

Transgenic Plants

Transgenic plants are ones, whose DNA is modified using genetic engineering techniques.

A transgenic plant contains a gene or genes that have been artificially inserted.

The inserted gene sequence is known as **Transgene** that may come from an unrelated plant or from a completely different species.

They are identified as a Genetically modified organism (GMO).

Methods for gene transfer

- DNA transfer by natural methods-
- 1. Conjugation
- 2. Bacterial transformation
- 3. Retroviral transduction
- 4. Agrobacterium mediated transfer

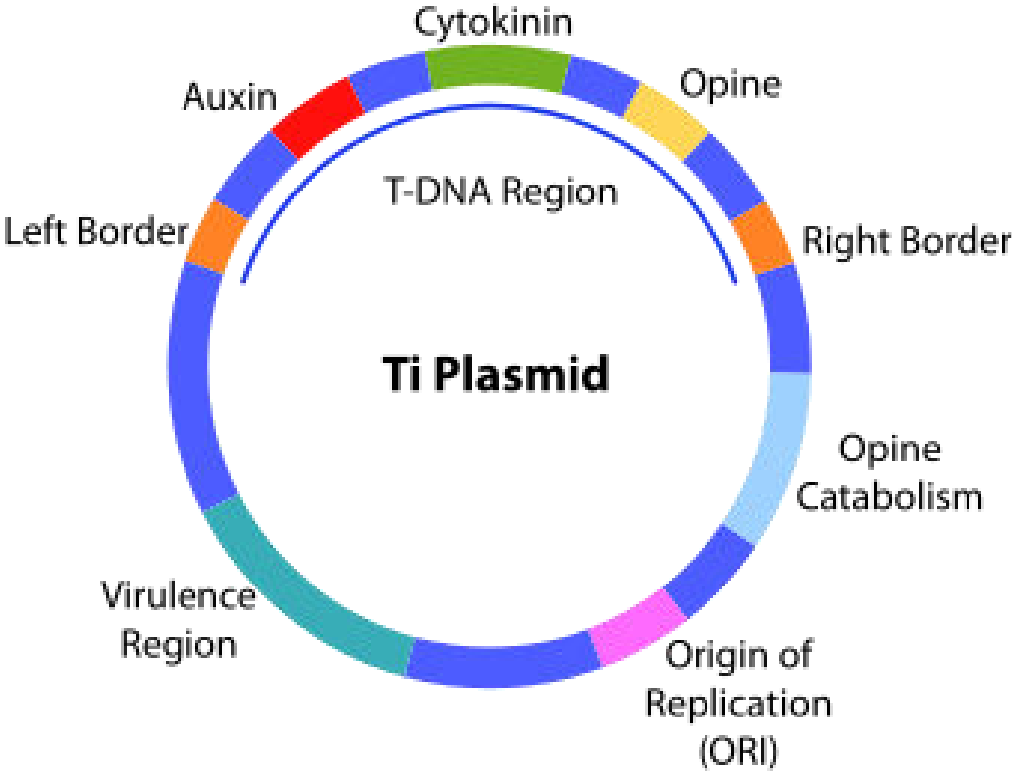
DNA transfer by artificial methods

- ***Physical methods***
 - 1. Microinjection
 - 2. Biolistic transformation
- ***Chemical methods***
 - 1. DNA transfer method by Calcium phosphate method
 - 2. Liposome mediated transfer
- ***Electrical methods***
 - 1. Electroporation

Agrobacterium mediated transfer

- *Agrobacterium tumefaciens* is a gram negative bacterium.
- It invades many dicot plants and causes Crown gall disease.
- The ability to cause crown gall disease is associated with presence of Ti plasmid within the bacterial cell.
- Ti Plasmid can be used to transfer new genes into plant cell.
- Ti plasmid can be used as a vector to transform plant cell.
- Ti plasmid has innate ability to transmit bacterial DNA into plant cell.
- By *Agrobacterium* infection, the donor genes can be transferred from recombinant Ti plasmid and integrated into the genotype of host plant.

Ti plasmid



Electroporation

- It uses electrical pulses to produce transient pores in plasma membrane thereby allowing DNA into the cell.
- These pores are known as Electropores.
- The cells are placed in solution containing DNA and subjected to electrical pulse to cause holes in the membrane.
- The foreign DNA fragments enter through holes into the cytoplasm and then to nucleus.
- ADVANTAGES of this method
- 1. Fast method and less costly.
- 2. Applied to number of cell types.

Microinjection method

- It is the process of transferring desirable DNA into the living cell , through the use of glass micropipette which is 0.5 to 5 micrometer, easily penetrate into the cell membrane and nuclear envelop.
- The desired gene is then injected into the subcellular compartment and needle is removed.
- **Limitations**
 - 1. Costly and requires skilled person.
 - 2. More useful for animal cells.

Biolistic or Microprojectile method

- It's a physical method which uses accelerated micro projectiles to deliver DNA or other molecules into tissues and cells.
- The gene gun is a device which literally fires DNA into target cells.
- The DNA to be transformed into the cell is coated with Tungston or gold particles.
- Some of the coated DNA pass through the cell wall into the cytoplasm of the target cells.

Liposome mediated gene transfer

- Introduction of DNA into cells via liposomes is known as lipofection.
- Liposomes are small, microscopic lipid molecules produced by dispersing phospholipids in aqueous phase.
- DNAs are entrapped into liposomes and they are induced to fuse with protoplast using PEG.
- Advantages
- Simplicity, long term stability, low toxicity, protection of nucleic acid from degradation.

PEG mediated transfection

- This is utilized only for protoplast.
- PEG stimulates endocytosis and therefore DNA uptake occurs.
- Protoplasts are kept in solution containing PEG.
- After transfer of DNA to the protoplast in the presence of PEG and other chemicals, PEG is allowed to get removed.