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BT 207

Plant expression vectors

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Plant expression vectors

- An **expression vector**, is usually a plasmid or virus designed for gene expression in cells. The vector is used to introduce a specific gene into a target cell, and can commandeer the cell's mechanism for protein synthesis to produce the protein encoded by the gene. Expression vectors are the basic tools in biotechnology for the production of proteins.
- The vector is engineered to contain regulatory sequences that act as enhancer and promoter regions and lead to efficient transcription of the gene carried on the expression vector.
- The goal of a well-designed expression vector is the efficient production of protein, and this may be achieved by the production of significant amount of stable messenger RNA, which can then be translated into protein.

PLANT GENE STRUCTURE

A typical plant gene has the following region beginning with the 5’end:

i). Promoter: For transcription initiation

ii) Enhancer/silencer: Concerned with regulation of gene

iii) Transcriptional start site or cap site: From here initiation of transcription take place

iv) Leader sequence: It is untranslated region

v) Initiation codon

vi) Exons

vii) Introns

viii) The stop codon

ix) A second untranslated region, and

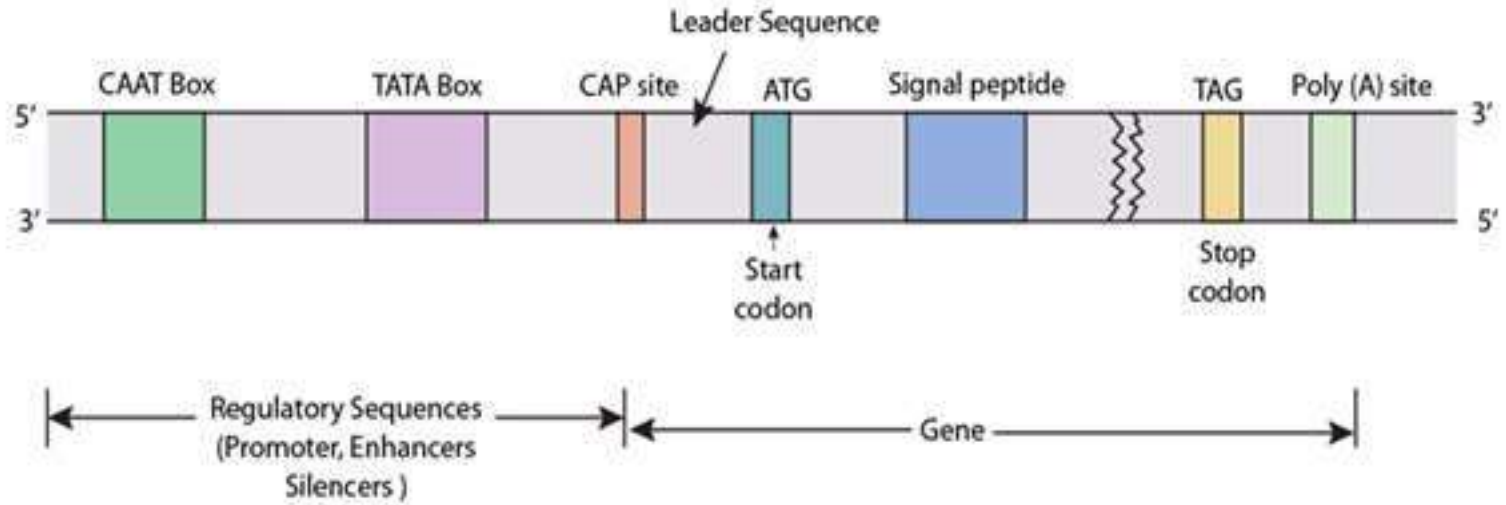
x) Poly A tail

Promoter is a region of DNA sequence which helps in the transcription of a particular gene. This contains specific DNA sequences as well as response elements which provide a secure initial binding site for RNA polymerase. These proteins called transcription factors that recruit RNA polymerase.

The CAAT and TATA boxes represent consensus sequences within promoter for RNA polymerase II.

