PLNT2530 Plant Biotechnology
2024
Unit 8a

Transformation with Agrobacterium

Plant Transformation

Transformation: The incorporation of foreign DNA into a chromosome.

***Agrobacterium* - mediated transformation**
Takes advantage of *Agrobacterium tumefacians* natural ability to transfer DNA into plants

Direct transfection of DNA
use of physical or chemical means to introduce foreign DNA into plant cells

Agrobacterium tumefaciens, a natural plant genetic engineer

- Soil bacterium, related to *Rhizobium*
- causes crown galls (tumors) on many dicots
- infects woody and herbaceous

plants belonging to 140 genera

and more than 60 families.

- Infection occurs at wound sites

Agrobacterium infection and tumorigenesis

- Infection occurs only at wound sites
- Involves recognition and chemotaxis of the bacterium toward wounded cells
- galls are tumors, which can be removed and grow indefinitely without hormones even in the absence of *Agrobacterium*
- Therefore, genetic information for tumor growth must be transferred to plant cells

Rationale:

T-DNA transformation is a form of parasitism. *Agrobacterium* transforms the plant to force it to produce opines, which serve as a carbon source for the bacterium.

The Ti-plasmid has all the necessary genes for this process:

- T-DNA mobilization
- opine synthesis
- opine catabolism

Tumor characteristics

- hormone (auxin & cytokinin) levels altered, explains abnormal growth

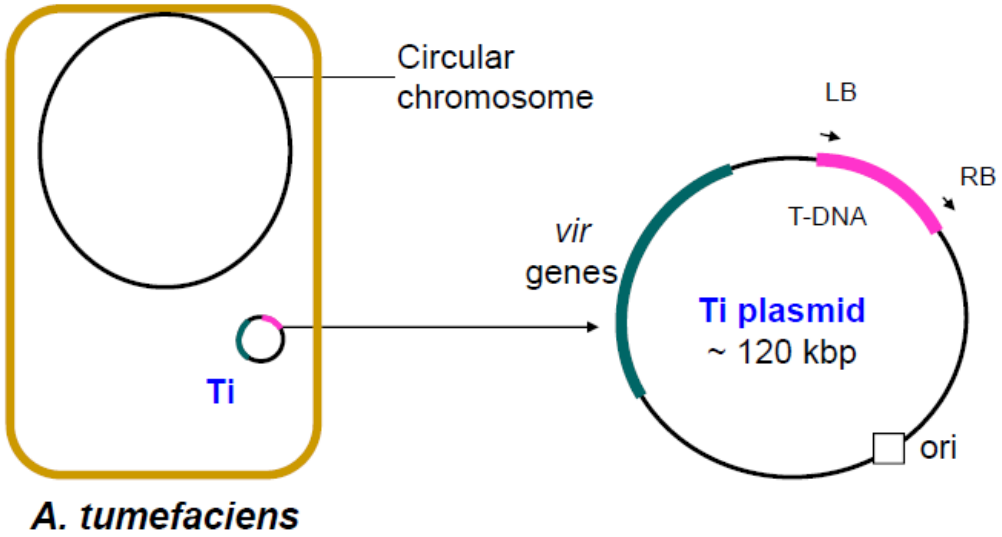
- synthesize unique amino acids, called “opines”
  - octopine and nopaline (derived from arginine)
  - agropine (derived from sugars)

- specific opine depends on the strain of *A. tumefaciens*

- opines are catabolized by the bacterium as a carbon source. Each strain of *Agrobacterium* has genes required for catabolizing the opine that it produces

