

Chapter 6.4

AGROBACTERIUM RHIZOGENES pRi TL-DNA INTEGRATION SYSTEM: A GENE VECTOR FOR *LOTUS JAPONICUS* TRANSFORMATION

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Transfer of T-DNA from binary vectors to transgenic “hairy roots” rely on co-transformation of the T-DNA from the binary vector and the T-DNA from the Ri plasmid into the same plant cell. The frequency of co-transformation varies, so for some experiments an alternative method integrating the gene construct under study directly into the Ri T-DNA segment may be useful.

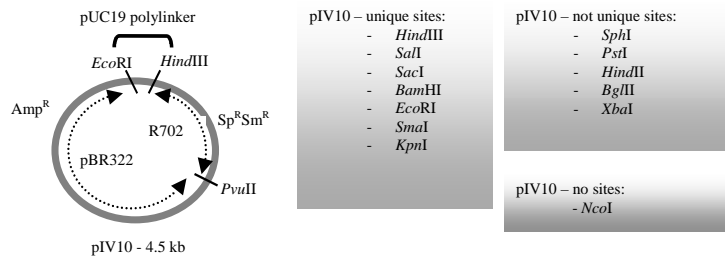
METHOD

This co-transformation methodology has been developed using the *A. rhizogenes* pRi15834 TL-DNA segment. Three *A. rhizogenes* strains, AR1193, AR12 and AR14 containing pBR322 plasmid sequences within the TL-DNA serve as acceptor strains for pBR322-derived plasmids unable to replicate in Agrobacterium. Integration into the TL-DNA acceptor region occurs by homologous recombination through the pBR322 sequences (Stougaard et al., 1987). In addition, AR12 contains a 35S::GUS reporter gene and AR14 a 35S::CAT reporter gene (Hansen et al., 1989).

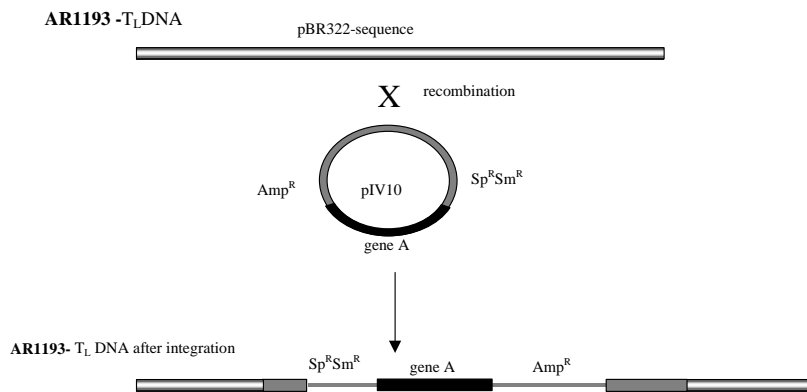
For conjugational transfer and integration of gene constructs into AR1193, AR12, and AR14, the pBR322 derived cloning vector, pIV10, can be used. The pIV10 plasmid is 4.5 kb, contains the *EcoRI* – *HindIII* polylinker cloning sites from pUC19, and for selection of integration events in Agrobacterium, the ampicillin resistance gene from pBR322 plasmid and spectinomycin/streptomycin resistance gene from R702 plasmid. Up to 15 kb of DNA has been cloned in pIV10, transferred to the acceptor strains by recombination and transformed into *Lotus*

japonicus hairy roots via the hypocotyl transformation procedure (Stougaard, J. 1995).

A)



B)



C)

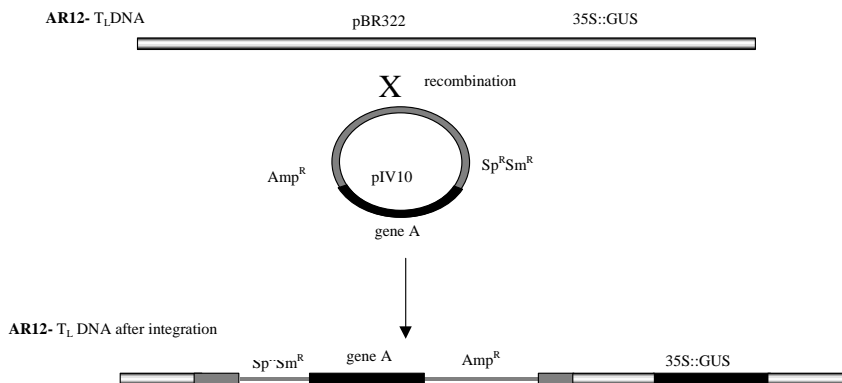


Figure 1. Gene integration system into *A. rhizogenes* TL-DNA. (A) pIV10 vector for gene cloning and integration into *A. rhizogenes* strains; (B) Structure of the TL segment before and after integration into *AR1193* strain; (C) Structure of the TL segment before and after integration into *AR12* strain.

A.J. Márquez (Editorial Director). 2005. *Lotus japonicus* Handbook. pp. 285-287.
<http://www.springer.com/life+sci/plant+sciences/book/978-1-4020-3734-4>

Selection of strains and plasmids

AR1193- Rifampicin 100 µg/ml

AR12 – Rifampicin 100 µg/ml

pIV10 – Ampicillin 100 µg/ml , Spectinomycin/Streptomycin 100 µg/ml

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