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🌐 Marchantia protoplast

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MATERIALS

8% (w/v) mannitol at pH 5.7

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We use this protocol and it's working

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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 μ m 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 μ m 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 μ l 50% glucose in the autoclaved liquid Gamborg B5 plus vitamins + mannitol solution

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