LSOP Title	In vitro screening of primary transformants (after floral dipping)
LSOP No.	LSOP19
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *UQ -waste* *OGTR*
Risk Assessments	
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1.0 Scope

To outline the procedures in vitro screening of primary transformants based on the floral dipping transformation by Zhang et al., 2006.

This LSOP does not cover floral dipping (see LSOP33).

2.0 Definitions

S - seconds

D - day

H – hour

3.0 Materials and Equipment

1. Selection plates with appropriate media/antibiotics/herbicide
2. Plates (150x150x25mm)
3. Agarose
4. Pipette & Pipette Tips
5. Laminar Flow

4.0 Prescribed Actions

1. Pour selection plates containing carbenicillin (to kill agrobacteria, does not work against plants) and the appropriate antibiotics or herbicide
2. Sterilize seeds using the regular procedure and re-suspend the sterilized seeds in sterile 0.05% agarose (40 ml seed per ml agarose)
3. Spread the seed-agarose suspension onto selection plates. Plate 3-4 ml seed-agarose mixture on each plate.
4. Dry plates under a laminar flow hood until agarose dries up and seeds become stable on the plate.
5. Vernalize seeds by placing them at 4^{o}C for 2-3 d.
6. Move plates to tissue culture room or growth chamber under long-day conditions.
7. After 10-14 d, transformants should be readily distinguished as seedlings with healthy green cotyledons and true leaves and roots that extend into the selective medium (Fig. 1f appendix)
8. Transplant plantlets to water-saturated soil and cover the tray with a plastic hood to maintain high humidity for 2-3 d. Move the tray to a growth chamber for seed collection. Confirm the uptake of the transgene via genotyping PCR.

5.0 Appendix

Read the full protocol before starting with this short version (the side notes are useful)

Zhang, X., et al. (2006) Agrobacterium-mediated transformation of Arabidopsis thaliana using the floral dip method.

https://www.nature.com/articles/nprot.2006.97

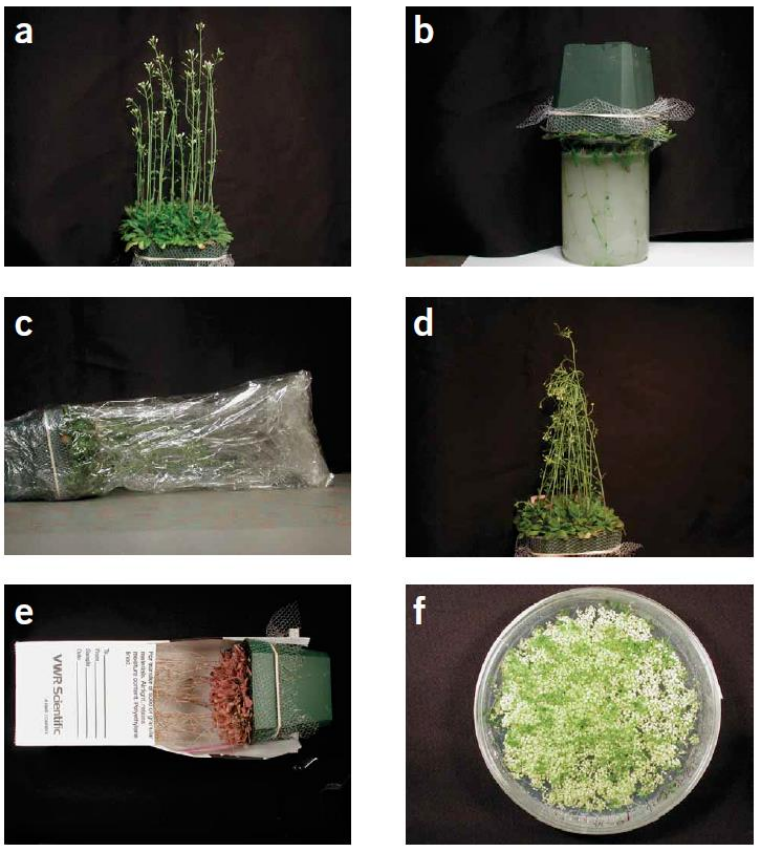

Figure 1 | Stages during the floral dip transformation method.

(a) A good stage for floral dipping is when a pot of healthy plants contain approximately 20–30 inflorescences and some maturing siliques. Siliques should be clipped off

(b) Invert plants and dip their aerial parts in an Agrobacterium cell suspension for 10 s.

(c) Wrap the dipped plants with plastic films to maintain high humidity for 16–24 h.

(d) Remove the plastic covers and grow plants in a growth chamber for 1 month.

(e) Dry and harvest seeds with a sample bag.

(f) Select primary transformants. Transgenic plantlets are readily distinguished from non-transgenic plants by their green true leaves and roots that penetrate the selection medium. In this experiment, we selected primary transformants using kanamycin (+ carbenicillin) and obtained more than 100 transgenic lines on a single selection plate. Note that non-transformed seedlings germinated as well but their cotyledons became chlorotic and bleached soon after germination,

whereas true transformants were very healthy, with green cotyledons and true leaves, and developed roots that penetrated the medium.

Comments on how to distinguish transformants vs non-transformants on various media:

- For kanamycin (+ carbenicillin) selection, non-transformed seedlings can germinate as well, but their cotyledons will become bleached very soon.
- For Basta (+ carbenicillin) selection, the cotyledons of non-transformants turn yellow or pale a little bit later (approximately 2–3 weeks after germination).
- For hygromycin (+ carbenicillin) selection plates, non-transformants germinate as well and their cotyledons can remain green for more than 1 month after germination; however, the cotyledons are smaller than those of true transformants, and normally roots develop properly only in the true transformants. All these selections share one thing in common: only true transformants can produce true leaves, whereas non-transformants will not.
- Carbenicillin inhibits growth of Agrobacterium cells and may have some effect on the growth of primary roots as well. Once plants are moved to MS plates without the antibiotics, or directly to soil, root growth will resume soon after transplantation