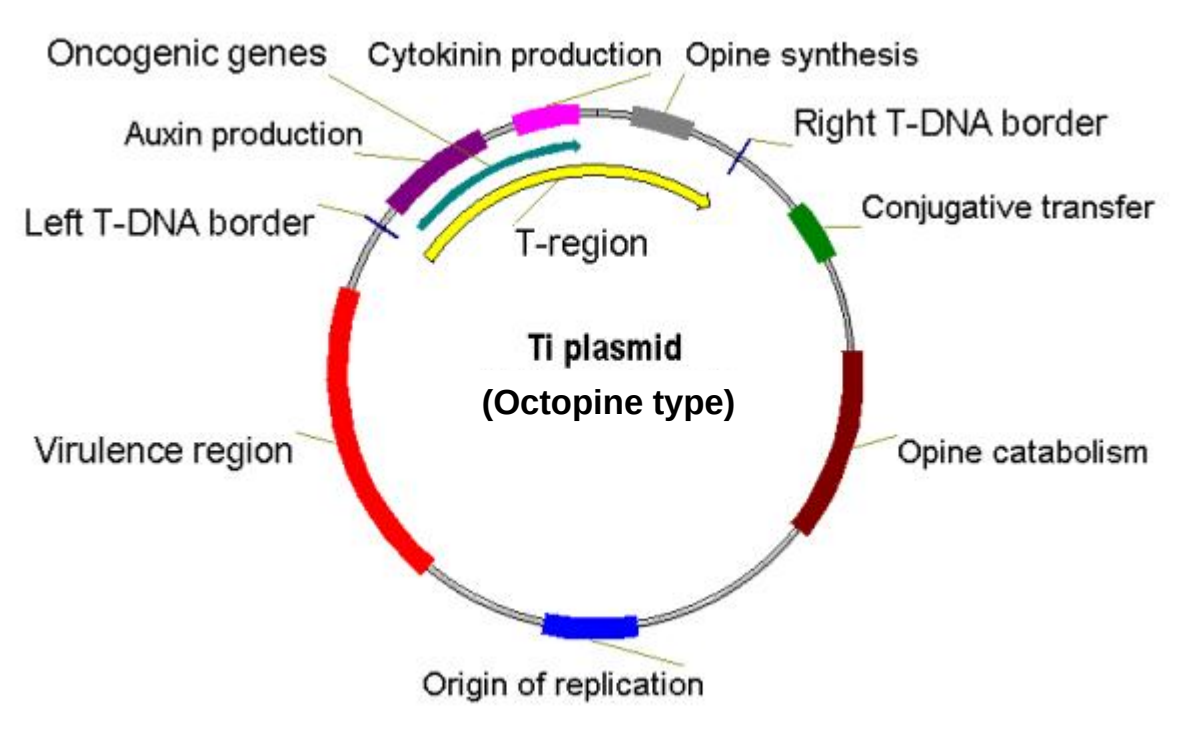
Agrobacterium tumefaciens: a natural tool for plant transformation

- Genes involved in crown gall disease are not present on the chromosome of *A. tumefaciens* but on a large **plasmid**, called the **Ti (tumor-inducing)** plasmid.



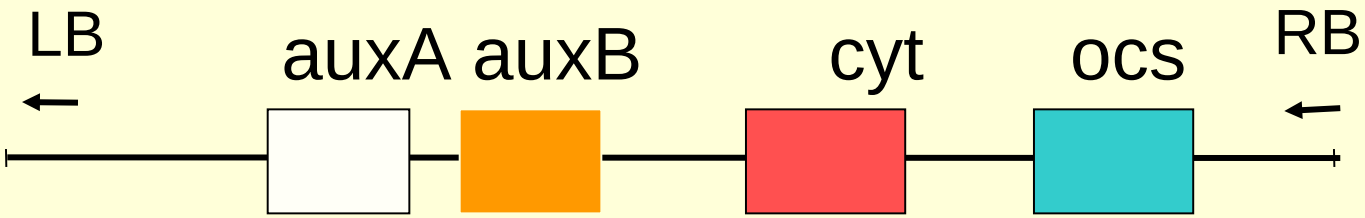
Ti Plasmid

- Large (-200-kb)
- Conjugative
- T-DNA region is transferred into plant cell and integrates semi-randomly into chromosomes
- Ti plasmid also encodes:
  1. enzymes involved in opine metabolism
  2. proteins involved in mobilizing T-DNA (*Vir* genes)



http://arabidopsis.info/students/paaras/ti_plasmid.jpg

T-DNA



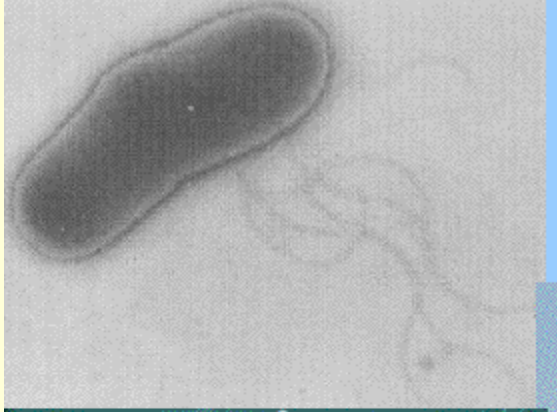
LB, RB – left and right borders (imperfect direct repeats)

