

Arabidopsis transformation (Christine)

Solutions required:

YN (1L)	3 g Beef Extract 5 g Bacto Peptone 8 g NaCl 10 g Yeast Extract Adjust pH to 7.3 with NaOH	IM (Infiltration media) (1L) 4.4 g of MS plus vitamins powder 50 g sucrose Adjust pH to 5.8 with KOH Just before use add 10 µl of BAP 10 µg/L (final conc)
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Things to be aware of:

- Contained as much as possible the transformation area
- Gloves, plant material etc go in bug bags (incinerated)
- *Agrobacterium* culture, glassware are treated with hypochlorite

Growing plants for transformation

- Vernalize seeds as usual and spread out on a small block of rock wool in a grid like manner

Day 1

- Set up 10 ml culture in 50 ml flasks. Use YN supplemented with the appropriate antibiotic.
For pART27 add 10 µl Spectinomycin (stock 100 mg/ml), 10 µl Gentamycin (stock 50 mg/ml), 10 µl Rifampicin (stock 50 mg/ml).
- Grow overnight at 28 °C with shaking.
- prepare 1L YN in a sterile 4L flask and autoclave
- prepare 500mL IM per 1L culture you want to grow and autoclave

Day 2

- In the afternoon (~2-3 pm) inoculate 1L of YN (+ antibiotics) with 2.5 ml of the overnight culture. Keep the remaining culture at 4°C.
- Optional: Perform DNA extraction to check presence of the right plasmid
- put the IM in the fridge

Day 3

- Check O.D when you arrive in the lab. Continue to grow the culture until the O.D reaches 0.8 at 600 nm.
- Centrifuge the culture at 4500 rpm, 4 °C, 20 min, switch on the pump in the greenhouse, it needs to run 30 min prior use. Follow strictly the instructions of “how to use the pump” that are on a sheet close to the pump.
- Resuspend the pellet (from 1L culture) in 500 mL of cold IM (may require the use of a 5 mL tip)
Remember to add 5 µl of BAP (stock solution is 1µg/µL) in the IM medium

In the greenhouse:

- Cut already existing siliques off the plants that will be transformed
- Add 100 µl of Silwet-L77/500 mL to 500mL of *Agrobacterium* culture in IM and mix.
- Transfer *Agrobacterium* solution into two (or more...) small beakers and place in the vacuum chamber.
- Take the rock wool blocks and place upside down onto the beakers. Check that most of the inflorescences are in the *Agrobacterium* solution.
- Apply vacuum for 10min
- Remove the plants and lie the blocks onto paper towels to let the *Agrobacterium* solution drip off
- Return the plants to the cabinet and keep them there until they start dropping seeds

If using kwick pots (Karine David's Protocol):

Carry out the procedures as above with some changes below:

Growing of Plants for Transformation

- For WT plants: cut 9 kwick-pots (3x3) and fill with soil to make a dome. Cover with mesh and fix the mesh with sellotape
- For *gi* mutants: Prepare 9 kwick-pots and transplant 1 *gi* plant per pot. There is no need to maintain the soil with mesh

Day-2

- In the afternoon (~3-4 pm) inoculate 1L of YN (+ antibiotics) with 1ml of the overnight culture. Keep the remaining culture at 4°C. Perform DNA extraction to check presence of the right plasmid
- put the IM in the fridge

Day-3

- Check O.D when you arrive in the lab. Continue to grow the culture until the O.D reaches 0.8 at 600 nm.
- Centrifuge the culture at 4500 rpm, 4 °C, 20 min, switch on the pump in the greenhouse, it needs to run 30 min prior use. Follow strictly the instructions of "how to use the pump" that are on a sheet close to the pump.
- Resuspend the pellet (from 1L culture) in 500 mL of cold IM (may require the use of a 5 mL tip)
Remember to add 10 µl of BAP in the IM medium

In the greenhouse:

- Cut already existing siliques off the plants that will be transformed
- Add 100 µl of Silwet-L77/500 mL to 500mL of *Agrobacterium* culture in IM and mix.
- Transfer *Agrobacterium* solution into two (or more...) small beakers and place in the vacuum chamber.
- Take the kwick-pot and place upside down onto the beakers. Check that most of the inflorescences are in the *Agrobacterium* solution.
- Apply vacuum for 15-20min
- Remove the plants and cover the plant with a plastic bag
- After 1 day cut few holes to increase ventilation
- Remove progressively the bag over the next few days to remove humidity