

## Simplified *Agrobacterium*-mediated Transformation of *Arabidopsis thaliana*

Li-Qing Chen

**[Abstract]** Acquiring transgenic lines is a fundamental strategy for plant gene/protein function studies. How to successfully obtain transgenic lines is an important consideration, particularly with regards to the selection of transformation methods. The *Arabidopsis* floral dip transformation method is routinely used in hundreds of laboratories because of easy operational steps and high rate of success. In the present protocol, a simplified *Arabidopsis* transformation protocol is described, which has been commonly used in our lab and developed/modified from several previous protocols.

### Materials and Reagents

1. *Arabidopsis* plants
2. *Agrobacterium* strain(s): ABI (refs. 16, 17), GV3101 (ref. 16), EHA105 (ref. 18), LBA 4404 (ref. 19) or others
3. Murshige&Skoog salts (MS) (Phytotechnology labs.™)
4. Tryptone
5. Yeast extract
6. NaCl
7. Carbenicillin
8. Hydrochloric acid
9. 5% (wt/vol) sucrose, prepared fresh (Sigma-Aldrich)
10. Silwet L-77 (Lehle Seeds)
11. Bleach (Thermo Fisher Scientific)
12. Appropriate antibiotics (Sigma-Aldrich/ Thermo Fisher Scientific)
13. 1/2 MS medium (see Recipes)
14. Liquid LB medium (see Recipes)

### Equipment

1. Growth chambers or light room or greenhouse, adjustable to long-day condition of 16 h light/8 h dark
2. Plant pots: e.g., 3.5 in x 3.5 in
3. Laminar-flow hood
4. Petri dishes (100 x 15 cm VWR)

5. Whatman filter paper
6. Desiccator jar

### **Procedure**

1. Grow healthy *Arabidopsis* plants until they are flowering. Grow under long day conditions in individual pots in soil (*Optional*: Soil can be covered with bridal veil, window screen or cheesecloth).
2. When constructs are not ready, the transformation experiment can be delayed by clipping the first bolts to proliferate secondary bolts. Plants will be ready roughly 4-7 days after clipping. Clipping is another option to get more immature flower clusters.
3. (*Optional*): If you want to increase the transformation efficiency, supply relatively more nutrients to new buds and try to remove the siliques on plants before transformation.
4. Prepare *Agrobacterium tumefaciens* strain harboring gene of interest on a binary vector. Inoculate one big colony into 5-10 ml liquid LB medium with the appropriate antibiotics for vector selection till saturation at 28 °C, or grow in other media. Normally it takes 1.5-2 days.
5. Subculture cells to 300 ml liquid LB with the appropriate antibiotics until cells grow to stationary phase ( $OD_{600}$ :1.5-2.0) at 28 °C.
6. Collect *Agrobacterium* cells by centrifugation at 4,000  $\times g$  for 15 min at room temperature (RT), and resuspend cells to  $OD_{600}$  =0.6-0.8 in 5% sucrose solution (made fresh, no need to autoclave). 100-200 ml for each two or three small pots to be dipped or 400-500 ml for each two or three 3.5" (9 cm) pots.
7. Sliwet L-77 needs to be added to a concentration of 0.02-0.05% (vol/vol) and mixed well before dipping. If there are problems with L-77 toxicity, use 0.02% or as low as 0.005%.
8. Pour the transformation solution with cells into an appropriately sized beaker. Next, invert plants and dip above-ground parts of plant in *Agrobacterium* solution for 2 to 3 sec with gentle agitation.
9. Use fingers or sticks to prevent loss of soil if the soil is not covered by bridal veil, window screen or cheesecloth. You can use a pipette to drop some solution on to some axillary floral buds that are too short to submerge into solution.
10. A film of liquid coating the plants should then be visible. After two spots are done, the solution needs to be stirred using one stick to avoid cells falling down.
11. Place dipped plants in the tray under a dome or covered by a big plastic trash bag for 16-24 h to maintain high humidity. Lay down the treated plants on their side. Do not expose to excessive sunlight (air under dome can get hot).

12. Remove the cover next day. Send the treated plants back to the greenhouse or the growth chamber. Water and grow plants normally, tying up loose bolts with wax paper, tape, stakes, twist-ties, or other means. Stop watering as seeds become mature.
13. Harvest dry seed.
14. For screening of primary transformants. Pour 1/2 MS plates containing carbenicillin to prevent bacterial contamination and the appropriate antibiotic or herbicide for positive selection of transformants.
15. Sterilize seeds by the vapor-phase sterilization method or other methods. Place a 250 ml beaker containing 50 ml bleach into the desiccator jar, carefully add 2.5 ml concentrated hydrochloric acid to the bleach prior to sealing the jar. Keep this overnight.
16. Place seeds on the medium evenly by gently shaking hands holding an autoclaved Whatman filter paper with seeds on.
17. Vernalize seeds at 4 °C for 3 days, then move to a normal growth chamber till transformants can be readily distinguished as seedlings with healthy green cotyledons and true leaves and roots that grow into the selective medium.
18. Transplant putative transformants to soil.  
For higher rates of transformation, plants may be dipped two or three times at seven day intervals. Do not dip less than 6 days apart. For many different background materials transformed with the same construct, spraying transformation method is more efficient.

### Recipes

1. 1/2 MS medium (pH 5.7-5.8)
  - 2.15 g Murshige&Skoog salts
  - 10 g sucrose
  - 0.8% agar per liter
  - Autoclaved
2. Liquid LB medium
  - 10 g tryptone
  - 5 g yeast extract
  - 10 g NaCl per liter

### References

1. [Additional commentary can be found by searching the Arabidopsis newsgroup archives.](#)

2. Bechtold, N., Ellis, J., and Pelletier, G. (1993). In planta *Agrobacterium*-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. C. R. Acad. Sci. Paris, Life Sciences 316:1194-1199.
3. Clough, S. J. and Bent, A. F. (1998). [Floral dip: a simplified method for \*Agrobacterium\*-mediated transformation of \*Arabidopsis thaliana\*](#). *Plant J* 16(6): 735-743.
4. Clough, S. J. and Bent, A. F. [Simplified Arabidopsis Transformation Protocol](#). (Brief version for those who are familiar with the method).
5. Zhang, X., Henriques, R., Lin, S. S., Niu, Q. W. and Chua, N. H. (2006). [Agrobacterium-mediated transformation of \*Arabidopsis thaliana\* using the floral dip method](#). *Nat Protoc* 1(2): 641-646.