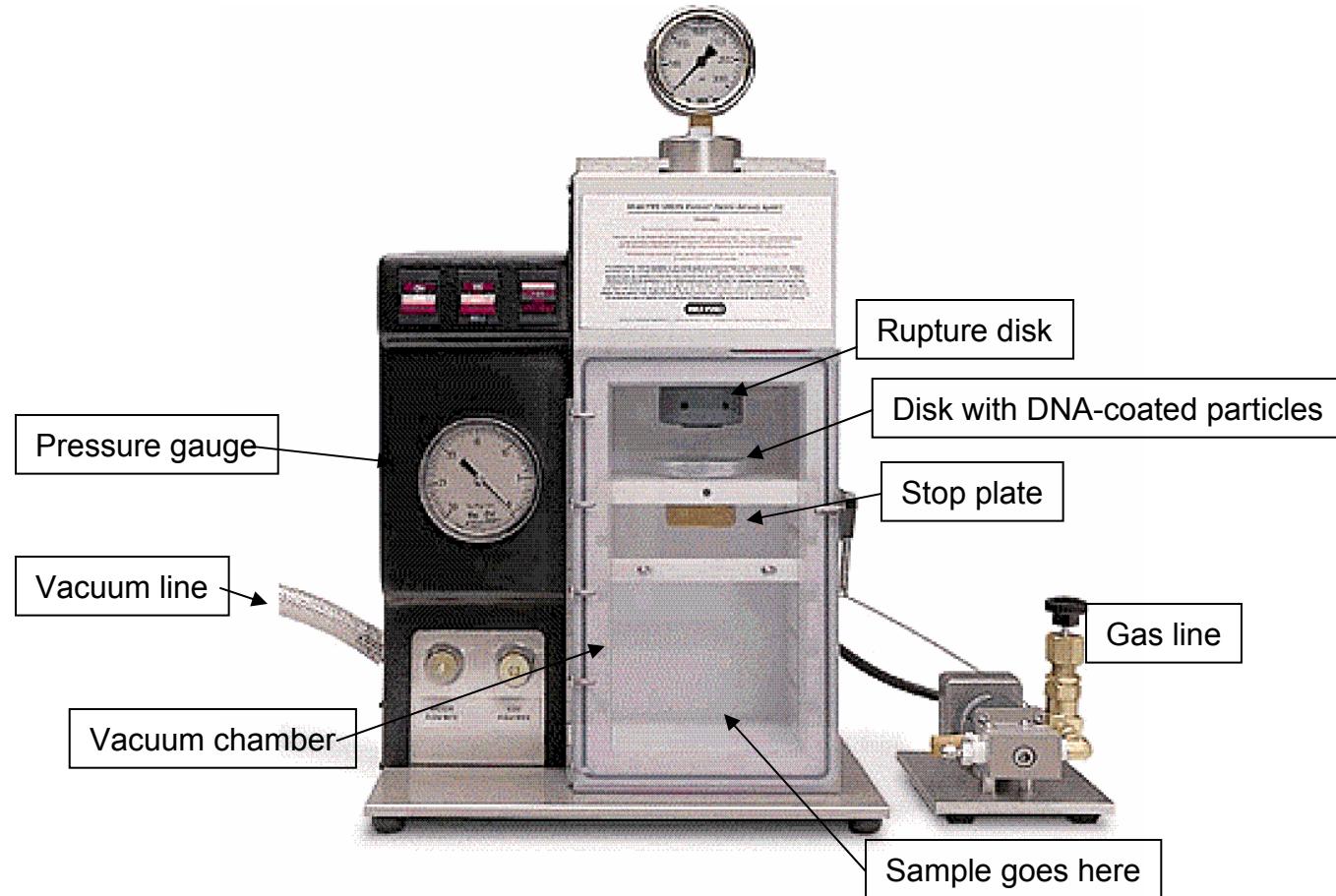


Gene gun



Particle delivery system





Biostatic® -PDS-1000/He Particle Delivery System

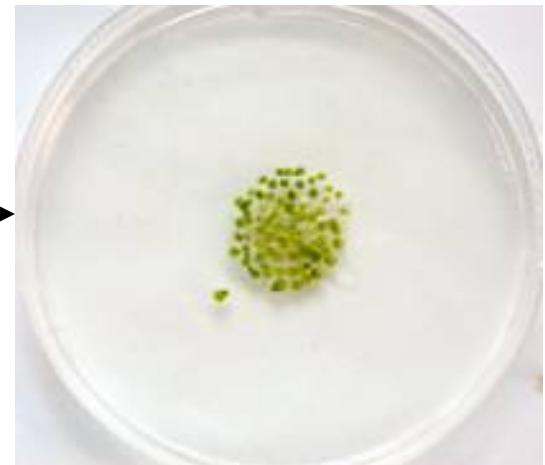
The model biolistic protocol



Binding DNA to
microprojectiles



Spread DNA over the macrocarrier



Plant tissue
preparation



Control pressure



Fit up

Vetiver inflorescence explants for Particle Bombardment (Test GUS)



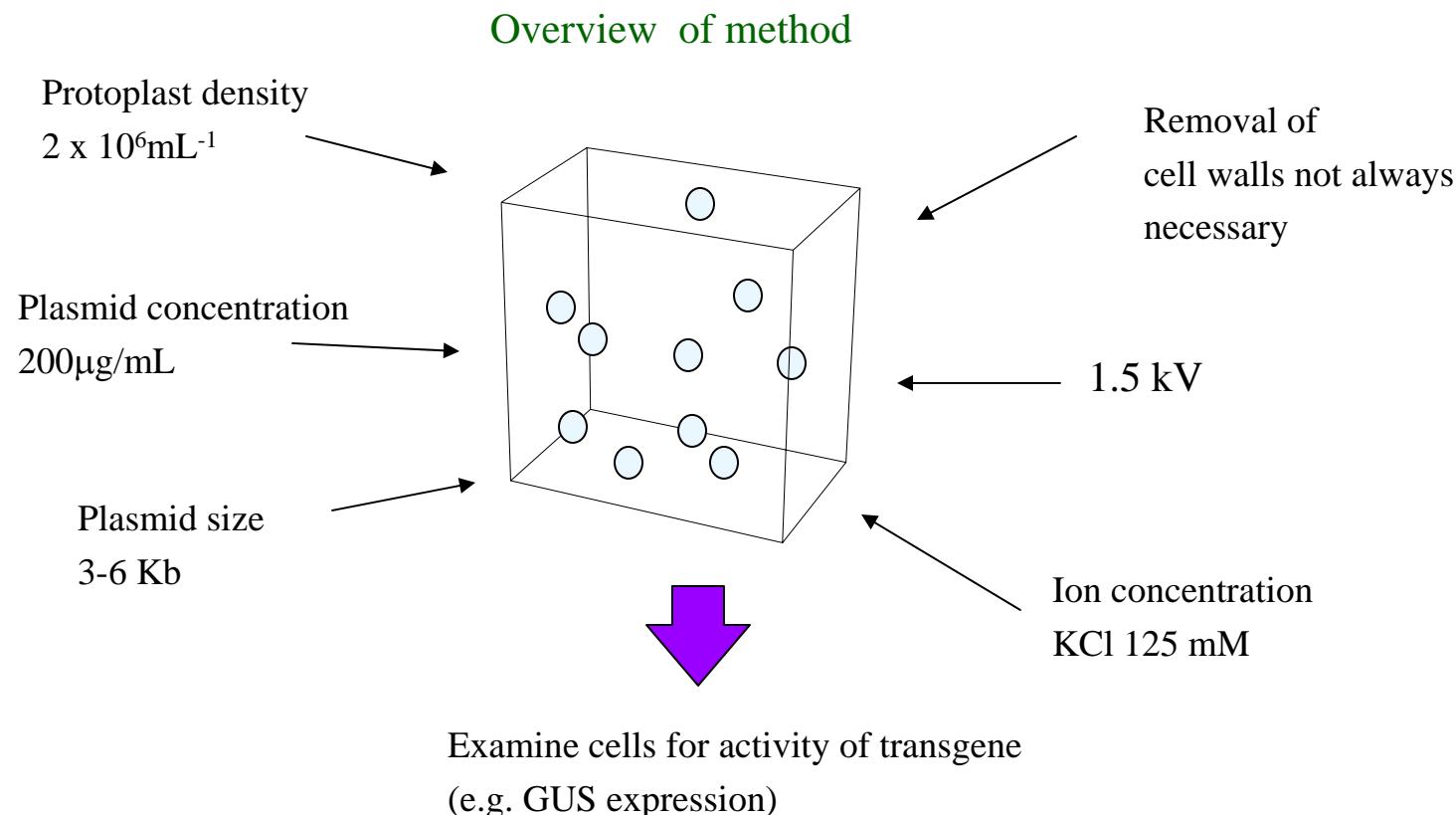
Protoplast polyethyleneglycol method

Protoplast polyethyleneglycol (PEG) method

- *It was the first technique to report the successful integration of foreign genes into a plant cell .*
- *PEG induces reversible permeabilization of the plasma membrane.*
- *Low transformation efficiency (only 0.0004%).*
- *Difficult to regenerate.*

