**Marchantia protoplast**

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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 30 min.

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 3 min. Remove the supernatant without disturbing the cells.

9. Wash the cells by re-suspending them in 6 ml 8% mannitol (Mix by swirling not shaking) and spin at 120 x g for 3 min. Repeat the wash 2 times.

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Add 300 μl 50% glucose in the autoclaved liquid Gamborg B5 plus vitamins + mannitol solution.