Construction and maps of binary vectors

T-DNA map for pCAMBIA vectors pC1105.1, pC1105.1r
T-DNA contains the hygromycin resistance gene (aph) for plant selection and the GUSPlus gene as a reporter to investigate gene transfer. P35S and Pe35S, promoter and enhancer+promoter from CaMV35S respectively; Tnos and T35S, terminators from A. tumefaciens nopaline synthase gene and CaMV35S respectively; cat, catalase intron; LB and RB, left and right T-DNA border sequence.

Multiple cloning site (MCS) sequences in pCAMBIA1105.1 used in A. tumefaciens, and pCAMBIA1105.1R, exclusively used in non-Agro bacterium bacterial species, respectively. Primers used to distinguish between pCAMBIA1105.1 (‘non-R’) and CAMBIA1105.1R (‘R’) are 5’-CTGGCACGACAGGTTTC-3’ and 5’-TACGGCGAGTTCTGTTAGGT-3’, encompassing the MCS region. These give PCR products of 491bp (pCAMBIA1105.1) and 572bp (pCAMBIA1105.1R).

pCAMBIA1105.1 (2638)ATTACGaattegact...N39 ...gcaagcttggCACTGG
pCAMBIA1105.1R (2638)ATTACGccaagcttg...N138...tattacaattCACTGG

- pCAMBIA1105.1 was derived from pCAMBIA1405.1 by removal of the kanamycin marker so only the spectinomycin/streptomycin marker was retained.

- pCAMBIA1105.1R was derived from pCAMBIA1105.1 by removing the Pvull-Pvull MCS fragment in pCAMBIA1105.1 and replacing it with the larger Pvull-Pvull MCS from pCRII, following re-ligation of the EcoRI sites.
Figure shows typical PCR confirming presence of Ti plasmid and binary vectors and absence of *Agrobacterium* contamination in rhizobial spp.

*S. meliloti*

*Rhizobium* sp. NGR234

PCR positive

Agrobacterium controls