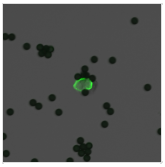

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Purification of Haustoria from Arabidopsis in Response to Infection by E. cichoracearum

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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*.

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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

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 filtrated 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 concial tube. Pellet filtrate at 1000g for 5 min.
10. Remove supernatant.
11. Resuspend pellete in 2mL 1x PBS buffer. Set asside 40ul for quantifying haustoria.
12. Investigate haustoria with confocal microscope and a hemacytometer before commiting to the the next steps.

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

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.

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

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

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