

## STERILIZATION OF ARABIDOPSIS SEED

1. Add the seed to a 15 ml Sarstedt tube.
2. Add 5 ml 70% ethanol and vortex for 3-5'.
3. Pipette off ethanol, add 5 ml 50% bleach, 0.1% Triton & vortex for 3-5'.
4. Pipette off the bleach, add 5 ml dH<sub>2</sub>O and vortex for 3-5'.
5. Repeat dH<sub>2</sub>O wash at least 3 times.
6. Pipette off the dH<sub>2</sub>O and in the laminar flow hood add 10 ml 0.15% Type "M" agar (made up in H<sub>2</sub>O and autoclaved).
7. Incubate at 4°C for ~3 days.
8. After 3 days, pour/pipette seed/agar onto **MS plates** in the laminar flow hood and seal the plates with Micropore tape.
9. Place plates in the growth chamber.
10. After 10 -14 days, plant out to soil and cover with saran wrap for a further 3-4 days.

### **MS Media (1L)**

4.3g MS Salts (Gibco)

10 g Sucrose (1%)

1 ml 1000 X Gamborg's vitamins (Sigma)

0.5 g MES (0.05%)

pH to 5.8 with KOH

Add 0.8% Type "M" Agar (8g/l) (Sigma)

Autoclave

Cool to 50°C before adding the appropriate antibiotic(s) and pouring out.