ATG (AUG in mRNA) is initiation codon for mRNA translation, and mark the beginning of coding sequence of the gene. A sequence between the cap site and ATG is not translated and form the 5'-leader sequence of mRNA. Codon TAG/TAA/TGA are chain terminating codon and it is followed by a stretch of nontranslated region.

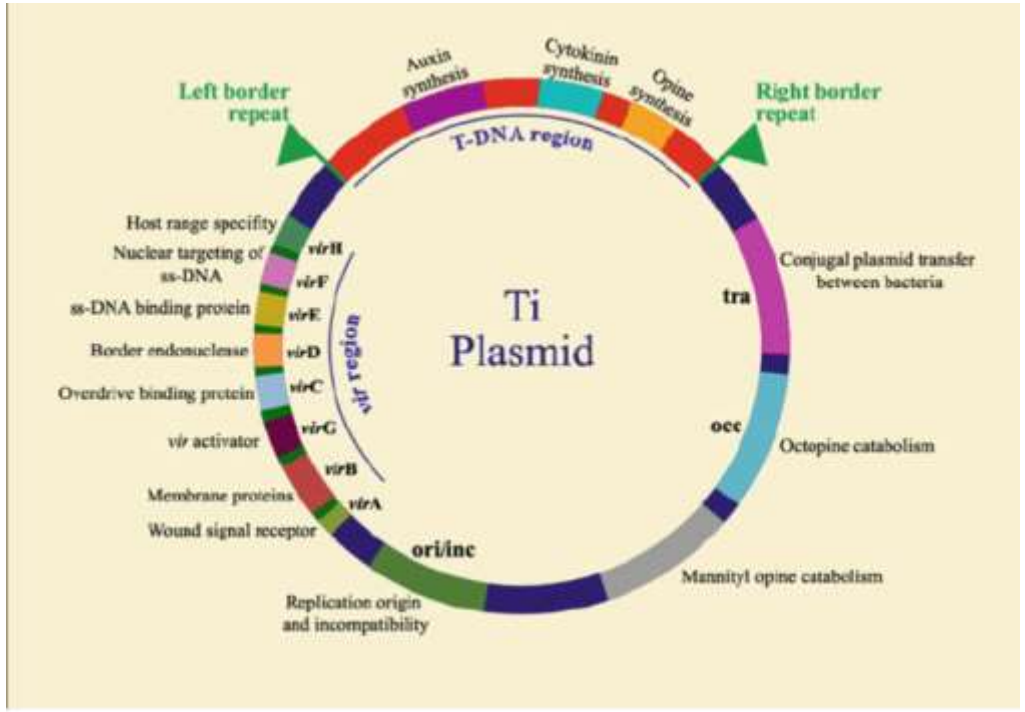
At the end, poly-adenylation site is present which denotes the end of transcription.



PLANT EXPRESSION VECTORS

Plant expression vectors are mainly based on –

Ti plasmid of *Agrobacterium tumefaciens*.