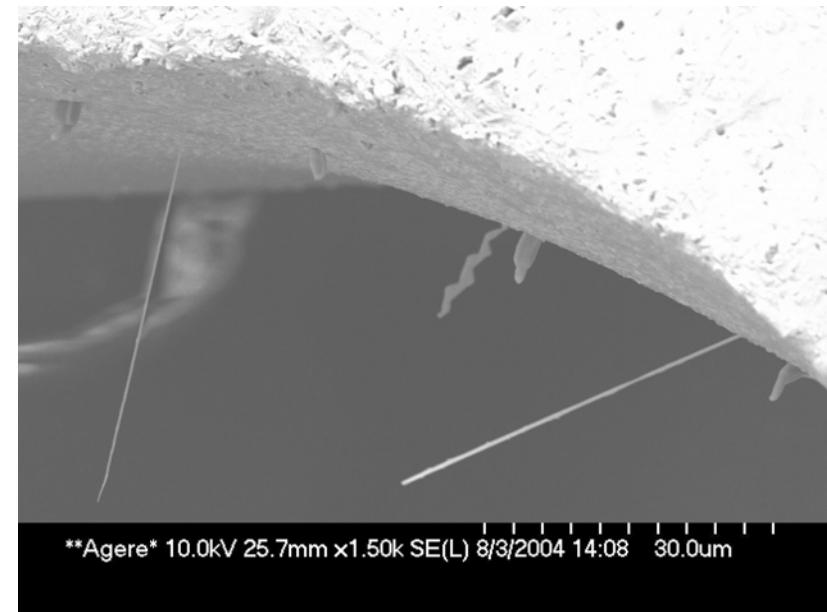
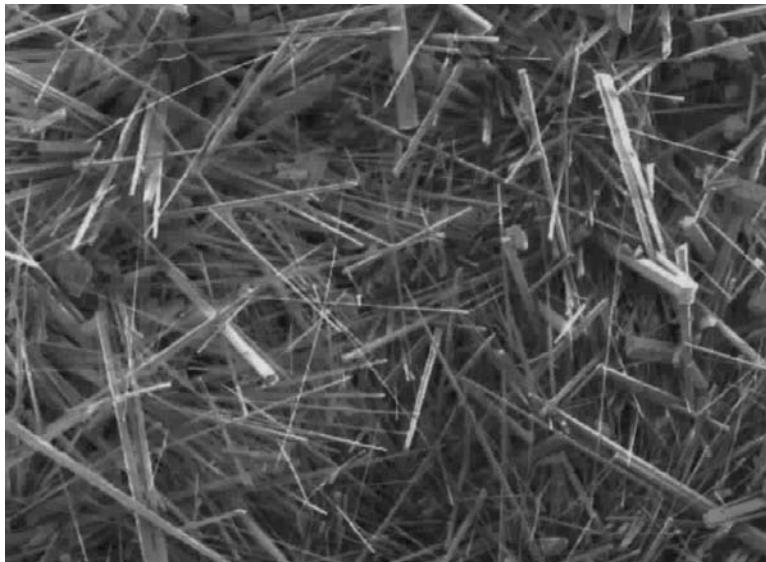
Protoplast electroporation method

- *Intensive electrical field leads to pores on plasma membrane, allowing DNA to enter*
- *Many variables need optimizing*



Silicone whiskers

- Use silicon carbide fibers to punch holes through cultured plant cells
- Silicon carbide fibers and cultured plant cells are added to a tube and vortexed vigorously
- The mechanical force generated by the vortex drives the fibers into the cell



3.3.2. Indirect methods (Agrobacterium-mediated transformation)

Agrobacterium tumefaciens and *Agrobacterium rhizogenes* are pathogenic soil bacteria that contain a *Ti* (tumor inducing) or *Ri* (root inducing) plasmid. A small piece of this plasmid, the *T-DNA* is transferred from the bacteria to the plant and integrates stably into the plant genome. The focus will primarily be on *A. tumefaciens* and the *Ti*-plasmid because it is more common.

Agrobacterium tumefaciens

- *Agrobacterium tumefaciens* is a Gram-negative soil phytopathogen.
- *Agrobacterium* affect most dicotyledonous plants in nature, resulting in crown gall tumors at the soil-air junction upon tissue wounding.
- Crown gall disease is not generally fatal, but it will reduce plant vigor and crop yield, and crown galls will attract other phytopathogens or pests.
- In some cases, necrosis or apoptosis is observed after *Agrobacterium* infection.

Tumor characteristics

- hormone (auxin & cytokinin) levels altered, explains abnormal growth
- synthesize a unique amino acid, called “opine”
 - octopine and nopaline (derived from arginine)
 - agropine (derived from glutamate)
- specific opine depends on the strain of *A. tumefaciens*
- opines are catabolized by the bacterium, which can use only the specific opine that it caused the plant to produce

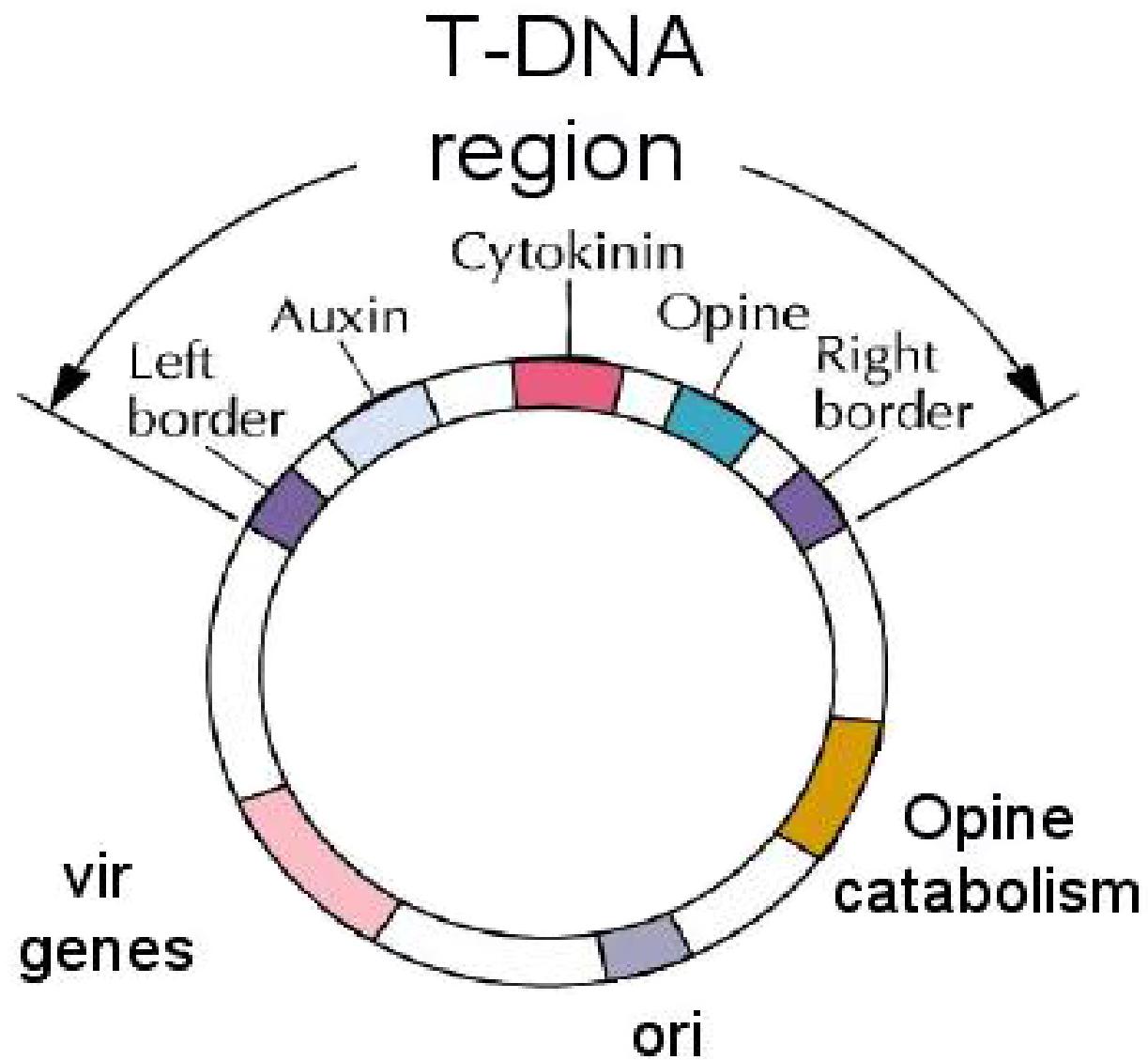
T-DNA

The T-DNA encodes genes that stimulate cell division (result in production of plant hormones) and the synthesis of opines (amino acid derivatives, either nopalines or octopines). These opines are used by the bacteria as carbon and nitrogen source. The Ti-plasmid contains genes that help in the break-down of these opines.

