E-content: Methods of Gene Transfer

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Chemically assisted transformation of protoplasts
Electroporation
Bombardment of plant material with DNA-coated microprojectiles
By exploiting the bacterium *Agrobacterium tumefaciens* and its T_i plasmid

Chemically-assisted transformation

Protoplasts take up DNA from surrounding medium
DNA gets stably integrated into the genome in a proportion of transfected cells
Polyethylene glycol (PEG) is the most widely used chemical

Problems:

Inability of the host species to regenerate from protoplasts
DNA inserted into cells in this way is not capable of independent replication
Random integration into any plant chromosome through non-homologous recombination

Bacterial Transformation

Problem:	*E. coli* cannot be transformed naturally
Solution:	Make *E. coli* competent in the lab
Problem:	Fate of the foreign DNA after entering bacteria
Solution:	Hook foreign DNA to a vector
Problem:	Locating transformants
Solution:	Use selection marker genes

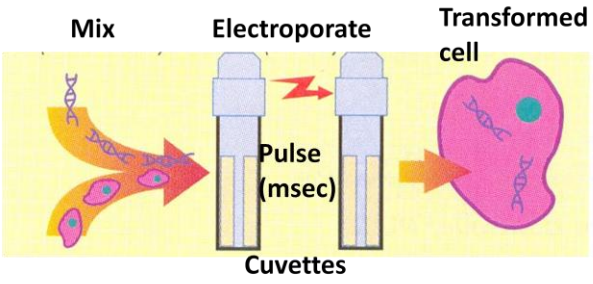
Preparation of Competent Cells

Grow bacteria in LB broth, 100 mL, 37 C x 3h (OD_{600} of approx. 0.4 = 10^8 viable cells/mL)
Transfer cells to sterile 50 mL tubes. Cool at 0 ^oC x 10 min
Centrifuge at 2700 x g, 4 ^oC, 10 min, decant supernatant
Resuspend pellet in 30 mL ice-cold $MgCl_2$ - $CaCl_2$ solution (80 mM $MgCl_2$, 20 mM $CaCl_2$)
Centrifuge at 2700 x g, 4 ^oC, 10 min, decant supernatant
Resuspend pellet in 2 mL ice-cold 0.1 M $CaCl_2$
Transform cells OR store at -70 ^oC

Transformation of Competent Cells

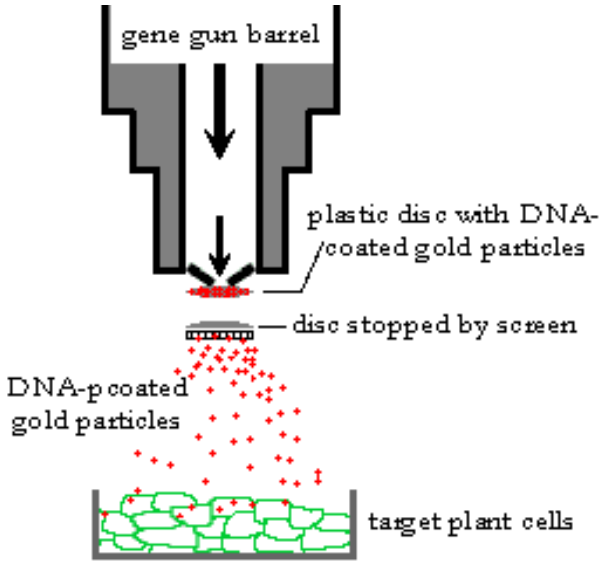
Take 200 µL of competent cells, add DNA, let stand on ice for 30 min
Transfer tubes to 42 ^{o}C in water bath, for 90 sec
Transfer immediately to ice-bath, chill for 1-2 min
Add 800 µL of LB/other medium, in water bath at 37 ^{o}C for 1 h
Plate on appropriate medium
Allow for liquid film to dry on medium
Incubate at 37 ^{o}C, colonies in approx 12-16 h

Electroporation



Passage of molecules through *electropores*
Pore formation extremely rapid – 1 µs
Electropores reseal simultaneously, resealing slower
Factors affecting electroporation: temperature, electrical field, topological form of DNA, host cell factors

Biolistics: Microprojectile Bombardment



DNA coated gold or tungsten spheres, 0.4 – 1.2 µm diam (microprojectiles)
Accelerated to 300-600 $msec^{-1}$ with a particle gun
Particle gun may use gunpowder, compressed air or compressed helium
Projectile hits stopping plate, microprojectiles released at high velocity, penetrate cells
DNA integrates randomly into plant DNA (how?)