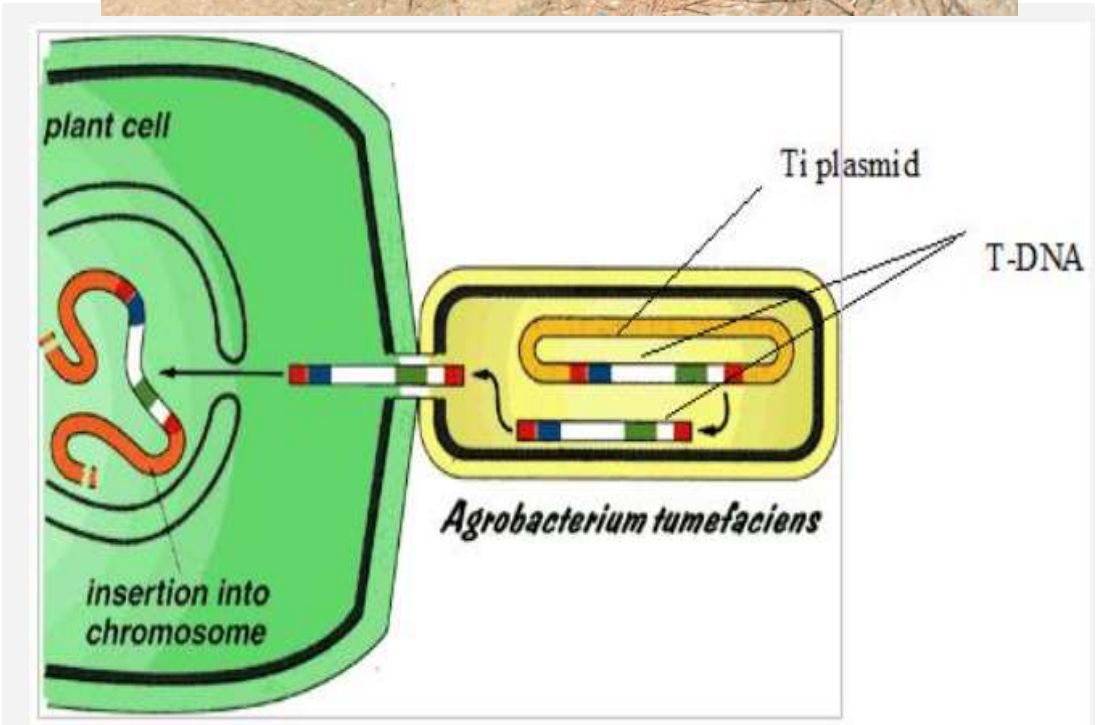


Plant viruses

1. Cauliflower mosaic virus (CMV)
2. Tobacco mosaic virus (TMV)
3. Potexviruses
4. Comoviruses

Ti plasmid of *Agrobacterium tumefaciens*.

- *Agrobacterium tumefaciens is a* common gram-negative soil borne bacteria causing induction of ‘crown gall' and ‘hairy root' diseases. These bacteria naturally insert their genes into the genome of higher plants.
- crown gall - *Agrobacterium tumefaciens*
- hairy root - *Agrobacterium rhizogenes*
- In crown gall formation the virulent strains of bacteria introduce a part of their genetic material into the infected cells where it gets integrated randomly with the genetic material of the host cell.
- *A. tumefaciens is* attracted to the wound site via chemotaxis, in response to chemicals (sugars and phenolic molecules) released from the damaged plant cells.



- The transferred DNA (named T-DNA) was originally part of a small molecule of DNA located outside the chromosome of the bacterium. This DNA molecule called Ti (tumor-inducing) plasmid

STRUCTURE OF Ti PLASMID

The Ti plasmid (size 200 kb) exist as independent replicating circular DNA molecules within the Agrobacterium cells.

The T-DNA is variable in length in the range of 12 to 24 kb.

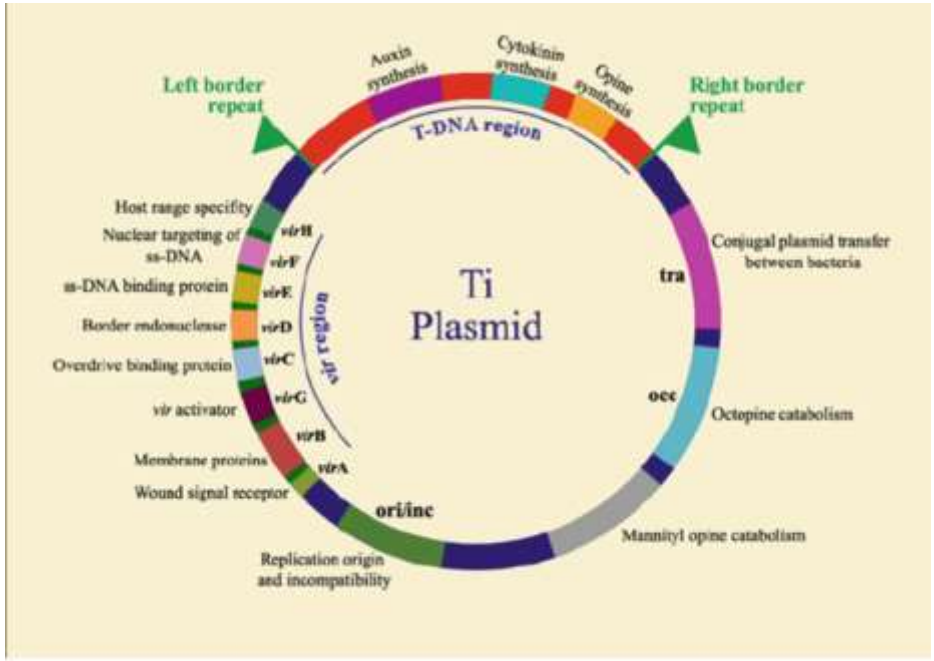
The Ti plasmid has three important region:

1) **T-DNA region**: The T-DNA region has four important genes- ***tms-1, tms-2, tmr and os .*** A modified T-DNA region of the Ti plasmid, in which the genes responsible for tumor formation are removed by genetic engineering and replaced by foreign genes of diverse origin, e.g., from plants, bacteria, virus

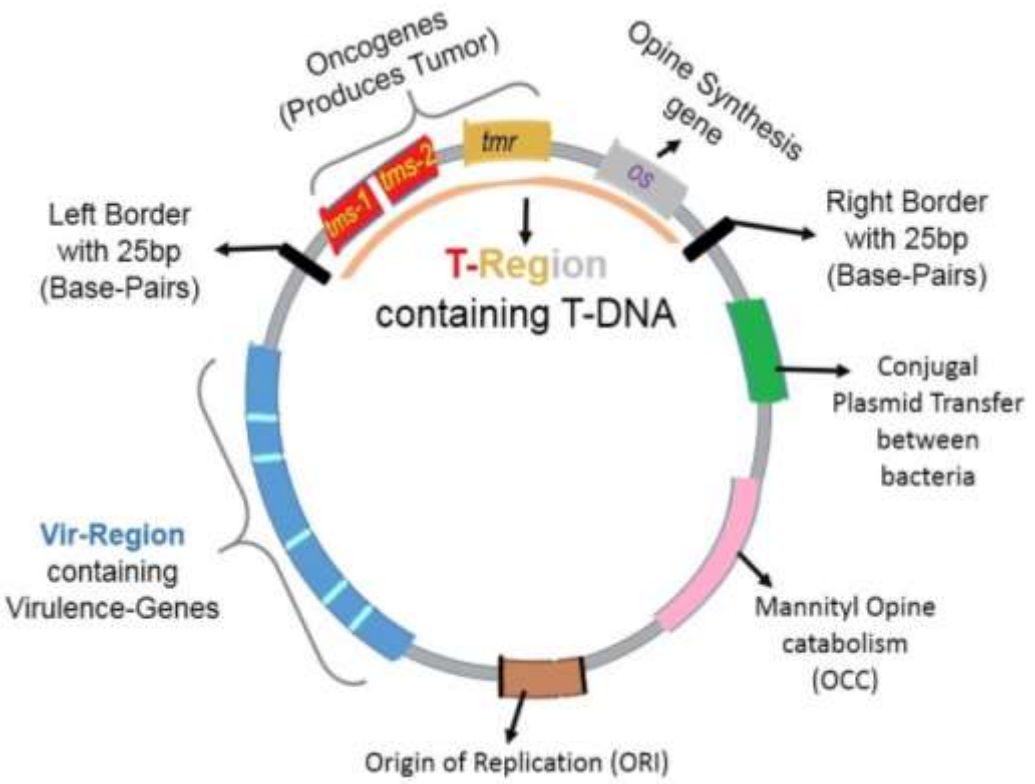
2) T-DNA borders- A set of 24 kb sequences present on either side (right & left) of TDNA are also transferred to the plant cells.