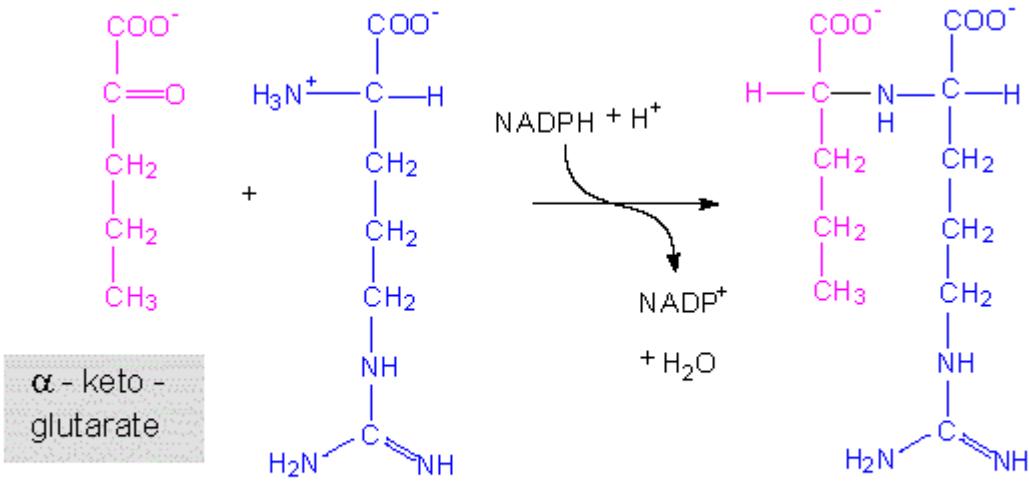
*The T-DNA, which is transferred to the plant cell, contains two types of genes: oncogenic genes, which are responsible for tumor formation, and genes encoding enzymes that synthesize opines, compounds synthesized and excreted by crown gall cells and consumed by *A. tumefaciens* as carbon and nitrogen sources.*

The vir genes are required for the transfer of the T-DNA from the bacterium to the plant cell. There are also genes involved in opine catabolism and in bacterium to bacterium plasmid transfer.

The 25 bp repeats flanking the T DNA mediate the transfer of this DNA from bacterium to host cell.