Advantages:
Can introduce DNA into many cell types
Works in monocots also
Linear DNA more efficiently integrated than circular
Can introduce DNA into chloroplasts and mitochondria

Disadvantages:
Multiple insertions require many breeding cycles to select best insertion
Can get transient gene expression without integration

***Agrobacterium*-mediated Gene Transfer**

Agrobacterium tumefaciens – causes Crown Gall disease in a wide variety of plants

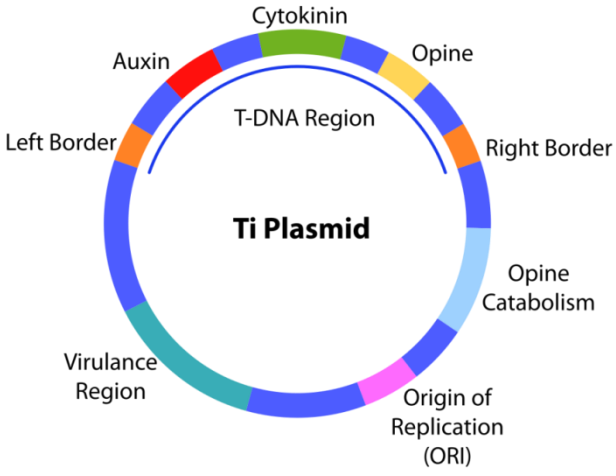
Crown gall tissue represents true oncogenic transformation, and tumor properties retained even after the destruction/death of infecting *Agrobacterium* :

Ability to form a tumor when grafted onto a healthy plant

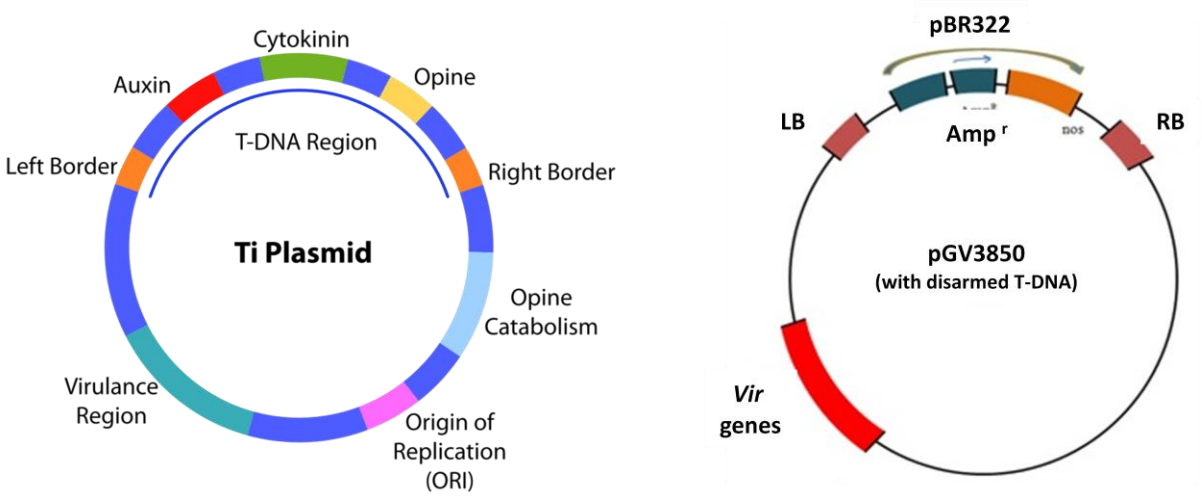
Unlimited growth as callus even in absence of phytohormones

Synthesis of opines (octopine and nopaline) that are unusual amino acid derivatives

