Simplified Arabidopsis Transformation Protocol
(Brief version for those who are familiar with the method)

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Our present protocol (Clough and Bent, 1998; modified from Bechtold et al. 1993) is extremely simple. We have found that the MS salts, hormone, etc. make no difference, that OD of bacteria doesn't make much of a difference, that vacuum doesn't even make much of a difference as long as you have a decent amount of surfactant present. Plant health is still a major factor - healthy fecund plants make a big difference! With this method you should be able to achieve transformation rates above 1% (one transformant for every 100 seed harvested from Agrobacterium-treated plants).

1. Grow healthy Arabidopsis plants until they are flowering. Grow under long days in pots in soil covered with bridal veil, window screen or cheesecloth.
2. (optional) Clip first bolts to encourage proliferation of many secondary bolts. Plants will be ready roughly 4-6 days after clipping. Clipping can be repeated to delay plants. Optimal plants have many immature flower clusters and not many fertilized siliques, although a range of plant stages can be successfully transformed.
3. Prepare *Agrobacterium tumefaciens* strain carrying gene of interest on a binary vector. Grow a large liquid culture @ 28°C in LB with antibiotics to select for the binary plasmid, or grow in other media. You can use mid-log cells or a recently stationary culture.
4. Spin down Agrobacterium, resuspend to OD$_{600}$ = 0.8 (can be higher or lower) in 5% Sucrose solution (if made fresh, no need to autoclave). You will need 100-200 ml for each two or three small pots to be dipped, or 400-500 ml for each two or three 3.5” (9cm) pots.
5. Before dipping, add Silwet L-77 to a concentration of 0.05% (500 ul/L) and mix well. If there are problems with L-77 toxicity, use 0.02% or as low as 0.005%.
6. Dip above-ground parts of plant in Agrobacterium solution for 2 to 3 seconds, with gentle agitation. You should then see a film of liquid coating plant. Some investigators dip inflorescence only, while others also dip rosette to hit the shorter axillary inflorescences.
7. Place dipped plants under a dome or cover for 16 to 24 hours to maintain high humidity (plants can be laid on their side if necessary). Do not expose to excessive sunlight (air under dome can get hot).
8. 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.
9. Harvest dry seed. Transformants are usually all independent, but are guaranteed to be independent if they come off of separate plants.
10. Select for transformants using antibiotic or herbicide selectable marker. For
example, vapor-phase sterilize and plate 40 mg = 2000 seed (resuspended in 4 ml 0.1% agarose) on 0.5X MS/0.8% tissue culture Agar plates with 50 ug/ml Kanamycin, cold treat for 2 days, and grow under continuous light (50-100 microEinsteins) for 7-10 days.
11. Transplant putative transformants to soil. Grow, test, and use!

For higher rates of transformation, plants may be dipped two or three times at seven day intervals. We suggest one dip two days after clipping, and a second dip one week later. Do not dip less than 6 days apart.

References:


Additional commentary can be found by searching the Arabidopsis newsgroup archives: http://genome-www.stanford.edu/cgi-bin/biosci_arabidopsis

NOTE: Obtain proper approval for transformation work from institutional authorities. Autoclave and properly dispose of all materials.

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