nopaline synthase
(NOS) reaction

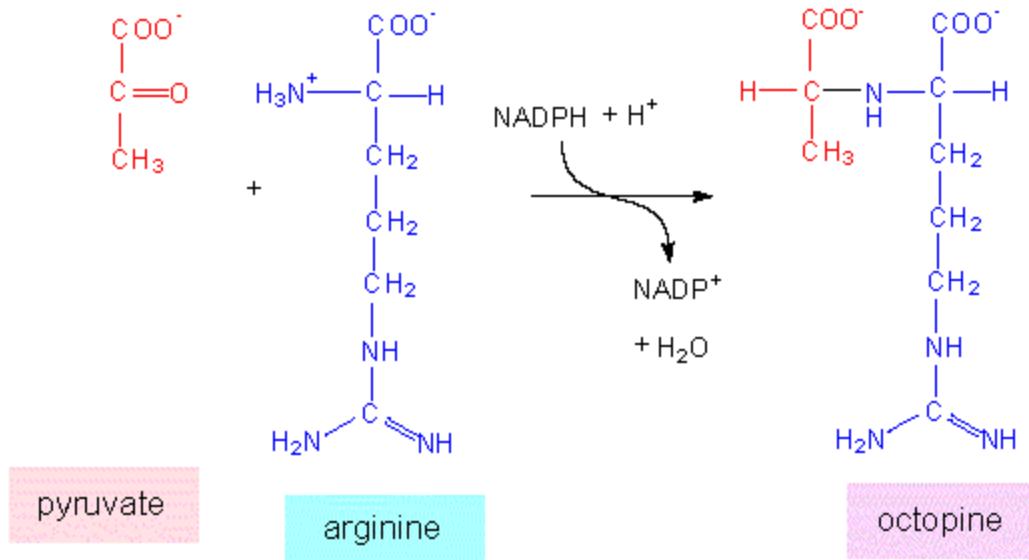


nopaline = arginine + *alpha*-ketoglutarate

octopine = arginine + pyruvate

mannopine = glutamine + mannose

octopine synthase
(OCS) reaction

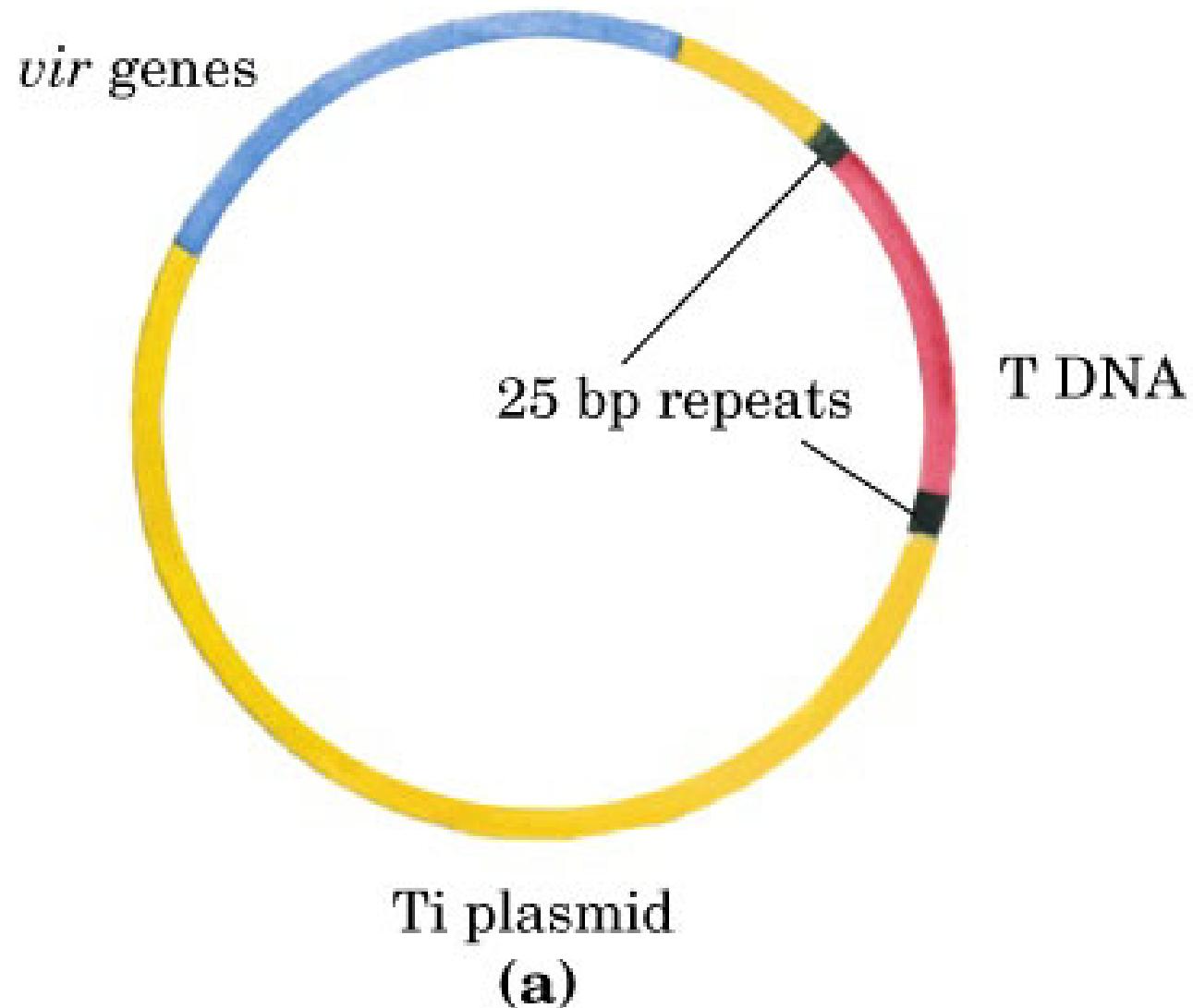


Biology of the *Agrobacterium*-plant interaction

Mechanism of *Agrobacterium*-plant cell interaction

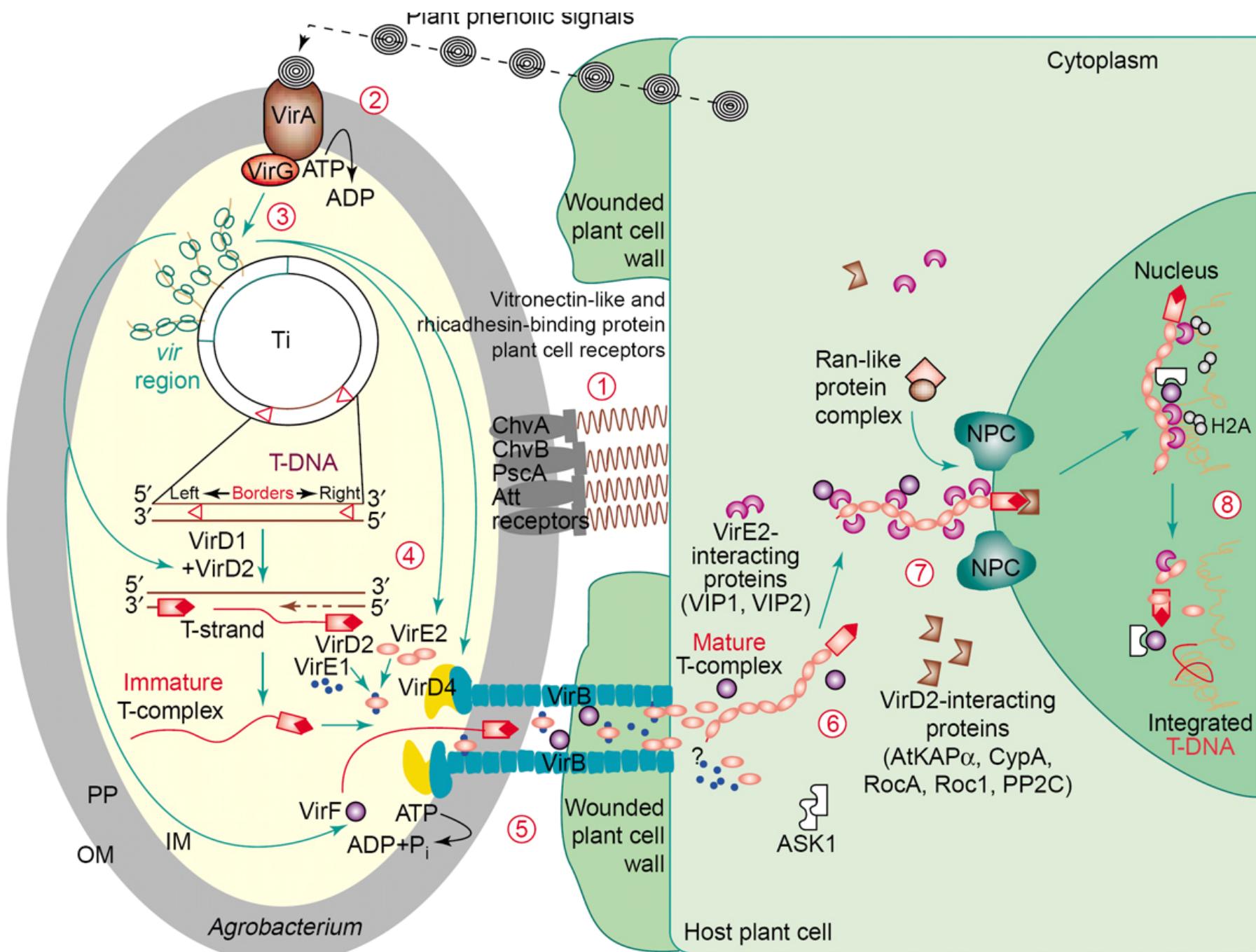
- *One of the earliest stages in the interaction between Agrobacterium and a plant is the attachment of the bacterium to the surface of the plant cell. A plant cell becomes susceptible to Agrobacterium when it is wounded. The wounded cells release phenolic compounds, such as acetosyringone, that activate the vir-region of the bacterial plasmid.*
- *It has been shown that the Agrobacterium plasmid carries three genetic components that are required for plant cell transformation.*

- *The first component, the T-DNA that is integrated into the plant cells, is a mobile DNA element.*
- *The second one is the virulence area (*vir*), which contains several *vir* genes. These genes do not enter the plant cell but, together with the chromosomal DNA (two loci), cause the transfer of T-DNA.*
- *The third component, the so-called border sequences (25 bp), resides in the Agrobacterium chromosome. The mobility of T-DNA is largely determined by these sequences, and they are the only *cis* elements necessary for direct T-DNA processing.*



Steps of plant-*Agrobacterium* interaction

1. *Cell-cell recognition*
2. *Signal transduction and transcriptional activation of vir genes*
3. *T-DNA transport*
4. *Nuclear import*
5. *T-DNA integration*

