

LSOP Title	Preparation of Thermo-Competent Agrobacterium
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## 1.0 Scope

*This procedure covers preparing thermo-competent agrobacterium based on the Hofgen and Willmitzer 1988 paper*

## 2.0 Definitions

LB – Luria Bertani Broth

Log Phase – logarithmic phase (for cell growth)

E. coli – Escherichia coli

Tris-HCL – trisaminomethane hydrochloride

EDTA - Ethylenediaminetetraacetic acid



## 3.0 Materials and Equipment

1. Shaking incubator
2. Centrifuge
3. Microcentrifuge tubes
4. Pipette and Pipette Tips

5. LB

6. 1X TE (10mM Tris-HCl, 1 mM EDTA pH8, autoclaved)



#### 4.0 Prescribed Actions

1. Inoculate 200 ml of LB with 1 ml of an overnight culture of the chosen strain of *Agrobacterium*. Incubate at 28°C with vigorous agitation. Start the culture in the late afternoon to be harvested the following morning.

*NB: if you are not sure of the strain you use to start the culture. Add the appropriate antibiotics (rifampicin and gentamicin for GV3101) – see appendix for list of *Agrobacterium* strains*

2. Grow the cells to log phase (OD<sub>550</sub> 0.5-0.8).
3. Pellet the cells in a benchtop centrifuge at 5000 rpm for 10 minutes at room temperature. *Agrobacterium* takes longer to pellet than *E. coli*.
4. Wash the pellet with sterile 1X TE (10mM Tris-HCl, 1 mM EDTA pH8, autoclaved).
5. Resuspend the cells in 0.1X the original volume of LB, and aliquot 250- or 500-µl fractions in microcentrifuge tubes.
6. Snap-freeze in liquid nitrogen and store at -80°C.



## 5.0 Appendix

List of Agrobacterium strains:

### Agrobacterium list

Strain	*Resistance	Antib. conc.	Solid / Liquid Medium	Reference Link
EHA105 (At564)	rif	15mg/L	LB / YEP	<a href="http://www.springerlink.com/content/t02h1486p1862715/">http://www.springerlink.com/content/t02h1486p1862715/</a>
GV3101	rif/gent	(r)10mg/(g)30mg/L	LB / LB	<a href="http://www.springerlink.com/content/lh0136u761823371/?MUD=MP">http://www.springerlink.com/content/lh0136u761823371/?MUD=MP</a>
C58C1	tetra	10mg/L	LB / LB	<a href="http://www.ncbi.nlm.nih.gov/pmc/articles/PMC211426/">http://www.ncbi.nlm.nih.gov/pmc/articles/PMC211426/</a>
LBA4404	rif	15mg/L	LB / YEP	<a href="http://www.nature.com/nature/journal/v303/n5913/abs/303179a0.html">http://www.nature.com/nature/journal/v303/n5913/abs/303179a0.html</a>
C58 (At11)	rif	15mg/L	LB / LB	<a href="http://www.springerlink.com/content/7p3506368u228p21/">http://www.springerlink.com/content/7p3506368u228p21/</a>
A208 (At10)	rif	15mg/L	LB / LB	<a href="http://web2.sbg.org.br/gmb/edicoesanteriores/v17n1/pdf/a13v17n1.pdf">http://web2.sbg.org.br/gmb/edicoesanteriores/v17n1/pdf/a13v17n1.pdf</a>
A281 (At77)	rif	15mg/L	LB / LB	<a href="http://www.ncbi.nlm.nih.gov/pubmed/3782037">http://www.ncbi.nlm.nih.gov/pubmed/3782037</a>
AGL1	carb	50mg/L	LB / YENB	<a href="http://www.ncbi.nlm.nih.gov/pubmed/1368724">http://www.ncbi.nlm.nih.gov/pubmed/1368724</a>

**\*Resistance:**

rif=Rifampicin

gent=gentamicin

tetra=tetracycline

carb=carbenicillin