LSOP Title	Arabidopsis growth on agar plates and seed sterilisation
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Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *OGTR*
Risk Assessments	
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1. Scope

To outline the procedures for growing Arabidopsis on agar for CK extraction

This LSOP does not cover the growth of Arabidopsis in soil or any other medium. It also does not include how to create agar or agar plates.

2. Definitions

EtOH – ethanol

Murashige and Skoog (MS) – a type of selective medium

3. Materials and Equipment

1. Square petri dishes
2. MS agar
3. Sucrose
4. Ethanol
5. Pipette
6. Microcentrifuge tube
7. Distilled water

8. Alfoil
9. Laminar Flow

4. Prescribed Actions

1. Prepare square petri dishes with 50 mL half-strength Murashige and Skoog (MS) medium containing 1% sucrose and 1% agar (pH 5.7).

   NB: autoclave 20% sucrose solution and MS agar separately, then add sucrose to the MS medium prior to pouring the plates.

2. Sterilise Arabidopsis seeds (see appendix)
3. Dispense seeds to be used into a new sterile microcentrifuge tube and add ~200 uL of sterile distilled water.
4. Using a pipette, suck up water until you get a single seed in the pipette tip, then dispense the liquid + seed onto the plate. Place seeds in a line across the center of the plate. Repeat until you have dispensed ~15-20 seeds per plate across all plates.
5. Wrap plates in alfoil and stratify by keeping at 4°C for two days.

   N.B. This should be done with plates flat.

6. Places plates upright (with the line of seeds horizontal) in a growth cabinet and grow for 4 weeks at desired growth conditions (i.e. 150, 25°C, 12h days)

5. Appendix

<u>Sterilise Arabidopsis seeds:</u>

1. Put the equivalent of ~10 µL worth of each seeds into microcentrifuge tubes.

2. Add 500 µL 70% EtOH then shake for 5 minutes in a vortex.
3. Discard supernatant, add 500 µL absolute EtOH then shake for 10 minutes in a vortex.
4. Remove supernatant in a laminar flow or class II biosafety cabinet to maintain seed sterility

5. Let seeds dry completely. This is easiest achieved by tapping the tube to spread seeds around the wall of the microcentrifuge tube; when the seeds are dry they will fall to the bottom. Seeds can be stored sterile (either at room temp or 4^{o}C for long term use) or used immediately.