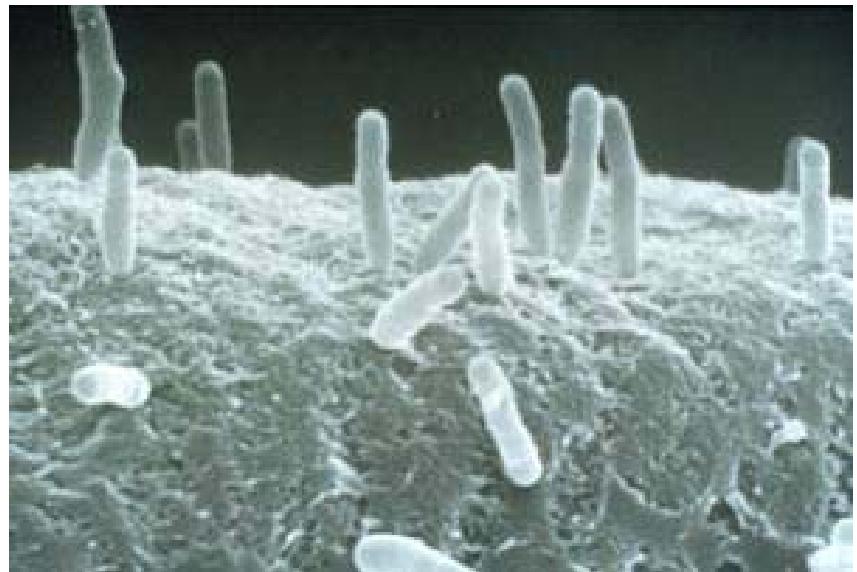


Cell-cell recognition

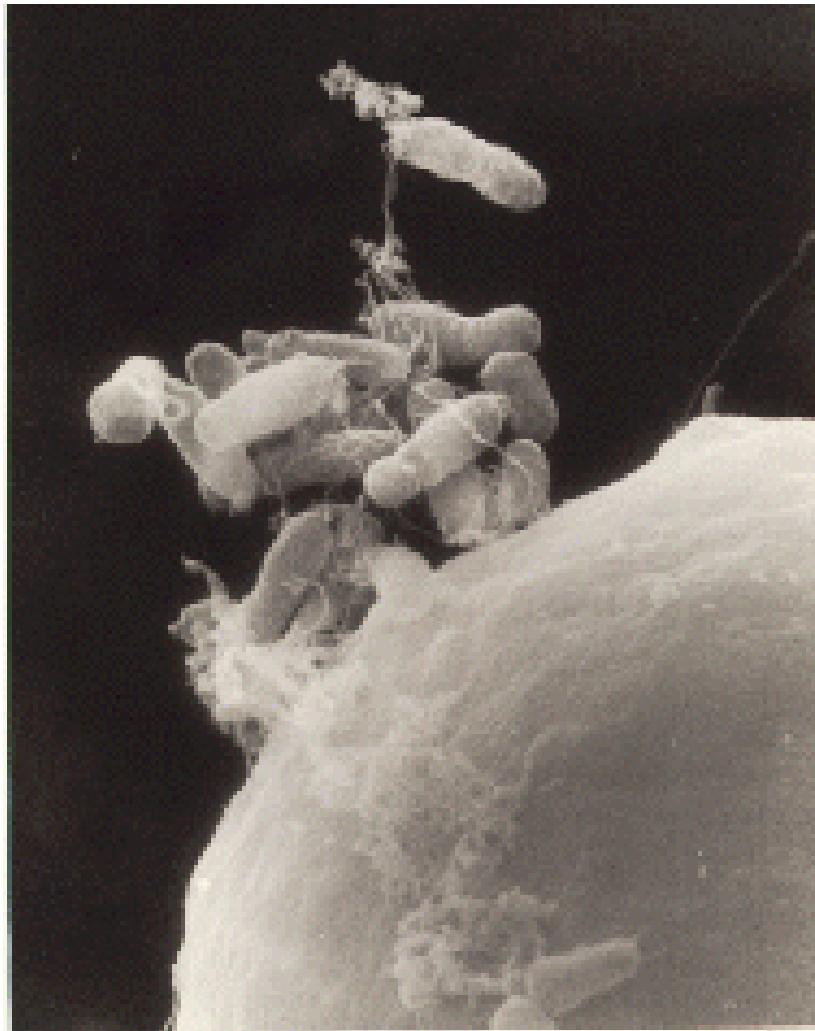
Agrobacterium-host cell recognition is a two-step process:

- ✓ *Loosely bound step: acetylated polysaccharides are synthesized.*
- ✓ *Strong binding step: bound bacteria synthesize cellulose filaments to stabilize the initial binding, resulting in a tight association between Agrobacterium and the host cell.*

Plant vitronectin-like protein (PVN, 55kDa) was found on the surface of plant cell. This protein is probably involved in initial bacteria/plant cell binding.



Source: www.wishart.biology.ualberta.ca



Source: www.en.wikipedia.com

Plant signals

- *Wounded plants secrete sap with acidic pH (5.0 to 5.8) and a high content of various phenolic compounds (lignin, flavonoid precursors) serving as chemical attractants to agrobacteria and stimulants for vir gene expression.*
- *Among these phenolic compounds, acetosyringone (AS) is the most effective.*
- *Low opine levels further enhance vir gene expression in the presence of AS .*

- ✓ Sugars like glucose and galactose also stimulate *vir* gene expression when AS is limited or absent.
- ✓ Sugars interact with *ChvE* (glucose/galactose binding protein) which interacts with *VirA* through its periplasmic domain.
- ✓ These sugars are probably acting through the *chvE* gene to activate *vir* genes.

Activation of vir genes

- *These compounds stimulate the autophosphorylation of a transmembrane receptor kinase VirA at its His-474. It in turn transfers its phosphate group to the Asp-52 of the cytoplasmic VirG protein.*
- *VirG then binds to the vir box enhancer elements in the promoters of the virA, virB, virC, virD, virE and virG operons, upregulating transcription.*

Production of T-strand

- *Every induced Agrobacterium cell produces one T-strand.*
- *VirD1 and VirD2 are involved in the initial T-strand processing, acting as site-and strand-specific endonucleases.*
- *After cleavage, VirD2 covalently attaches to the 5' end of the T-strand at the right border nick and to the 5'-end of the remaining bottom strand of the Ti plasmid at the left border nick by its tyrosine 29.*
- *VirC1 enhances T-strand production by binding to overdrive.*
- *Overdrive is a cis-active 24-base pair sequence adjacent to the right border of the T-DNA. It stimulates tumor formation by increasing the level of T-DNA processing.*

- *VirC1 enhances T-strand production by binding to overdrive.*
- *Overdrive is a cis-active 24-base pair sequence adjacent to the right border of the T-DNA. It stimulates tumor formation by increasing the level of T-DNA processing.*

Formation of the T-complex

The T-complex is composed of at least three components:

- *one T-strand DNA molecule, one VirD2 protein, and around 600 VirE2 proteins.*
- *Whether VirE2 associates with T-strand before or after the intercellular transport is not clear.*
- *If VirE2 associates with the T-strand after interbinding, VirE1 is probably involved in preventing VirE2-T-strand*
- *Judging from the size of the mature T-complex (13nm in diameter) and the inner dimension of T-pilus (10nm width), the T-strand is probably associated with VirE2 after intercellular transport.*

Intercellular transport

Transport of the T-complex into the host cell most likely occurs through a type IV secretion system.

In Agrobacterium, the type IV transporter (called T-pilus) comprises proteins encoded by virD4 and by the 11 open reading frames of the virB operon.

Intercellular transport of T-DNA is probably energy dependent, requiring ATPase activities from VirB4 and VirB11.

Physical contact between Agrobacterium and the plant cell is required to initiate T-complex export. Without recipient plant cells, T-strands accumulate when vir genes are induced

Nuclear Import

Because of the large size of T-complex (50,000 kD, ~13nm in diameter), the nuclear import of T-complex requires active nuclear import.

The T-complex nuclear import is presumably mediated by the T-complex proteins, VirD2 and VirE2. Both of them have nuclear-localizing activities.

VirD2 is imported into the cell nucleus by a mechanism conserved between animal, yeast and plant cells (bipartite consensus motif).

VirE2 has a plant-specific nuclear localization mechanism. It does not localize to the nucleus of yeast or animal cells

In host plant cells VirD2 and VirE2 likely cooperate with cellular factors to mediate T-complex nuclear import and integration into the host genome.

These host factors have been identified through two-hybrid screens; however, their functions are not clear

T-DNA integration

T-DNA integration is not highly sequence-specific. (About 40% of the integrations are in genes and more of them are in introns.)

Non-homologous end-joining occurs during T-DNA integration.

- Integration is initiated by the 3'(LB) of the T-DNA invading a poly T-rich site of the host DNA.
- A duplex is formed between the upstream region of the 3'-end of T-DNA and the top strand of the host DNA.
- The 3'-end of T-DNA is ligated to the host DNA after a region downstream of the duplex is degraded.
- A nick in the upper host DNA strand is created downstream of the duplex and used to initiate the synthesis of the complementary strand of the invading T-DNA.
- The right end of the T-DNA is ligated to the bottom strand of the host DNA. This pairing frequently involves a G and another nucleotide upstream of it.

Why is *Agrobacterium* used for producing transgenic plants?

The T-DNA element is defined by its borders but not the sequences within. So researchers can substitute the T-DNA coding region with any DNA sequence without any effect on its transfer from Agrobacterium into the plant.

