LSOP Title	Split-plate bud assay
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Location	UQ Node/Centre-wide
Policy/Procedure Link	*OGTR*
Risk Assessments	
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1.0 Scope

This procedure covers the protocols for split-plate bud assays.
This LSOP does not cover split-plate for Arabidopsis (see LSOP20).

2.0 Definitions

GR24 – synthetic strigolactone

NAA - 1-Naphthaleneacetic acid (synthetic auxin)

BA - 6-Benzylaminopurine (synthetic cytokinin)

MS – Murashige and Skoog medium

MES - 2-(N-morpholino)ethanesulfonic acid agar

3.0 Materials and Equipment

1. Pots (2L)
2. UQ23 potting mix
3. GR24 in acetone

4. NAA and BA in 100% ethanol

5. AM Media (0.5% Sucrose, 1.15g MS Salts and 0.25g MES per 500ml bottle; pH 5.9, 5g agar).
6. 500mL Schott bottles
7. Autoclave
8. Square Plates (10cm x 10cm)
9. Laminar Flow
10. Scalpel
11. Spatula
12. Micropipette & Tips
13. Razor blade
14. Tweezers
15. Digital callipers
16. Camera

4.0 Prescribed Actions

<u>Plant Material, Growth Conditions and Hormone Treatments</u>

1. Sow seeds 4 or 5 per pot (2L) in UQ23 potting mix and place in growth cabinet at 22°C with a photoperiod of 18hrs.

   NB: Number of plants required was generally calculated as 12 x treatment groups + 10% extra. More may be required for mutants with poor germination.

2. For hormone treatments dissolve GR24 in acetone and NAA and BA in 100% ethanol. The concentration of stock hormone treatment is calculated using the final volume of 36cm^3 for the agar block and injection volumes of <u>20µL for NAA</u> and <u>40µL for GR24 and BA</u>.

   NB: A new stock solution of 100mM NAA was prepared for each experiment and 10mM stock solutions were used to prepared treatment stocks of GR24 and BA.

Split-Plate Bud Assay Protocol

Preparation of Split-plates

1. Prepare AM media in 500ml bottles and autoclave. (0.5% Sucrose, 1.15g MS Salts and 0.25g MES per 500ml bottle; pH 5.9, 5g agar).

   NB: Generally 6 bottles of media were used per experiment giving 6 treatment groups of 6 plates and 12 nodal segments.

2. Pour square plates (10cm x 10cm) to a depth of approx. 9mm (6 plates per 500ml bottle) and leave to dry open in Laminar Flow for 60 mins.

3. Cut a 2cm wide trough from the centre of each plate using a scalpel with handle to cut along lines of template and spatula to scoop centre agar out.

   NB: All agar needs to be removed from trough to make sure there are no bridges between the agar blocks.

4. Leave plates to dry for a further 30mins with trough parallel to air flow in Laminar Flow.

5. Once dried, use a micro-pipette to inject either 20 (NAA) or 40 µL (GR24 and BA) of the treatment solutions into the centre of each media block (best to use template).

6. Resealed plates in plastic sleeves and leave for 72 hrs at 4°C to ensure the hormone treatment is evenly diffused throughout the media.

Excising the Nodes and Set-up of Assay

1. Excise nodes from plants 10 days after sowing. Use a single-sided razor blade and ruler to cut a 3 cm section (~1.5cm either side of node) at node 3.

   NB Only plants with fully expanded leaves at node 4 should be used to ensure the stem at node 3 has finished elongating and the ends won't push out of the media.

2. Remove leaf and stipules using razor blade leaving only ~ 10mm of petiole.

3. Transfer nodal section to the prepared split plates using fine tweezers and position so that ~ 5mm of the cut stem is embedded into the agar at each end. Two nodal sections should be placed in each plate.

   NB: This step should be performed as quickly as possible to avoid desiccation of the excised node and only the ends of the stem should come in contact with the media.

4. Place plates horizontal in growth cabinet under the same growth conditions as the original plants.

Bud Growth Measurements

1. Measure length of buds in a Laminar flow every 24 hrs (excluding weekends) for 7 days starting with initial length at 0 hrs.
2. Measure bud length using digital callipers from the base of the bud to 2/3 the length of stipules when visible.

   NB: Excised nodes should be excluded if the stem has elongated out of the media, the growing buds have come in contact with the media or nodal segment is contaminated.

5.0 Appendix

Recommended Timetable

Week 1	Thursday	Plant Peas.
	Friday	
Week 2	Monday	Rotate Peas in growth cabinet.
	Tuesday	
	Wednesday	Make up media and autoclave. Rotate peas in growth cabinet.
	Thursday	Pour plates and make split. Place in coldroom. *(This can be done on Friday if enough time).*
	Friday	Inject hormone treatments into plates and place in coldroom. Remove plastic covers off Peas, rotate and water.
Week 3	Monday	Water pots first thing in the morning. Excise nodes and add to split-plates. Measure buds.

	Tuesday	Measure buds.
	Wednesday	Measure buds.
	Thursday	Measure buds.
	Friday	Measure buds.
Week 4	Monday	Final bud measurement.