Transformation Protocol for *Arabidopsis* – Abbreviated

Germinate seed in pots

↓ 4 weeks

Streak bacteria onto YM/MinA

↓ 2-3 days 28°C

Spray/dip bacterial suspension onto plants

↓ 1 day in box

Let plants set seed

↓ 3-4 weeks

Plate seed to GM + selection

↓

Transfer transformed plants to the glasshouse

Transformation Protocol for *Arabidopsis*

Method modified from Clough and Bent (1988)

Plant material:

- Thinly sow *Arabidopsis thaliana* cv C24 seed into soil. Grow at 20°C under 16h light until plants are beginning to flower. Cut off any seed pods that have formed prior to transformation.

Prepare bacteria:

- Streak **YM plus antibiotic** plates with bacteria, or **Minimal A** plus antibiotic plates for *Agrobacterium.*
- Incubate plates for 2-3 days at 28°C.

Transformation:

- Measure about 20mL **Infiltration Medium** for each bacterial strain.
- Scrape or wash bacteria from plate with sterile loop and suspend in 20mL of Infiltration media.
- Adjust density to an OD_{600} 0.9-1.0.
- Dip flowers and unopened buds of *Arabidopsis* into the bacterial suspension, or alternatively the plants can be sprayed with bacterial suspension using a plant mister.
- Cover plants to maintain humidity overnight. Grow at 20°C in light.
- Repeat dipping/spraying after 7 days.
- Collect seed, store at 4°C

Selection:

- Weigh seed to estimate number (100 seed ≈ 21.8mg)
- Sterilise for 2 mins in 70% (v/v) ethanol followed by 20 mins in 20% (v/v) bleach (Zixo brand) + 0.02% Triton X-100. Keep the seeds moving in the bleach solution by putting tubes on a rotator wheel.
- Wash seeds 5 times with sterile distilled water (in laminar flow hood)
- Plate to **Germination Medium** + selection plates, seal with Micropore tape.
- Incubate overnight at 4°C then in light at 20°C. After 10 days select transformants, transplant to soil.

Media and solutions for *Arabidopsis* transformation

YM Media

	1L
Mannitol	10g
Yeast extract	0.4g
K_2HPO_4 (10% w/v stock)	1 ml
KH_2PO_4 (10% w/v stock)	4 ml
NaCl (10% w/v stock)	1 ml
$MgSO_4.7H_2O$ (10% w/v stock)	2 ml

- pH 6.8
- Agar 15g/L
- Autoclave
- When ready to pour add:
    - Antibiotic selection if required

Keep poured plates for 2 days at room temperature to visualise any contamination, then store at 4°C.

Minimal A Medium

(Svab, et al, 1975)

Liquid	1L
Stock solution A	490mL
Stock solution C	11mL
Milli-Q H_2O	499mL

Solid	1L
Stock solution A	490mL
Stock solution B	499mL
Stock solution C	11mL

- Store 4°C

Minimal A Stock Solution A

	g/490mL	Final concentration 1 x mM
K_2HPO_4	10.5	60
KH_2PO_4	4.5	33
$(NH_4)_2SO_4$	1.0	7.6
tri-Sodium citrate.$2H_20$	0.5	1.7

- Autoclave in 490mL aliquots
- Store 4°C
- Substituting chemicals
    - $K_2HPO_4.3H_2O$ 13.8g/490mL

Minimal A Stock Solution B

Dissolve 15g agar in Milli-Q H_2O, make up to 500mL

- Autoclave
- Store room temperature

Minimal A Stock Solution C

Add 1mL 1M $MgSO_4$ to 10mL 40% sucrose

- Store room temperature

Infiltration media

	200ml	Final conc.
10X MS salts	20ml	1xMS salts
Sucrose	10g	5%
MES	0.2g	0.1%
Silwet-L77	200□l	0.1%

- pH 5.7

Alterations to media:
- Low Silwet media (0.02%)
- Low sucrose media (1%)
- pH7.0

Germination media (GM) plates

	1L
10X MS salts	100ml
Sucrose	30g
MES (potassium salt)	0.5g

- pH 5.7
- Phytagel 8g/L
- Autoclave
- When ready to pour add:
    - 1ml Hygromycin (50mg/ml)
    - 0.5ml Timentin (200mg/ml)

References:

Clough SJ and Bent AF, 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. **Plant J** 16:735-43.

Svab, Z., P. Hajdukiewicz and P. Maliga. 1975. Transgenic tobacco plants by co-cultivation of leaf disks with pPZP *Agrobacterium* binary vectors. In “Methods in Plant Molecular Biology-A Laboratory Manual”, P. Maliga, D. Klessig, A.. Cashmore, W. Gruissem and J. Varner, eds. Cold Spring Harbor Press: 55-77.