

LSOP Title	Adventitious Rooting Protocol
LSOP No.	LSOP01
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	UQ- Equipment OGTR
Risk Assessments	
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1.0 Scope

This procedure covers the adventitious rooting protocol for Arabidopsis.

This LSOP does not cover Arabidopsis growth methods or protocols, seed removal from mature Arabidopsis plants or anything unrelated adventitious rooting protocol.

2.0 Definitions

MES - 2-(N-morpholino)ethanesulfonic acid

MS – Murashige and Skoog

H – hour

Col – Columbia (a type of *Arabidopsis thaliana*)

3.0 Materials and Equipment

1. Eppendorf Tubes
2. 0.2% Agar
3. Media Plates
4. Pipette (and Pipette Tips)
5. Masking Tape
6. Scalpel



7. Black Trays
8. Paper Towel
9. Alfoil
10. Laminar Flow
11. Growth Cabinets
12. Dissecting Microscope

4.0 Prescribed Actions

1. Prepare media (10 g/L agar, 5 g/L sucrose (may vary), 1.15 g/L MS, 0.5 g/L MES) and autoclave

Inside laminar flow:

2. Add any required hormones/treatments to media prior to pouring into square plates. Allow media to set.
3. Surface sterilize Arabidopsis seeds in Eppendorf tubes (labelled with pencil; see LSOP05)
4. Add 0.2% agar (sterile; autoclaved; see LSOP05 appendix) to Arabidopsis seeds in Eppendorf tubes
5. Plate seeds using 1 mL pipette with blue tips (or clear filter tips) onto plates as one row of ~ 15 seeds, approximately 3.5cm from the top of the plate (use a guide)
6. Place all plates in a stack, and place masking tape along two sides across all plates
7. Put trays in 4°C for 3 days

Outside Laminar Flow (at lab bench)

1. Between each plate, slit the tape with a scalpel such that plates are no longer connected to each other. Randomize plates, and place spacer plates between each plate.
2. Line base of big black trays with white paper towel. Place spaced plates in each black tray. Tilt plates slightly (use a pipette tip at base of end plate of each row).

3. Place plates in the light for 6 hours (growth cabinet at 22°C).

NB: A light treatment is important to stimulate germination

4. Cover black trays with another black tray, and cover with alfoil so in complete darkness.
5. Keep plates in darkness in the growth cabinet for 5 days
6. After 5 days in the growth cabinet, remove the covering tray and alfoil, and allow seedlings to grow for a further 7 days or so (growth cabinet; 16 h, 22°C day /8h, 18°C night).
7. Count the number of adventitious roots along the elongated hypocotyls using a dissecting microscope

NB: Col control plants should have an average of 1 adventitious root/seedling.