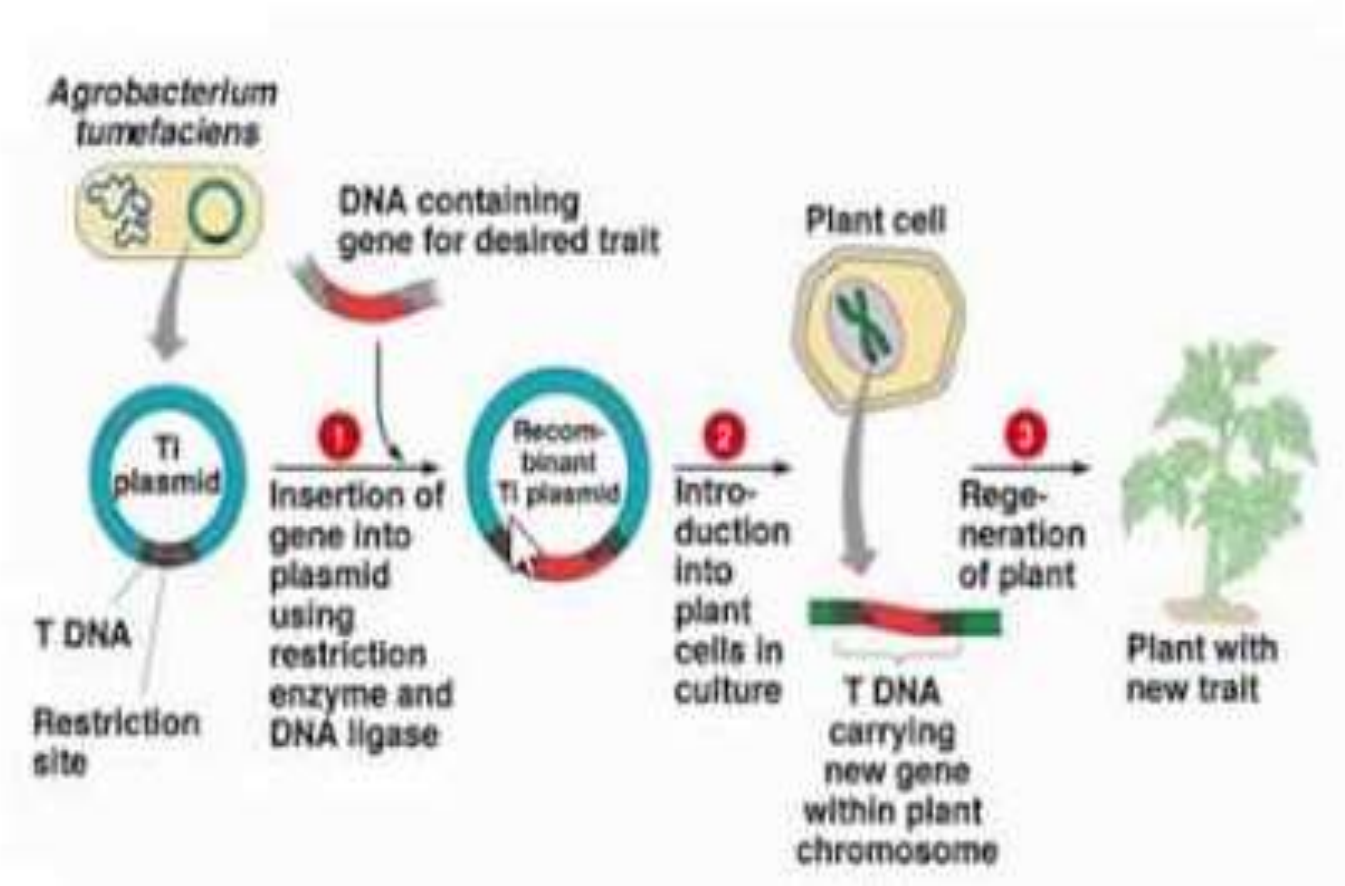
It is clearly established that the right border is more critical for T-DNA transfer.

3) Virulence region: The genes responsible for the transfer of T-DNA into host plant are located outside T-DNA and the region is referred to as *vir* or virulence region . At least nine *vir*-gene operons have been identified. These include *vir* A, *vir* G, *vir* B1, *vir* C1, *vir* D1, D2, *vir* D4 and *vir* E1,E2.



STRUCTURE OF Ti PLASMID





Some limitations of Ti-plasmid as vector:-

i. Ti-plasmid are large in size (200-800 kb) whereas smaller vectors are preferred for recombinant experiment.

ii. Absence of unique restriction enzyme sites on Ti plasmids.