auxA + *auxB* – enzymes that produce auxin

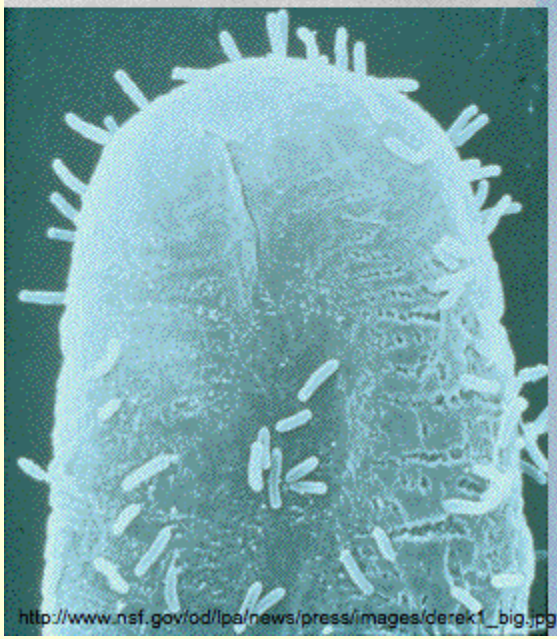
cyt – enzyme that produces cytokinin

Ocs – octopine synthase, produces octopine

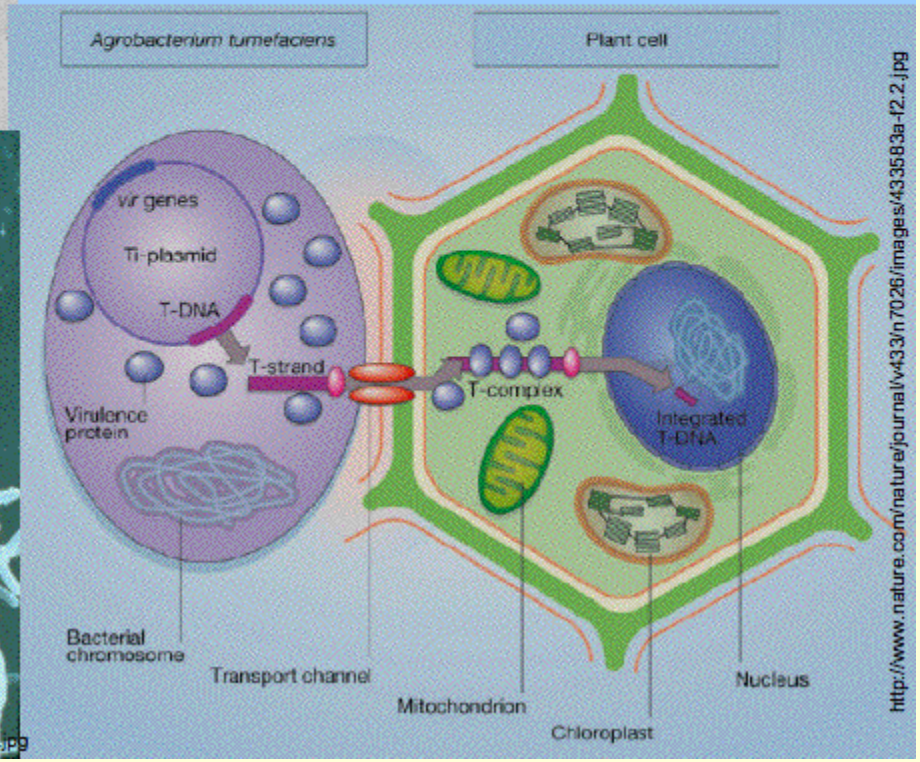
http://arabidopsis.info/students/agrobacterium/agrobact.gif



Agrobacterium tumiefaciens has a specially-recombinant portion (Ti) of a plasmid that can integrate into a plant cell's nuclear genome.