Ti-based plasmid vectors for plant transformation

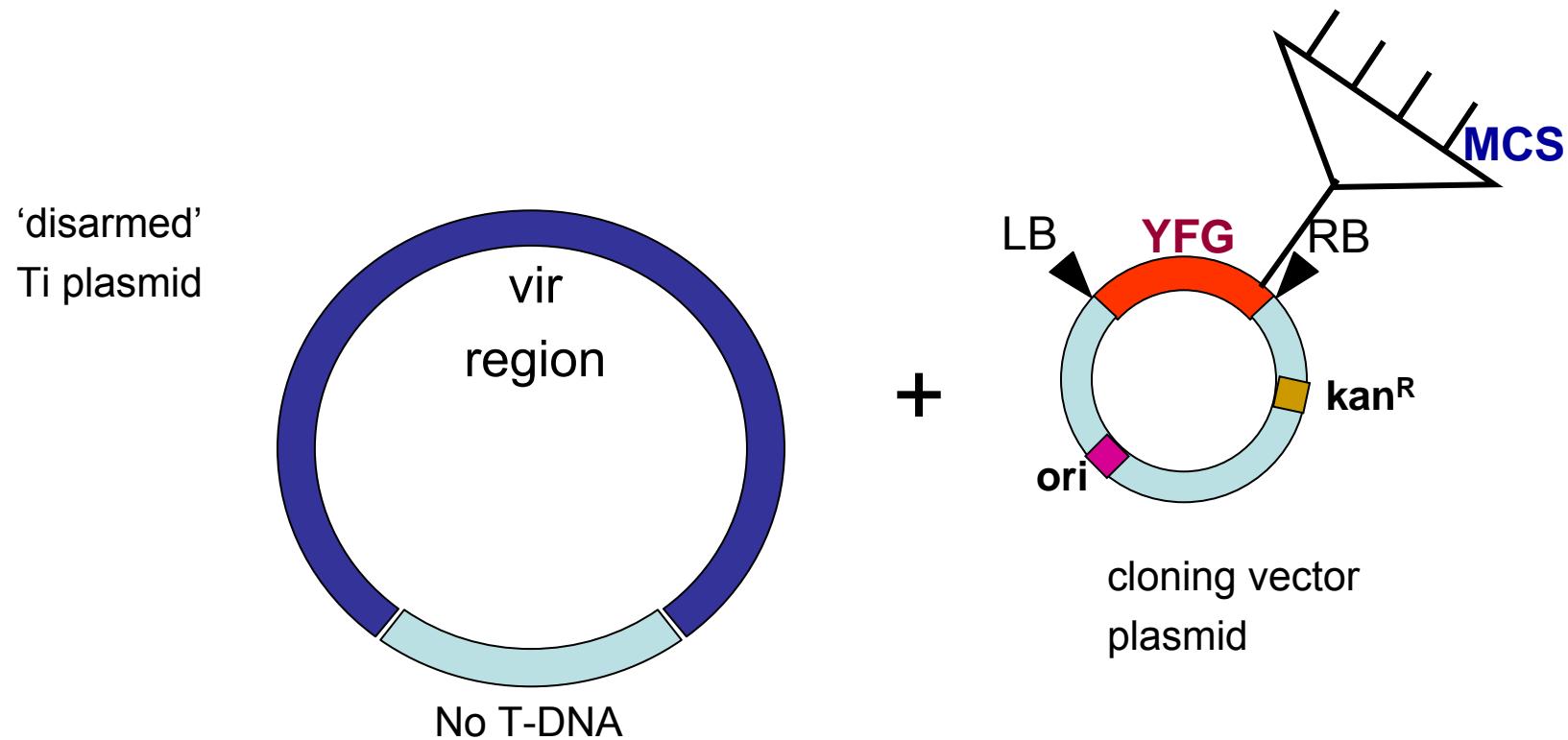
In 1983, Hoekema et. al. and de Frammond et al. found that the T-region and the Vir genes could be separated into two different replicons.

Disarmed vectors do not produce tumors and can be used to regenerate normal plants containing the foreign gene.

The Vir helper plasmid generally contains a complete or partial deletion of the T-region, rendering strains containing this plasmid unable to incite tumors.

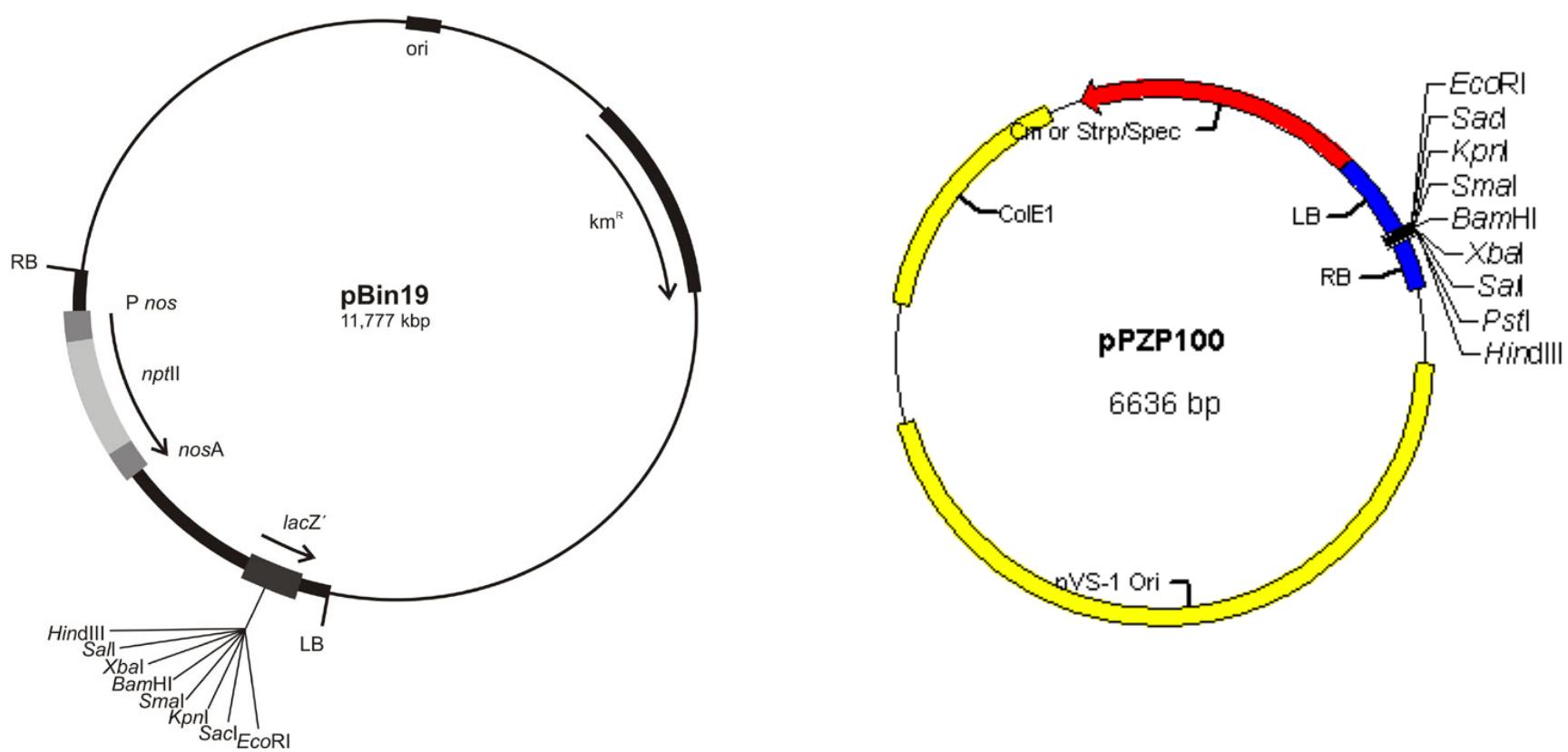
Agrobacterium strains like LBA4404, GV3101 MP90, AGLO, EHA101, EHA105 (derivative of EHA101), and NT1 all contain a Vir helper plasmid.

System binary vectors



One of the earliest binary vectors constructed was pBIN19, made by M. Bevan in 1984. After that, modifications were made in such vectors to expand the range of their utility and to improve their transformation efficiency.

