

Arabidopsis Transformation

(Floral dip method)

Supplies & Reagents:

Sucrose:

Silwet L-77:

Germination medium (GM):

½ Murashige and Skoog salt mixture

1% Sucrose

1X Gamborg's B-5 vitamins

Agar 0.8%

Autoclave for 20mins.

Protocol:

1. Grow Arabidopsis plants in a growth chamber at 12/12 light/dark condition.
2. (Optional) Clip first bots to encourage proliferation of secondary bots. After 5 days, plant can be used for floral dip.
3. Culture Agrobacterium overnight in 5ml LB medium, containing antibiotic (Kan 50 mg/ml and Gentomycin 50 mg/ml for C58 harboring pBI121-based constructs). Inoculum in 200-400ml LB with antibiotics.
4. Agrobacterium is spin down at 2500Xg, RT for 15min, and suspended in 5% Sucrose solution (pH5.7), and add Silwet L-77 to 0.03%.
5. Dip the floral into the infiltration solution for 20sec-2min. Lay the plant on its side, cover with Saran wrap, and put in dark for 24h.
6. Grow plant in long day condition (2-3 weeks), and harvest seeds.
7. Sterilize the seeds in 10-20% clorox for 10 mins, wash with 4x sterile water.
8. Place seeds on GM medium containing antibiotics (Kan 50mg/ml and Timentin 200mg/ml), and incubate at 4°C for two days.
9. Move to a growth chamber. After 7 days, the transformant leaves are dark green.