

Sequences of genotyping primers and sizes of PCR products

Bacterial strains were genotyped by PCR using the following primer sets for the chromosomes and megaplasms.

Genotyping of bacterial strains

Bacterial species	Selective growth condition (antibiotic concentration in µg/ml)	Genotyping by PCR	Size of PCR product (bp)
<i>Rhizobium</i> sp. NGR234 strain ANU240	YM + Streptomycin (200)	Sme16SrRNA (chromos.) NodD1-NGR234 (pSym)	820 350
<i>Sinorhizobium meliloti</i> 1021	YM + Streptomycin (200)	Sme16SrRNA (chromos.) NodQ (pSymA) NodQ2 (pExo)	820 420 360
<i>Mesorhizobium loti</i> strain MAFF303099	YM + Gentamycin (20)	Mlo16SrRNA (chromos.) RepCa (pMLa) RepCb (pMLb)	500 200 320
<i>A. tumefaciens</i> EHA105	LB + Rifampicin (20)	Atu16SrRNA (chromos.) AttScirc (circular chromos.) VirJ (Ti plasmid) MoaA (Ti plasmid) AttS-pAT (pAT megaplasms)	410 436 948 433 436

Sequence of primers used to distinguish bacterial strains

Bacterial strain	Genome location	Targeted gene	Sequence 5'-3'
<i>Rhizobium</i> sp. NGR234	Chromosomal	16SrDNA ¹	TGTGCTAATACCGTATGAGC CAGCCGAACTGAAGGATACG
	pSym	<i>NodD1</i>	GCCAGAAATGTTTCATGTTCGCACA AATGGGTTGCGGAAGTTCGGT
<i>S. meliloti</i> 1021	Chromosomal	16SrDNA ¹	TGTGCTAATACCGTATGAGC CAGCCGAACTGAAGGATACG
	pSym	<i>NodQ</i>	GACAGGATCCTCCACGCTCA CGCCAGGTCGTTTCGGTTGG
	pExo	<i>NodQ2</i>	GACAGGATCCTCCACGCTCA GCTCATAGGGCGAGGATACA
<i>M. loti</i> MAFF303099	Chromosomal	16SrDNA	CCCATCTCTACGGAACAAC ACTCACCTCTTCCGGACTCG
	pMLa	<i>RepC</i>	GACGGCCGAGCCAAGGACGA CACATGGCAAGCCTCCTCA
	pMlb	<i>RepC</i>	GATGCTGGAAAGCTTCACAAGT CACATGGCAAGCCTCCTCA
<i>A. tumefaciens</i> EHA105	Chromosomal	16SrDNA	GAATAGCTCTGGGAAACTGGAAT CGGGGCTTCTTCTCCGACT
	Chromosomal	<i>attScirc</i>	CAGGCTCAAACCGCATTTC GTAAGTCCAGCCTCTTTCTCA
	pAT	<i>attS</i> (pAT)	GTGCTTCGGATCGACGAAAC GGAGAATGGGAGTGACCTGA
	pTi	<i>VirJ</i> ² <i>MoaA</i> ²	TGACCTTGGCCAGGGAATTG TCCTGTCATTGGCGTCAGTT CTCCAAGAGGGTCGTTGAC ATGGATCCTGCCGTGGTCTCGTGTCT GG

¹ These primers amplify the 16S rDNA genes from both *S. meliloti* and *Rhizobium* sp. NGR234

² Location on the pTiBo542 plasmid map at 8 o'clock and 4 o'clock for *VirJ* and *MoaA* respectively

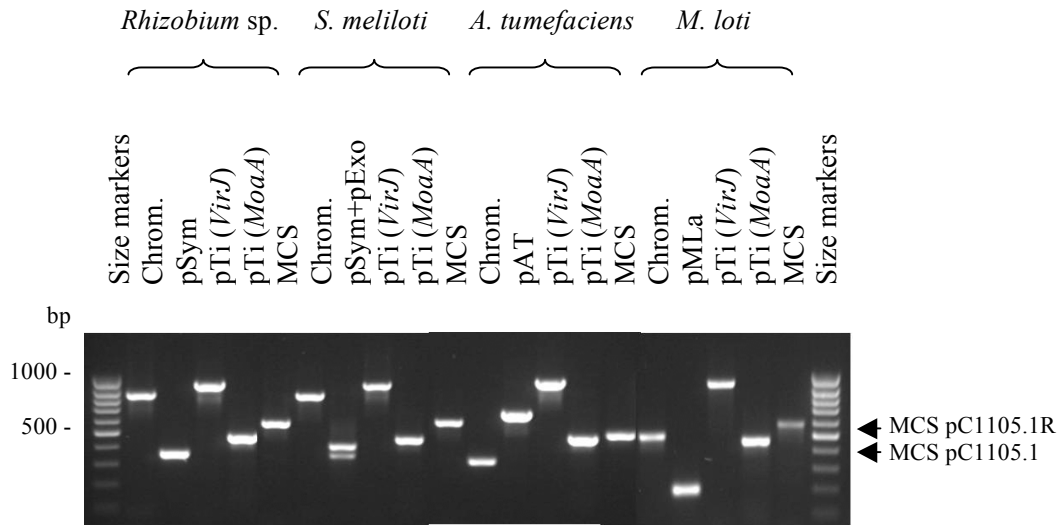


Figure shows genotyping of gene transfer competent bacterial strains containing pTiWB3 by selective PCR amplification of chromosomal and megaplasmid markers, and the differently sized MCS in pCAMBIA1105.1R (pC1105.1R) and pCAMBIA1105.1 (pC1105.1) vectors (arrows). Figure from Broothaerts *et al.*, 2005 Nature.