TRANSIENT INFILTRATION OF NICOTIANA LEAVES

Infiltration medium

	Stock	100 mL	200 mL	400 mL
10 mM MgCl2	1 M	1 mL	2 mL	4 mL
10 mM MES pH5.7	0.2 M	5 mL	10 mL	20 mL
200 uM acetosyringone	100 uL	200uL	400 uL	800 uL

100 mM acetosyringone (0.1962 g dissolved in 5 mL DMSO, final to 10 mL)

1. Grow 10 mL GV2260 or GV3101 agrobacterium cultures harbouring the plasmid of interest in appropriate selection medium for 36 h to OD600 1.0-1.2. You may also wish to start a culture harbouring a plasmid expressing a P19 suppressor of silencing.
2. Transfer cultures to 15 mL Falcon tubes. Spin down culture at 3000 rpm for 20-30 min in the table top centrifuge. Discard supernatant.
3. Re-suspend pellet in 10 mL infiltration medium. Incubate at room temperature with low rotation speed for at least 3 h (or overnight). If you are hurry, you can use it immediately).
4. After incubation, spin down at 3000 rpm for 15-20 min. Discard supernatants. Re-suspend in 5-10 mL of fresh infiltration medium.
5. Read the OD 600 and adjust to 0.8 for target vector (and 0.2 for P19 if using).
6. If using multiple agro strains, mix at a 1: 1 ratio to make a ready-to-go solution (the final concentration for each target vector is 0.4 and for P19 is 0.1).
7. Infiltrate leaves with the agro mixture. Usually 3-4 large leaves per constructs are used, starting from the top of the plants. Use 2-3 week old healthy plants, grown in medium-sized pots. For infiltration, puncture the leaf and slowly push the solution using 1ml syringe (without the needle!) from the bottom side of the leaf while closing the hole with finger from the top side of the leaf.
8. Normally, expression will reach its maximum at ~ 3-5 days.
9. If DNA/protein/RNA is required, harvest the tissues (it is possible to cut them with borer), place in tubes and freeze in liquid nitrogen immediately and then store them at 80^0C.