http://www.nsf.gov/od/lpa/news/press/images/derek1_big.jpg



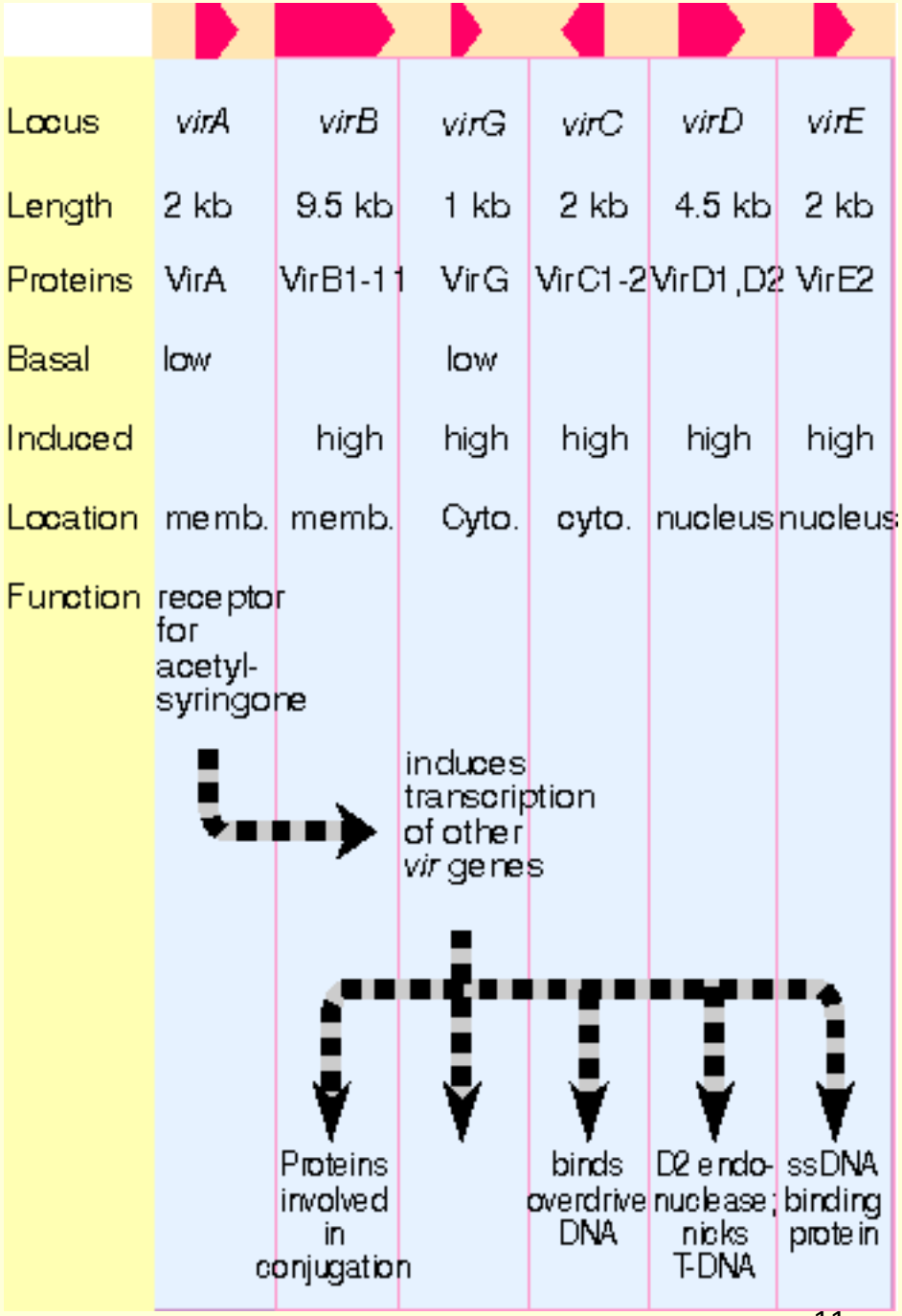
http://www.nature.com/nature/journal/v433/n7026/images/433583a-f2.2.jpg

Steps in crown gall transformation:

- a) **Wounding**
- i) In nature, *A.tumefaciens* only infects wounds.
- 1) entry into intercellular space
- 2) wound-induced elicitors are needed to trigger the transformation mechanism.

