

## DIPPING PROTOCOL FOR *ARABIDOPSIS* TRANSFORMATION

1. Grow *Arabidopsis* (no more than 2-3 per pot).
2. Prune inflorescence shoot on appearance of flowers.
3. Wait until there are a good number of flowers but few siliques formed (normally about 2 weeks from clipping).
4. Grow up 5 ml culture of *Agrobacterium* containing your construct for transformation shaking overnight at 28°C in LB with 10 µl 50 mgml<sup>-1</sup> rifampicin and 10 µl 50 mgml<sup>-1</sup> kanamycin.
5. Add to 500 ml LB/Rif/Kan prewarmed and shake overnight at 28°C.
6. Spin down at 5K for 20'.
7. Whilst spinning down *Agrobacterium*, prepare dipping solution
  - 25 g sucrose
  - 250 µl Tween 20
  - make up to 500ml with H<sub>2</sub>O
8. Pour off supernate leaving pellet.
9. Add dipping solution to *Agrobacterium* pellet and mix gently with pipette tip until pellet completely dispersed.
10. Pour half of mix into dipping tray (usually bottom half of empty tip box) and dip the plants for 3-5 seconds. Vary how much of the plant you dip - from inflorescence only to all above-ground tissues. Use second half of dipping solution for second half of plants to be dipped.

11. Lay dipped plants on tray on top of paper towel and cover with saranwrap overnight.
12. Remove film and place plants back in pot holders (vertical again).
13. Repeat steps **4-12** one week later on the same plants.
14. Harvest seed when mature siliques ready.
15. Select for transformants on MS Kan plates.