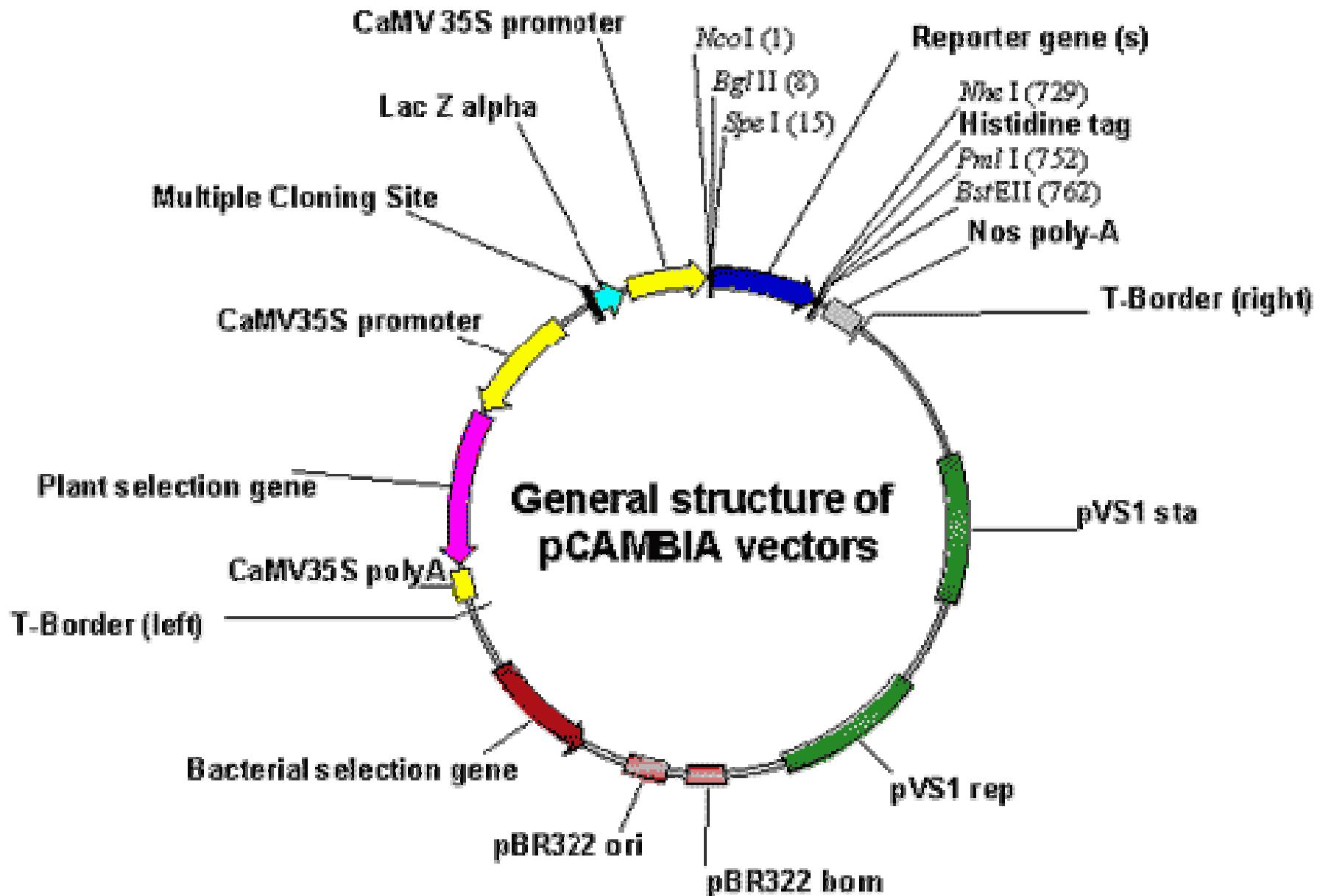
Smaller binary vectors like the pPZP series are stable in Agrobacterium and easy to handle.



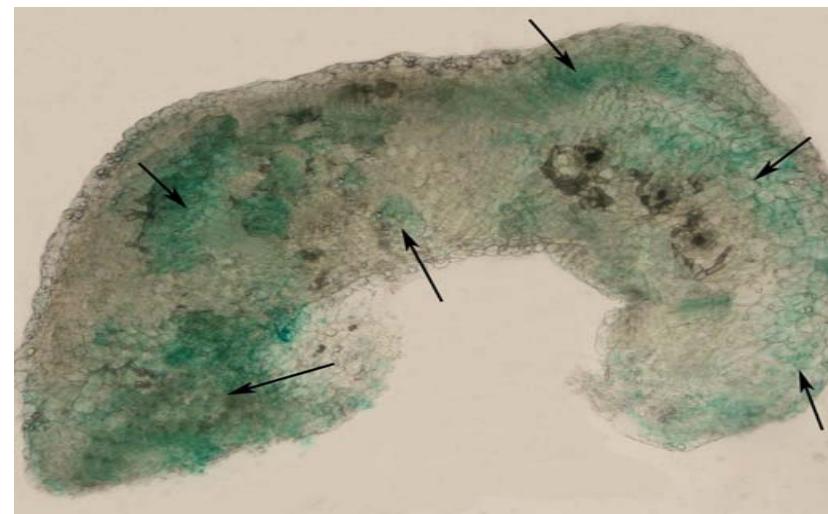
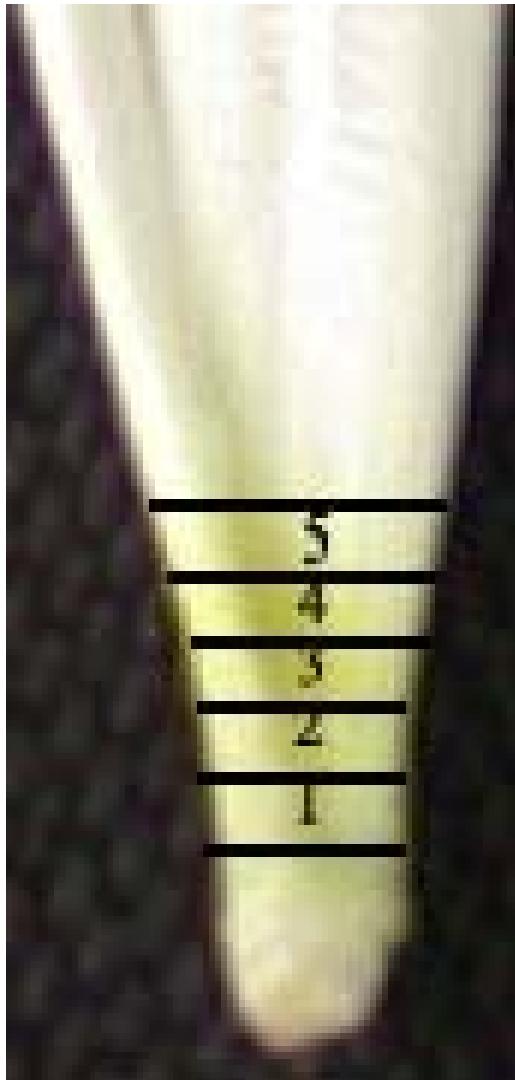
*Binary vectors like the pCAMBIA series were constructed from pPZP with more choices of selection markers (*nptII*, *bar*, or *hpt*) and reporters (*gus* or *gfp*).*

*Superbinary vectors contain copies of *virB*, *virG* and *virC*, and have made Agrobacterium-mediated transformation of monocots such as japonica rice possible.*

Binary BAC (BIBAC) system can transfer 150 kb DNA into plants.

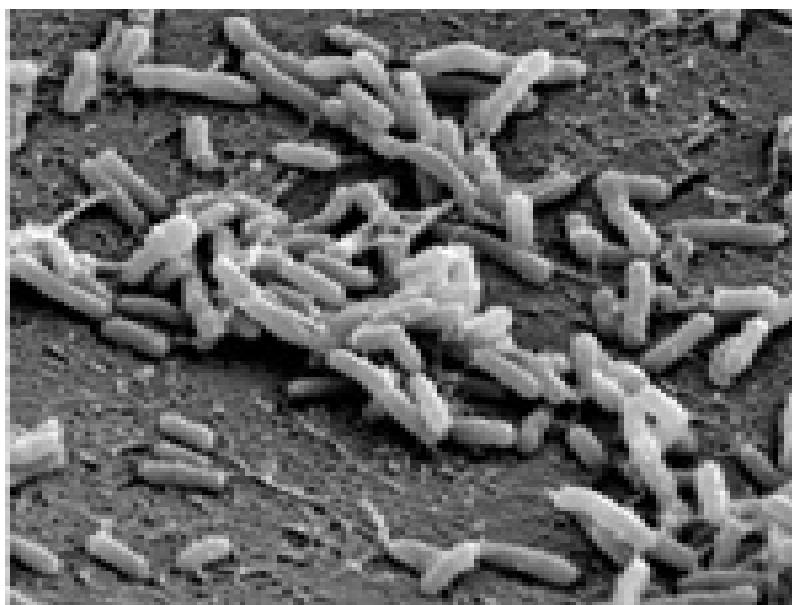


e.g. Chrysanthemum explants for *Agrobacterium* mediated-transformation (Test GUS)



Mechanism of *Agrobacterium rhizogenes*

- *A. rhizogenes* carries a different (Ri) large plasmid but possesses most of the same functionality.
- The Ri T-DNA appears to lack a gene for cytokinin biosynthesis, which probably accounts for the “rooty” morphology of *A. rhizogenes*-induced tumours