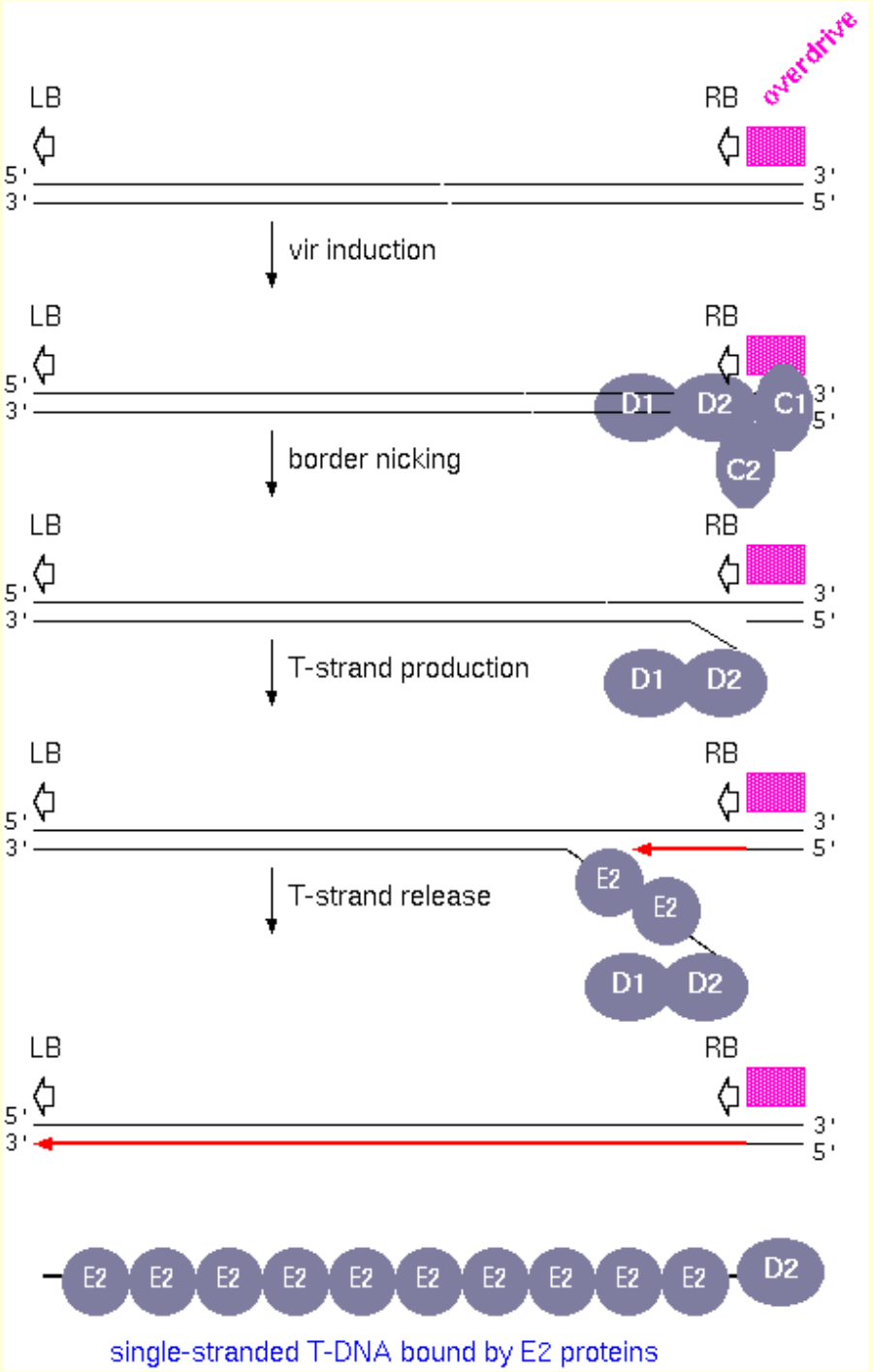
- b) **Bacterial attachment**
- c) Concurrent receipt of **wound signals** (eg. acetosyringone)
- VirA gene product is a receptor that detects plant-derived wound signals. Sends an intracellular signal that activates the VirG protein.
- d) **Induction of vir genes** - Vir G is a transcriptional activator that turns on the other genes in the Ti vir region.
- e) **Excision, transfer, and integration** of T-DNA

The Vir (virulence) region of the Ti plasmid



from http://genes.atspace.org/17.8.html

Excision of T-DNA by vir proteins



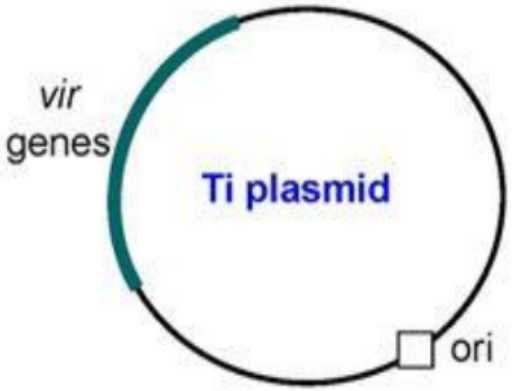
Engineering plants with *Agrobacterium:*

Ti plasmid can't be used directly for cloning:

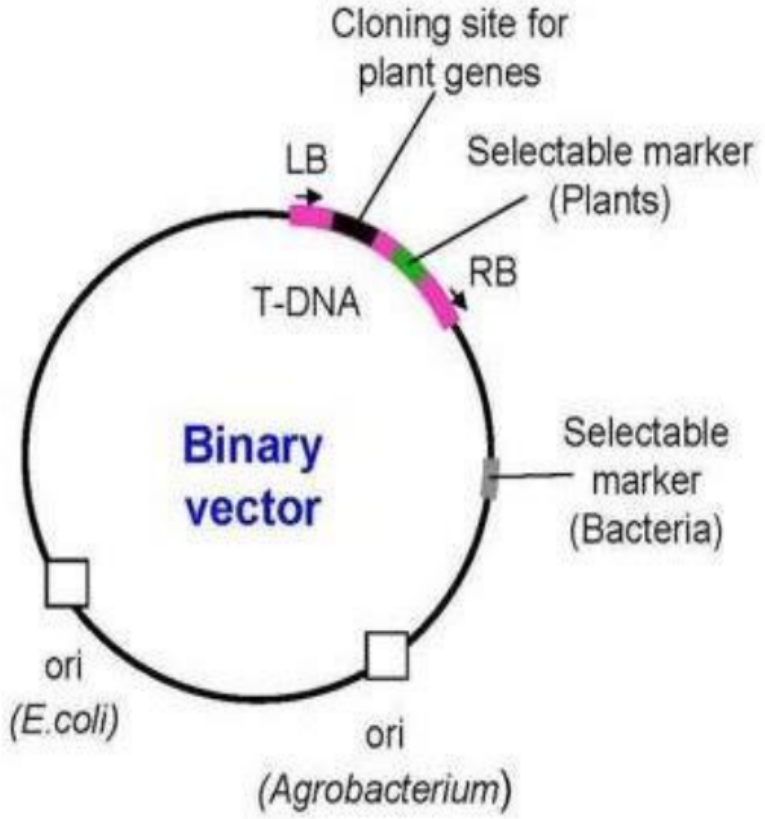
- must be disarmed ie. phytohormone production genes disabled to allow regeneration of normal plants

