Grind Plants Containing Haustoria

Citation: Harley King (10/02/2020). Purification of Haustoria from Arabidopsis in Response to Infection by E. cichoracearum. https://dx.doi.org/10.17504/protocols.io.tqkemuw

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1. Weigh out 5-10g frozen Arabidopsis leaves. Set aside 2-3 leaves for quantifying haustoria with a confocal microscope before purification.

2. Grind leaves in ice-cold 8 mL 1x PBS buffer pH 7.4 for 1 min in kitchen blender in cold room.

3. Filter grindate through 100um Nylon mesh. Haustoria are about 10-20um in diameter.

4. With a glass rod, remove unfiltered debris to blender. PBS buffer can be used to wash the membrane with a transfer pipette into the blender.

5. Add 5 mL 1x PBS buffer and grind for another 45 seconds.

6. Filter through 100um Nylon mesh.

7. Filter the collected filtrate from steps 3 and 6 and pass through a 40um mesh. A vacuum and modified filter assembly can be used to expedite the purification. Wash the membrane with PBS buffer into the trash.

8. Pass filtrate through 40um mesh again.

9. Transfer ~13-15mL to a 15mL conical tube. Pellet filtrate at 1000g for 5 min.

10. Remove supernatant.

11. Resuspend pellet in 2mL 1x PBS buffer. Set aside 40ul for quantifying haustoria.

12. Investigate haustoria with confocal microscope and a hemacytometer before committing to the next steps.

Col-O plants should be processed concurrently as a negative control especially if haustoria will be filtered by FACS.

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**Purify Haustoria Using Percoll Cushion**

2. Using Percoll, make a 60% solution and a 40% solution with 1x PBS.

Layer 4mL of the 60% solution on the bottom of a 15mL conical tube.

Layer 6mL of the 40% solution on the top of the 60% solution.

Gently add 1mL of resuspended pellet containing the haustoria to the top 40% Percoll solution.

Centrifuge at 2500g for 10 minutes.

The haustoria will penetrate the 40% layer but not the 60% layer.

Remove the 40% layer. Mix, then pellet at 1000g for 5 min.

Resuspend in 2mL. Removed 40ul aliquot for quantifying haustoria.

If sufficient, proceed to next step.

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**Incubate Haustoria with anti-GFP (Chromotek) Conjugated to Dynabeads**

3. Conjugate anti-GFP Trap antibody to Dynabeads following manufacturer's recommended protocol.

2. Incubate beads with haustoria with end-over-end rotation overnight.

3. Wash beads 5x with 1x PBS pH7.4.

4. Disrupt haustoria-GFP Trap binding following manufacturer's protocol.

5. Proceed to FACS purification

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4. Follow previous experiments to use as a baseline in purifying haustoria with FACS. Use col-o plants as negative control.

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