

## 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<sub>600</sub> 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 <sub>2</sub> HPO <sub>4</sub> (10% w/v stock)	1 ml
KH <sub>2</sub> PO <sub>4</sub> (10% w/v stock)	4 ml
NaCl (10% w/v stock)	1 ml
MgSO <sub>4</sub> .7H <sub>2</sub> O (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](#))

<u>Liquid</u>	1L
Stock solution A	490mL
Stock solution C	11mL
Milli-Q H <sub>2</sub> O	499mL

<u>Solid</u>	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 <sub>2</sub> HPO <sub>4</sub>	10.5	60
KH <sub>2</sub> PO <sub>4</sub>	4.5	33
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.0	7.6
tri-Sodium citrate.2H <sub>2</sub> O	0.5	1.7

- Autoclave in 490mL aliquots
- Store 4°C
- Substituting chemicals
  - K<sub>2</sub>HPO<sub>4</sub>.3H<sub>2</sub>O 13.8g/490mL

### **Minimal A Stock Solution B**

Dissolve 15g agar in Milli-Q H<sub>2</sub>O, make up to 500mL

- Autoclave
- Store room temperature

### **Minimal A Stock Solution C**

Add 1mL 1M MgSO<sub>4</sub> to 10mL 40% sucrose

- Store room temperature

### **Infiltration media**

	<u>200ml</u>	<u>Final conc.</u>
10X MS salts	20ml	1xMS salts
Sucrose	10g	5%
MES	0.2g	0.1%
Silwet-L77	200µl	0.1%

- pH 5.7

Alterations to media:

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

### **Germination media (GM) plates**

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

- pH 5.7
- Phytigel 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.