- Ti plasmid is too big (~200kb) for most cloning purposes (ie. hard to find unique RE sites, hard to isolate intact plasmid)

Binary vector strategy: two vector strategy



Helper plasmid - This is a “disarmed” Ti plasmid from which T-DNA and opine catabolism genes have been deleted. Carries vir genes which transfer the T-DNA into the plant.

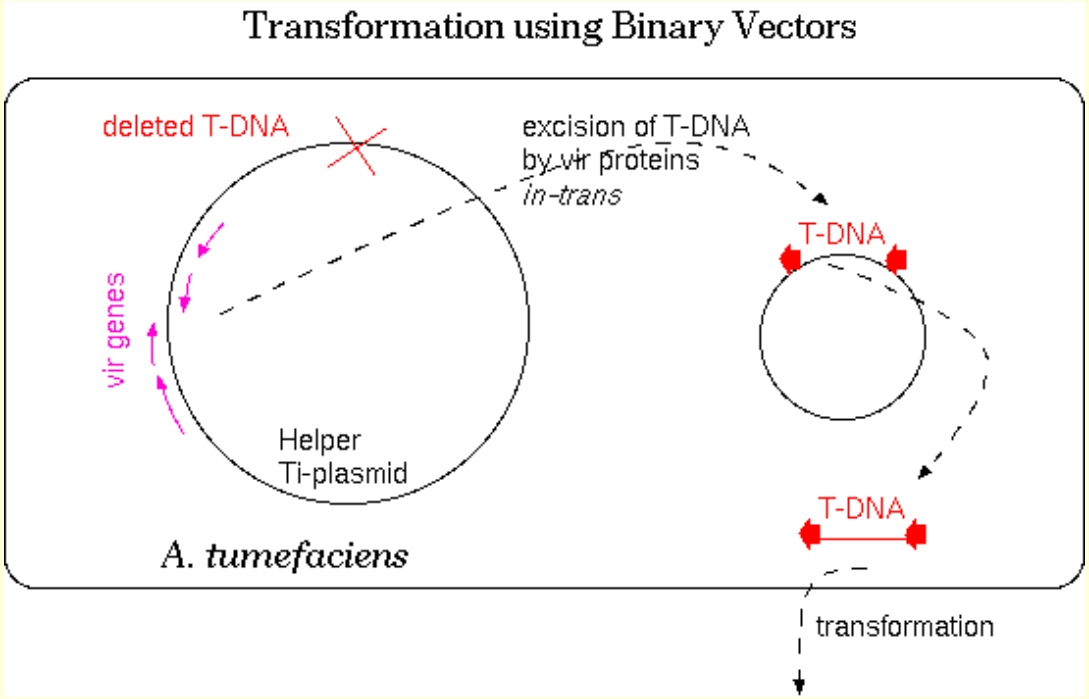


Micro-Ti plasmid - small vector. Do cloning steps in E. coli and then conjugate into Agro. strain carrying helper. T-DNA is transferred by vir genes into plant.

