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Marchantia protoplast

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Works for me

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OpenPlant Project



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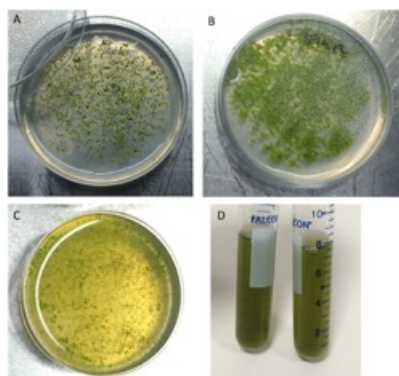
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MATERIALS TEXT

8% (w/v) mannitol at pH 5.7

- 1
Grow gemmae on ½ Gamborg B5 plus vitamins media plates (the plates should be covered like Fig. 1A) for 4 days (Fig. 1B).
- 2
Add 10 ml of 8% (w/v) mannitol at pH 5.7 on the plate and incubate at room temperature 30min.
- 3
Prepare 2% Driselase solution: add 0.2 g Driselase in 10 ml 8% mannitol solution and incubate in dark for 20 min at RT. Gently invert to mix at intervals. Spin at 3.3k for 3 min. Filter sterilize supernatant using a 0.2 µm filter attached to a syringe.
- 4
Replace mannitol solution with 5 ml 2% Driselase and incubate at RT for 4 hours with gentle shaking (50 rpm).
- 5
Using a tip gently release protoblasts from gemmae (Fig. 1C).
- 6
Filter the solution through a 70 µm cell strainer into a 50 ml falcon tube and then transfer in a round bottom falcon tube (Fig. 1D).
- 7
Incubate for further 10 min.
- 8
Spin at 120 x g for 3min. Remove the supernatant without disturbing the cells.
- 9
Wash the cells by re-suspending them in 6ml 8% mannitol (Mix by swirling not shaking) and spin at 120 x g for 3 min. Repeat the wash 2 times.
- 10
Add 300 µl 50% glucose in the autoclaved liquid Gamborg B5 plus vitamins + mannitol solution



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