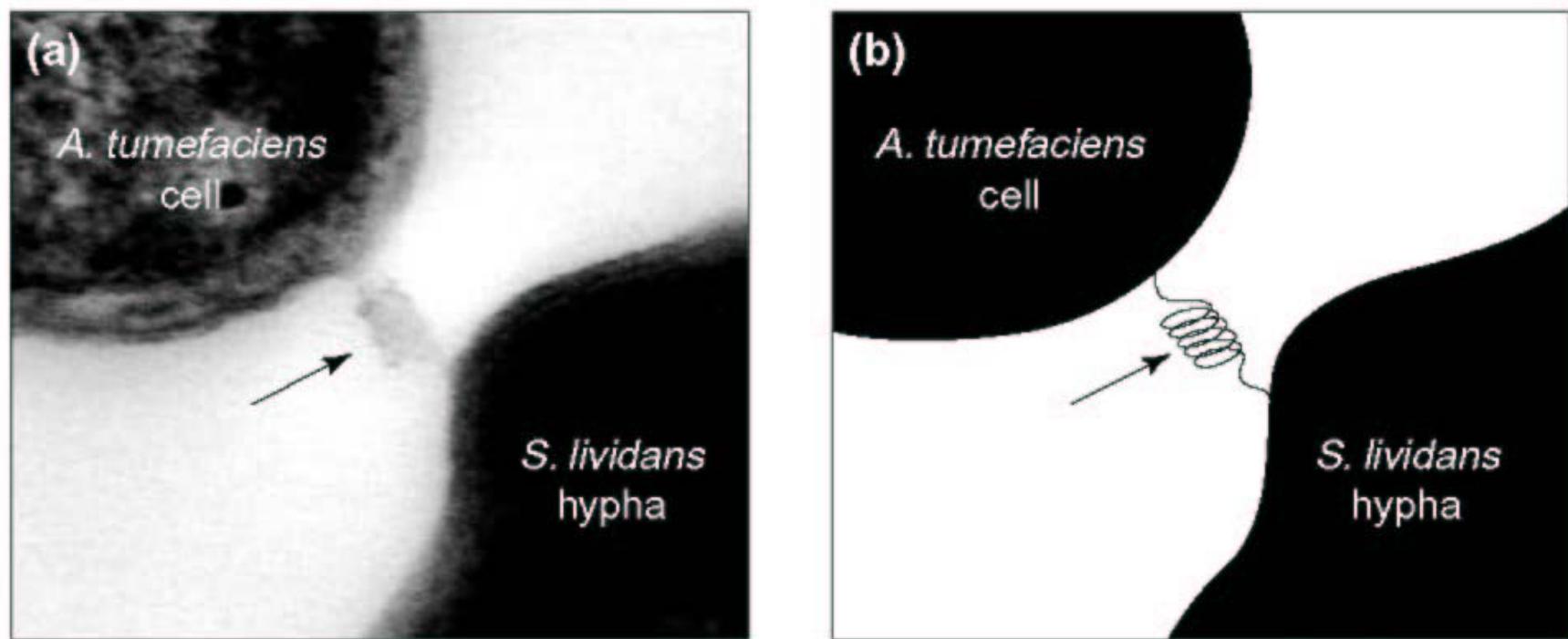


Source: www.bio.davidson.edu

The genus *Agrobacterium* has a wide host range:

- ✓ Overall, *Agrobacterium* can transfer T-DNA to a broad group of plants.
- ✓ Yet, individual *Agrobacterium* strains have a limited host range.
- ✓ The molecular basis for the strain-specific host range is unknown.
- ✓ Many monocot plants can be transformed (now) although they do not form crown gall tumors.
- ✓ Under lab conditions, T-DNA can be transferred to yeast, other fungi, and even animal and human cells.

T-pilus usually wind into compact coils to bring the bacterium and host cell closer



trends in Microbiology

Fig. 1. (a) Planar thin section showing attachment of an *Agrobacterium tumefaciens* cell to a hypha of *Streptomyces lividans* by means of a coiled T-pilus filament. A diagram depicting the association is shown in (b).

3.4. IN PLANTA TRANSFORMATION

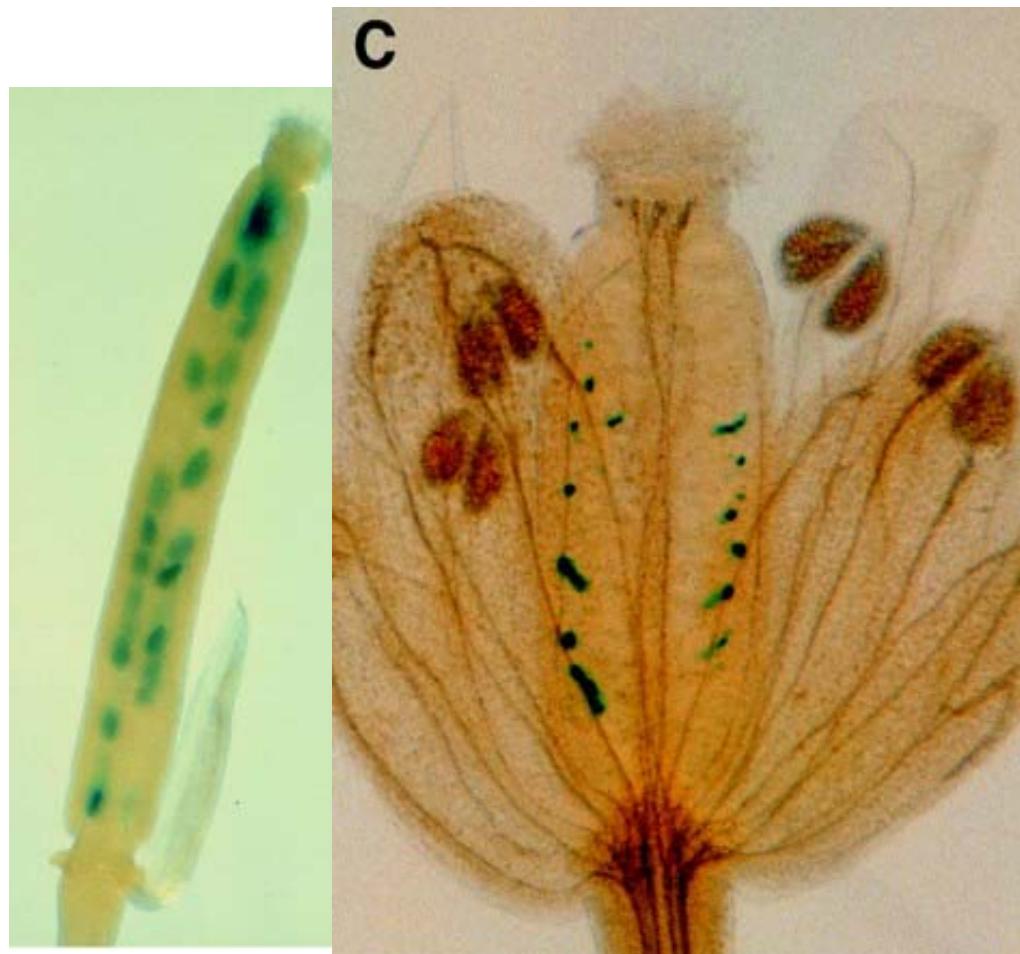
The latest Agrobacterium-based transformation technique in Arabidopsis is referred to as “floral dip”. In this case the tissue culture stage has been eliminated. Instead, the Arabidopsis plant is dipped into a suspension of Agrobacterium at the stage where it has the maximum number of unopened floral bud clusters. The suspension also contains a detergent to reduce surface tension, and some sugar. The bacteria get into the developing flowers and transfer the T-DNA. This then results in the formation of transgenic seeds that can be planted out on medium with a selectable marker. The plants that survive on the selectable medium get transplanted and can be studies or screened.

Arabidopsis
floral
transformation

Source: www.plantmethod.com

- *All of the methods transform female reproductive tissue.*
- *Reports showed that only the application of Agrobacterium to pollen acceptor will produce transformants.*
- *Reports also showed that GUS expression can only be observed in ovaries after flowers were infected with Agrobacterium carrying ACT11-gusA-Intron.*

Agrobacterium infect the ovaries of flowers



GUS staining can only be observed in ovaries 5 days after inoculation and is vanished 12 days after inoculation.

How can *Agrobacterium* infects the ovaries of flowers?



- *Because the gynoecium of *Arabidopsis* were formed by two carpels and they remained separated until three days before anthesis (flowering).*

Agrobacterium vs particle bombardment

In general *Agrobacterium* is considered the method of choice for transformation.

Advantages include:

- ✓ *low copy number of the transgene;*
- ✓ *higher proportion of stable transformants;*
- ✓ *larger DNA segments can be transferred;*
- ✓ *more time-efficient.*