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Marchantia Cryopreservation of Gemmae

Forked from [Marchantia cryopreservation of gemmae](#)

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We use this protocol and it's working

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ABSTRACT

Simplified cryopreservation protocol for *Marchantia polymorpha* gemmae, based on ([Tanaka et al. 2016, Plant and Cell Physiology](#)). Enables long term storage of gemmae at -80°C.

MATERIALS

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☒ Agar **Melford Catalog #A20021**

☒ Absciscic acid **Sigma Aldrich Catalog #A1049**

☒ Sodium Alginate **Duchefa Biochemie Catalog #S1320**

☒ Calcium chloride **VWR Scientific Catalog #22328.262**

☒ Sucrose **Fisher Scientific Catalog #10634932**

☒ Glycerol **Sigma Aldrich Catalog #G5516**

☒ Gamborg B5 Medium with Vitamins **Duchefa Biochemie Catalog #G0210**

- Petridish, 9cm/4.5cm or 6/12-well transparent multi-well plates
- Liquid nitrogen (N₂)

Reagent setup:

Preculture plates

1/2 strength Gamborg B5, pH 5.8 + 1.2% agar + 0.3 M sucrose + 10μM ABA
Autoclave media without ABA, add ABA from a filter sterilised stock solution just before pouring plates. 9cm/4.5cm petridishes or 6/12-well transparent multi-well plates can be used for plates, depending on desired throughput.

Alginate Solution

1/2 strength Gamborg B5 + 3% Sodium alginate

CaCl₂ Solution

1/2 strength Gamborg B5 + 0.1M CaCl₂

Loading solution

1/2 strength Gamborg B5 + 2M glycerol, + 1M sucrose

Thawing solution

1/2 strength Gamborg B5 + 1M sucrose

Rinse solution

1/2 strength Gamborg B5 + 1% sucrose

Preculture

- 1 Collect fresh gemmae and plate on **preculture plates**, incubate 1-3 days under normal growth conditions (e.g. 21°C constant light)

Encapsulation

- 2 Place small drops (approx. 40 μ L) of **alginate solution** on an empty 4.5cm petridish/multi-well plate to form beads.

- 3 Use forceps to transfer gemmae from preculture plates, place 1-5 gemmae inside each bead.
Proceed quickly to step 4 to avoid gemmae sinking to the bottom or edge of the bead.

4 Add a drop of **CaCl₂ solution** to each bead, make sure to not displace gemmae from the bead.

4.1 Allow beads to solidify for 10min.

- 5 Use a serological pipette to fill the plate with **loading solution**, submerging the beads. Soak beads for 30min.
- 6 Use a serological pipette to remove the loading solution without disrupting the beads.

- 6.1 Remove lid and air dry beads in flowhood for >2h.

- 7 Use forceps or scalpels to transfer beads into 1.5mL Eppendorf tubes (max. 10 beads/tube).
- 7.1 Flash freeze tubes in liquid nitrogen for >2 minutes.
- 7.2 Move tubes to -80°C for long term storage.

Thawing and Recovery

- 8 Remove tubes from freezer and immediately place in a 37°C water bath or heat block for 2 minutes.
- 9 Add 1.5mL **thawing solution** to each tube, incubate at room temperature for 10 minutes.
- 10 Replace thawing solution with **rinse solution**. Incubate at room temperature for 10 minutes.
- 11 Remove rinse solution, transfer beads on 1/2 Gamborg plates, incubate under normal growth conditions (e.g. 21°C constant light) until thalli grow