

Arabidopsis Transformation by floral dip

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1. Grow very healthy plants. Use plants with bolt height of 2-10 cm if possible. If plants are not very healthy transformation will probably not work.
2. Grow 50 mL LB culture of Agrobacterium with selection at ≤ 28 degrees C, 200-300 rpm shaking overnight.
3. Spin cell down in 50 mL tubes for 10 min., 3000xg.
4. Resuspend Agrobacterium in 50 mL of 5% sucrose, 0.05% Silwet L-77 (2.5g sucrose, 25 μ L Silwet L-77 volume to 50mL with water).
5. Pour Agro solution into a shallow box (can be a lid from pipet tips). Bend Arabidopsis inflorescence into the solution so that the flowers are covered with the suspension.
6. Cover plants with a dome to maintain high humidity. Add water to the bottom of the flat so that there is a small amount of residual water in the bottom of the flat. Place the plants in normal growth conditions for (≤ 24 degrees C, 80 uEin) for 1 day.
7. After 24 hrs., slightly open the dome for 1 day. Then completely remove the dome.
8. Harvest the seeds several weeks later once the flowers that were dipped have siliques that are beginning to yellow. It is best to harvest seeds from individual plants separately so that you can be certain that you have different transgenic events.
9. Dry the seeds for one week with good air flow (I dry plants in a paper envelop in our seed storage facility which has fans that circulate the cold air).
10. Clean the seeds by passing through a mesh several times and by rolling on paper.
11. For BASTA selection (kanamycin and hygromycin selection should be performed on sterile MS plates): Plant the seeds by sprinkling them on wet soil at high density. Cold treat the entire flat for 1-3 days. Then grow in normal conditions. If things work well you can expect about 1% of your seeds to be transgenic.
12. Once plants have true leaves that are about equal in size to the cotyledons, spray the plants with the selective herbicide. Repeat herbicide spray 7-14 days later if needed to discern transgenic plants.

See video here:

<https://rahulpatharkar.000webhostapp.com/2018/11/transforming-arabidopsis-by-agrobacterium-floral-dip>