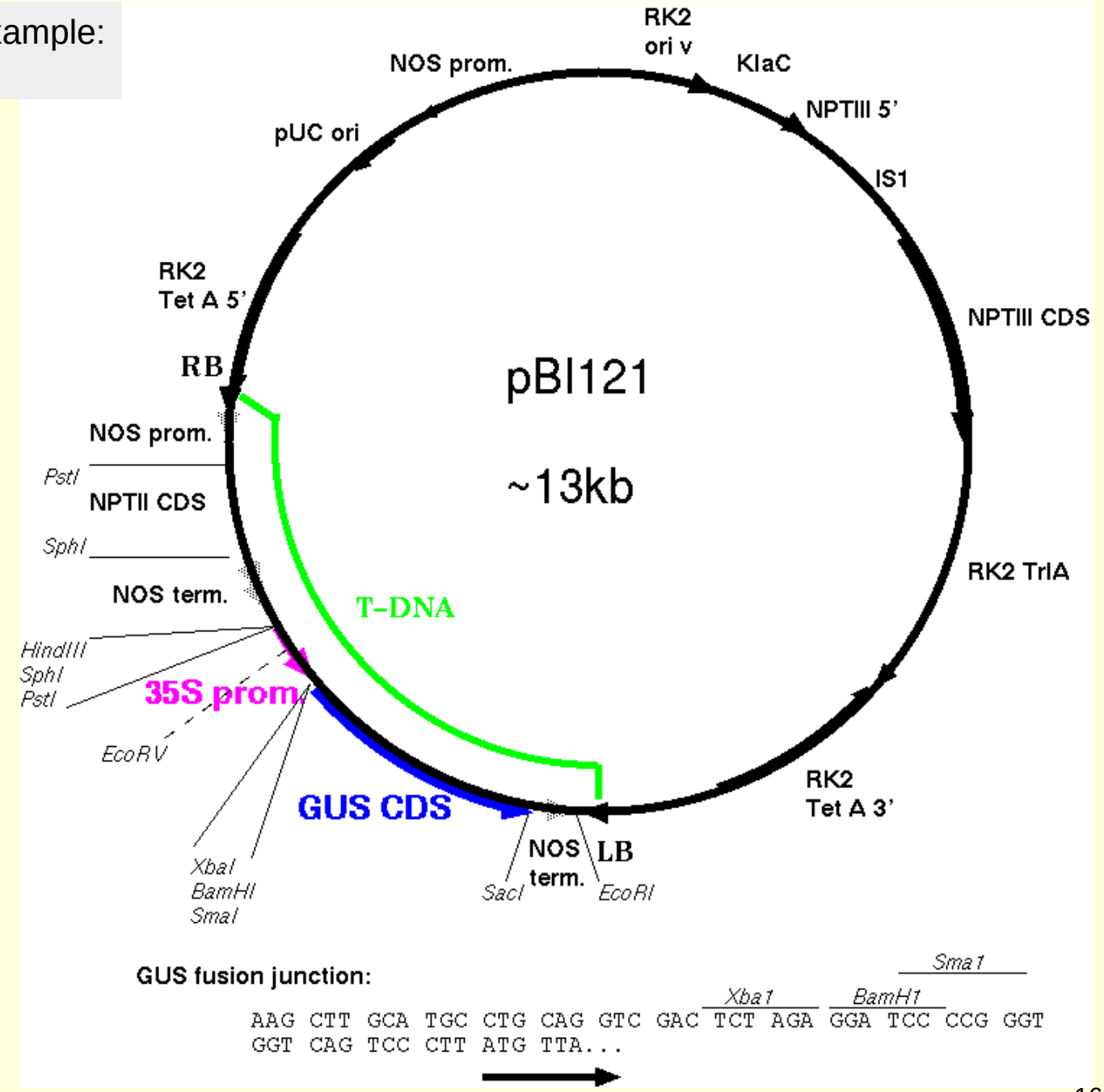
from https://miteshshrestha.wordpress.com

Binary vectors work in tandem with disarmed Ti helper plasmids

vir genes can act in trans to excise the T-DNA from the binary vector and transfer it to the plant cell. The only sequences that appear to be required for mobilization are the left and right T-DNA borders.



