Agrobacterium-mediated transient expression in tobacco leaves

- using standard molecular biology techniques clone a GFP construct of choice into a binary vector and mobilize into a suitable *Agrobacterium tumefaciens* strain
- culture Agrobacterium to stationary phase (overnight culture) in MGL medium at 25 °C
- pellet 1 ml of the culture at 5000 rpm (2200 g) for 5 minutes in a microcentrifuge at room temperature
- resuspend the pellet in 1 ml of the infiltration buffer and centrifuge as above
- repeat once
- dilute the bacterial suspension with infiltration buffer to adjust the inoculum to a concentration that gives an absorbance OD_{600} of approximately 0.1 (depends on case). Usually, the overnight culture is diluted 5 fold for OD measurements, but this depends on how well the culture grew.
- The infiltration is easier when the leaf stomata are opened. It helps to put your plants under a lamp for a couple of hours before infiltration and wet them (Figure 1). Using a yellow tip, create small holes in the leaves. Press the nozzle of a 1 ml syringe (no needle) against the lower (abaxial) epidermis of a tobacco leaf, covering the small hole with the nozzle and holding a gloved finger to the other side of the leaf and inject slowly (Figure 2). The infiltrated area turns dark. Mark its limits with a permanent pen (Figure 3).
- Incubate plants under normal growing conditions for 2-3 days
- Excise a marked area and examine under the confocal microscope (Figure 4), or extract proteins and analyse as appropriate.

MGL medium	
Yeast extract	2.5 g/L
Tryptone	5.0 g/L
NaCl	5.0 g/L
Mannitol	5.0 g/L
Monosodium Glutamate	1.16 g/L
KH2PO4	0.25 g/L
$MgSO_4 \cdot 7H_2O$	0.1 g/L
Biotin	1.0 µg/L
pH 7.0	

Infiltration Buffer
50 mM MES (pH 5.6)
2 mM Na_3PO_4
0.5% glucose
100 µM Acetosyringone

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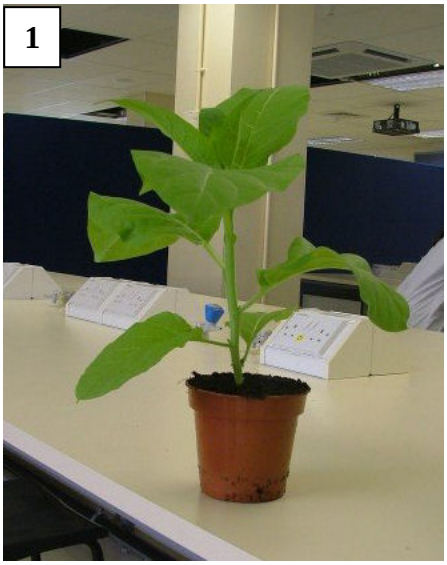

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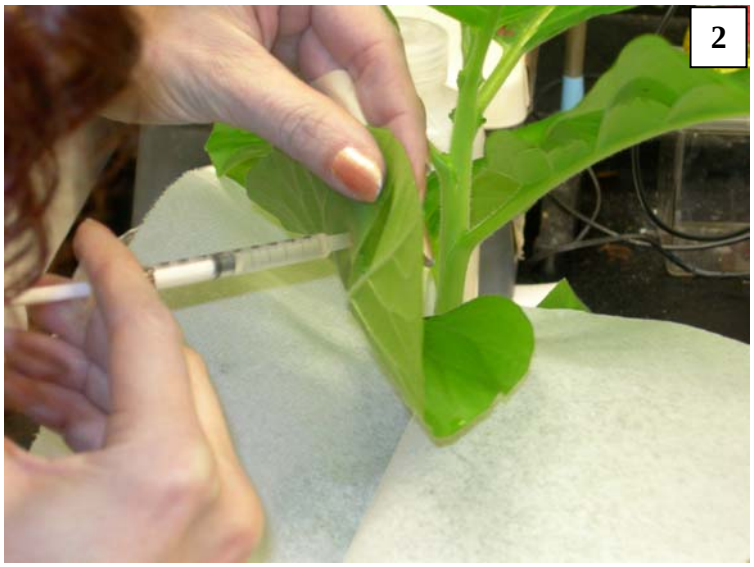

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