T_i Plasmid
200 kbp
Has two major regions: VIR, 35 kbp
T-DNA, 10 kbp

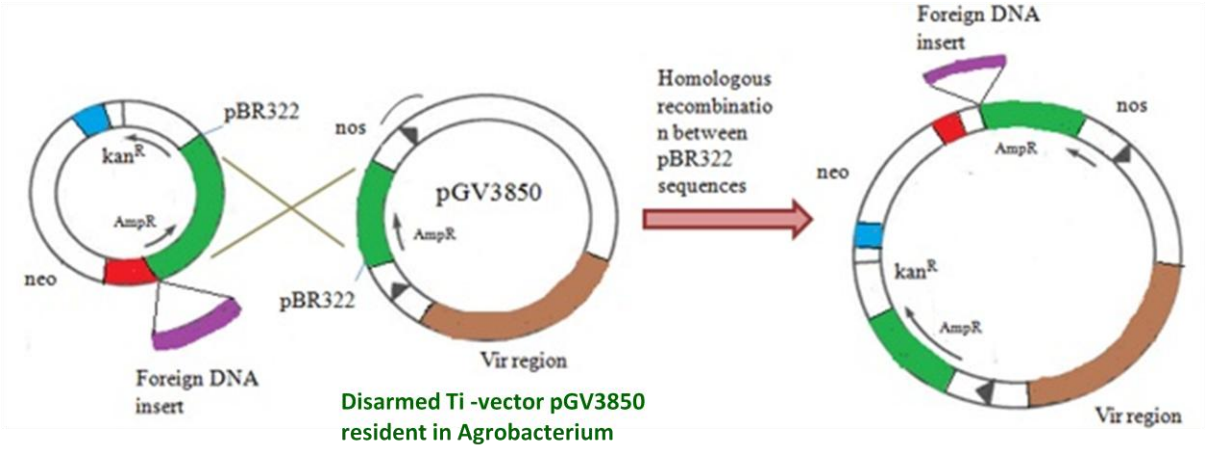


Disarmed Ti Plasmid



Co-integrate Vector

Intermediate vector transferred into *Agrobacterium* by conjugation
Unable to replicate autonomously in *Agrobacterium*



Co-integration in *A. tumefaciens* between homologous regions on modified Ti plasmid (disarmed) and a small *E. coli* cloning vector (intermediate vector) which contains a selectable marker gene that will function in plant cells and unique sites for the insertion of foreign DNA

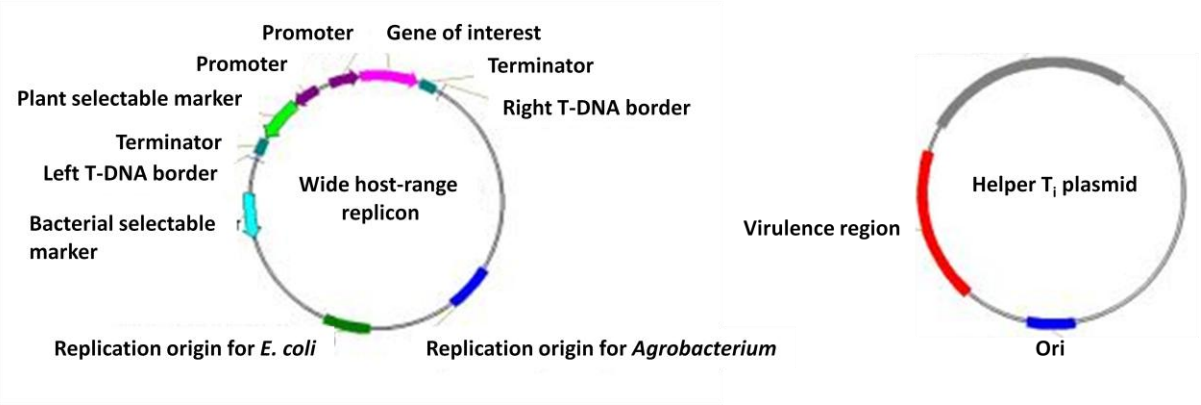
Vir genes carried on the SAME plasmid as the insert

T_i Plasmid Based Binary Vector

Based on plasmids that can replicate both in *E. coli* and *A. tumefaciens*, and which contain T-DNA borders
Border sequences flank MCS to allow insertion of foreign DNA and markers for direct selection of transformed cells
Vector system consists of two plasmids: one carrying the MCS, and the other carrying the *Vir* genes to function in trans

Binary vector (trans vector)

Vir genes and T-DNA borders with MCS on SEPARATE plasmids



Recombinant plasmid transferred to *A. tumefaciens* carrying helper *Ti* plasmid with *Vir* genes
Plant cells co-cultivated with *Agrobacterium* to allow transfer of recombinant T-DNA into the plant genome
Transformed plant cells selected, and grown in through tissue culture