

Culturing information for TransBacter strains shipped as stab cultures

Enclosed in this package are stabs of bacteria grown on YM media (*Mesorhizobium*, *Rhizobium*, *Sinorhizobium*) or LB (*Agrobacterium*). These should be streaked on plates as soon as possible after receipt, or stored at 4°C. We can't warrant how long they will remain viable at 4°C.

Please be reminded that by growing and using these bacteria and plasmids, you are indicating your acceptance of the terms of the BiOS license under which they have been made available. Information on the license can be found at www.bioforge.org, where you should register your agreement. Registration also allows us to send you updates on improved protocols, strains, vectors, etc., and provides you a way to share your improvements with others as required by the terms of the license.

If you do not intend to register and comply with the terms of the license, please do not use these bacteria and plasmids.

The strains grow well in 2 days on YM plates or TY liquid media at 29°C.

Antibiotics required:

Modified Ti plasmids (pTi1 and pTi3) – kanamycin 50µg/mL

Binary vector (pCAMBIA1105.1R) - spectinomycin 100µg/mL

Binary vectors can be inserted into these strains either by triparental mating, or by electroporation using methods that are standard for preparing *Agrobacterium* competent cells.

Methods for verifying strains and plasmids, and for checking against *Agrobacterium* contamination, and a map of the binary vector pCAMBIA1105.1R can be found at www.bioforge.org in the TransBacter project.

Details of plasmid construction can be found in our February 10, 2005 paper: Broothaerts *et al* (2005) Gene transfer to plants by diverse species of bacteria. Nature 433:629-633 which is available in full text via Nature's site or in pdf format at [www.bioforge.org/forge/servlet/KbServlet/download/1-102-6/Gene Transfer to Plants.taf.pdf](http://www.bioforge.org/forge/servlet/KbServlet/download/1-102-6/Gene%20Transfer%20to%20Plants.taf.pdf).

<u>YM Media</u>	<u>1L</u>
Mannitol	10g
Yeast extract	0.4g
K ₂ HPO ₄ (10% w/v stock)	1 mL
KH ₂ PO ₄ (10% w/v stock)	4 mL
NaCl (10% w/v stock)	1 mL
MgSO ₄ .7H ₂ O (10% w/v stock)	2 mL

Adjust to pH 6.8, for solid medium add agar 15g/L, autoclave.
When ready to pour add antibiotic selection if required

Keep poured plates for 2 days at room temperature and inspect for growth of contaminating microbes, then store at 4°C.

<u>TY-medium</u>	<u>1L</u>
Tryptone	5 g
Yeast extract	3 g

After autoclaving, add 10 mL sterile CaCl₂ (700 mM) per litre. CaCl₂ can be autoclaved separately.