Micro-Ti plasmid example:
pBI121



pBI121 features

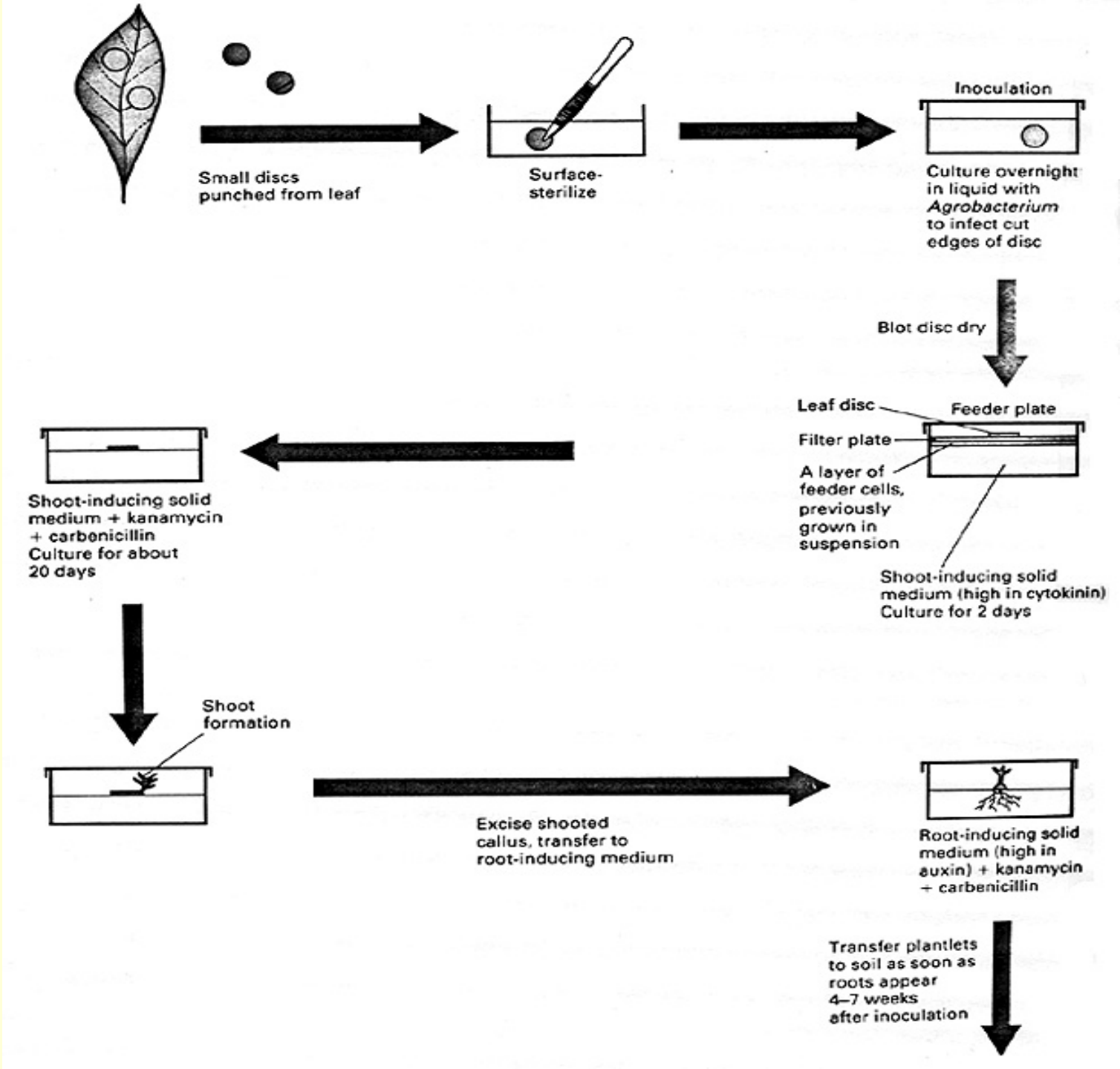
T-DNA

- **RB, LB** - nopaline T-DNA right borders
- **NOS-NPTII-NOS** - Chimeric gene for kanamycin resistance . Neomycin phosphotransferase gene under the control of nopaline synthase promoter and terminator. Can be used to assay for presence of construct in transformant plant.
- **35S/GUS CDS** - E. coli β-glucuronidase reporter gene (GUS) protein coding sequence (CDS) controlled by the constitutive 35S promoter from CaMV.
- **multiple cloning site**

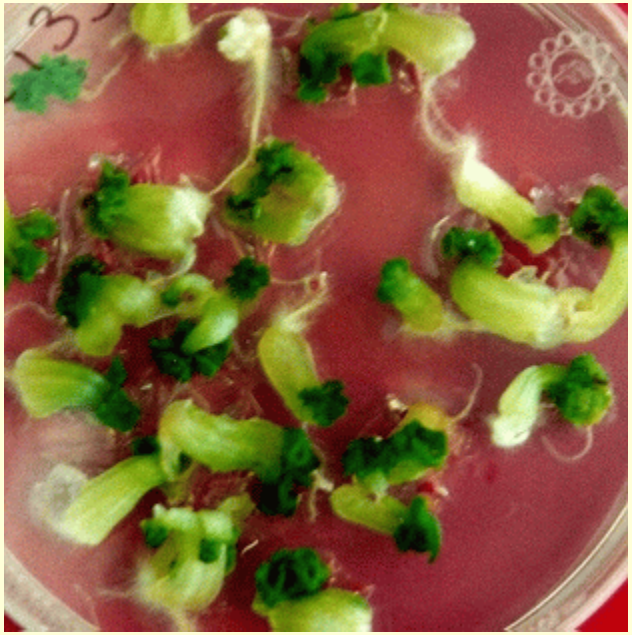
NON-T-DNA REGION

- **pUC ori** - E. coli origin of replication derived from vector pUC9
- **RK2 oriV** - origin of replication from plasmid RK2. Allows replication in *Agrobacterium*.
- **NPTII5', NPTII CDS** - neomycin phosphotransferase controlled by prokaryotic promoter for expression of kanamycin resistance in E. coli and A. tumefaciens.

Making a transgenic plant by leaf-disc transformation with *Agro.*



Brassica napus plantlets transformed with pBI1121-based construct regenerating in the presence of kanamycin, using the method of Moloney M. M., Walker M.J. and Sharma K.K. 1989. High efficiency transformation of *Brassica napus* using *Agrobacterium* vectors. Plant Cell Reports 8:238-242.[P. Zhang (1999) Ph.D. thesis, University of Manitoba]



Regenerated *B. napus* plant in soil [P. Zhang (1999) Ph.D. thesis, University of Manitoba]