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Purification of Haustoria from Arabidopsis in Response to Infection by *E. cichoracearum*

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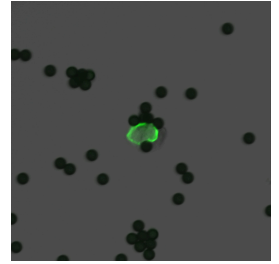
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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

This procedure describes steps for the purification of *Arabidopthis thaliana* haustoria in response to powdery mildew infection by fungus *Golovinomyces cichoracearum*.

Grind Plants Containing Haustoria

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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.

Note

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

Purify Haustoria Using Percoll Cushion

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 - 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. Remove 40ul aliquot for quantifying haustoria.
 - If sufficient, proceed to next step.



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

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 1. 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
- 4 Follow previous experiments to use as a baseline in purifying haustoria with FACS. Use col-o plants as negative control.