LSOP Title	Arabidopsis Seed Surface Sterilisation
LSOP No.	LSOP05
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *OGTR*
Risk Assessments	
Approved by	*Franziska Fichtner*
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1.0 Scope

This procedure covers the sterilisation protocol for Arabidopsis seeds surfaces to reduce contamination and growth of bacteria.

This LSOP does not cover Arabidopsis growth methods or protocols, seed removal from mature Arabidopsis plants or anything unrelated to seed surface sterilisation.

2.0 Definitions

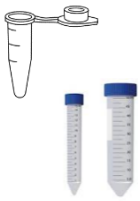
Arabidopsis – Arabidopsis thaliana

EtOH – Ethanol

milliQ water – high purity water from the milliQ system

Tween20 – a specific type of polysorbate 20 surfactant (i.e. detergent)

3.0 Materials and Equipment



1. Eppendorf tube
2. Falcon tube
3. Pencil

4. Bleach
5. Distilled water
6. Tween20

7. Ethanol
8. Pipette
9. Centrifuge
10. Laminar Flow

4.0 Prescribed Actions

1. Decant *Arabidopsis* seeds into Eppendorf tubes, labeled with pencil (doesn't wash off in ethanol)

2. Make sterilizing solution (must be made fresh each time) in a falcon tube as follows:
    a. 25 mL bleach (12.5% strength; kept under sink)
    b. 25 mL distilled water
    c. Drop of Tween20 (dispense drop using a 200uL pipette)
    d. Invert to mix.

3. In the laminar flow (or lab), add 1 mL 70% EtOH to Eppendorf tube with Arabidopsis seeds. Wash down ethanol is fine to use. Invert tube a few times to rinse the seeds, then quickly discard EtOH (seeds in ethanol too long will start to die).

4. Add 1 mL of sterilizing solution to seeds in Eppendorf tube. Invert to mix at least four times over a 10 min timed period (period may be extended up to 15 minutes if there are greater than 500uL of seeds or if the seeds came from plants with a lot of dirt or fungus, but do not exceed 10-15 minutes as seeds will die).

5. In laminar flow, remove sterilizing solution, then rinse seeds with 1 mL sterile distilled water (autoclaved) at least four times, using fresh water each rinse, and at least until detergent bubbles are not visible.

   NB: Can use a brief centrifuge to help stop seeds from floating during rinses if required.

6. Add 0.2% agar (sterile; autoclaved; see appendix) to Arabidopsis seeds in Eppendorf tubes, brief centrifuge to remove any air bubbles if needed.

5.0 Appendix

0.2% Agar

1. In a small Schott bottle, add 0.2 g agar to 100 mL milliQ water.
2. Autoclave.
3. Add ~ 1.5 mL (depending on number of seeds) sterile 0.2% agar solution to Arabidopsis seeds in Eppendorf to vernalise / plate