

Floral dipping: Agrobacterium-mediated transformation (germ-line stable transformation)

**** Developmental stage of the plants: Inflorescences have developed and floral buds are evident with very few open flowers. The more unopened floral buds the better.**

The following is for transforming Arabidopsis with one construct (at least two independent Agro colonies used per construct):

1. Obtain an autoclaved 125ml bottle of 100ml of LB and add the following antibiotics: typically kanamycin, gentamycin, and rifampicin to concentrations of 50:40:100 µg/ml. Store in fridge. Also autoclave 2x25mls LB in 125 ml flask, and 2x500mL in 2L flask)
2. Obtain 3 sterile 15ml glass test tubes and add 5ml of the above LB + AB's. (one of these tubes will be a control and should be put aside).
3. Obtain the plated out Agrobacteria you have transformed and select two single colonies and inoculate in separate 5ml of LB + AB's tubes prepared in step 2.
4. Place both the control and experimental tubes in an incubated shaker at 28°C with moderate rotation overnight. Make sure nothing grows in un-inoculated control
5. Obtain the cultures grown over night in step 4 and use 5ml of each to inoculate separate 125ml flasks with 25ml of LB + AB's prepared above.
6. Place the 125ml flask into an incubated shaker at 28°C at 250 rpm for 1 night (until well saturated). Room temp in growth room downstairs generally sufficient.
7. Add 12.5 ml of each above culture to 500ml LB kan/gent/rif 50:40:100µg/ml to grow overnight or until cells are grown to stationary phase ($OD_{600} \approx 2.0$). (This may take 2+ days, but not usually).
8. Spin down each culture in 2x250ml GSA bottles at 7000rpm for 10 minutes.
9. Discard the supernatant and resuspend the pellets in 500ml of 5% sucrose and 0.05% Silwet L-77 (combine contents in 2 GSA bottles from step 8).

□ In 500ml of water add 25g of sugar and 250µL of Silwet L-77.
10. Put Agro suspension in shallow vessel for dipping.

- 11 Dip plants (e.g. Col-WT) directly into media for 2-3 seconds. Usually dip 2-4 round pots for each colony; can use 500ml suspension repeatedly.
- 12 Place plants on side to drain excess liquid.
- 13 Place a clear plastic dome over plants (or clear garbage bag) to retain humidity for 12-24hrs. Plants should be kept in low light or dark until the following day and out of direct sunlight thereafter.
- 14 For more transformants, repeat dipping procedure on same set of plants 2-3 more times (often not necessary if using Col-WT plants; Ler-WT has lower efficiency as do certain mutants). Make sure pot of plants are dipped with the same colony! Don't mix things up.
- 15 Kill Agrobacteria with bleach before discarding down sink. You don't want the whole world to be transgenic!