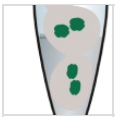

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Marchantia cryopreservation of gemmae

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1 Works for me dx.doi.org/10.17504/protocols.io.46hgzb6

OpenPlant Project

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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

NAME	CATALOG #	VENDOR
Agar	A20021	Melford
Gamborg B5 Medium	G0209	Duchefa Biochemie
Abscisic acid	A1049	Sigma Aldrich
Sodium Alginate	S1320	Duchefa Biochemie
Calcium chloride	22328.262	VWR Scientific
Sucrose	10634932	Fisher Scientific
Glycerol	G5516	Sigma Aldrich

MATERIALS TEXT

- Petridish, 9cm/4.5cm or 6/12-well transparent multi-well plates
- Liquid nitrogen (N_2)

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

$CaCl_2$ Solution

1/2 strength Gamborg B5 + 0.1M $CaCl_2$

Dehydration buffer

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

Thawing solution

1/2 strength Gamborg B5 + 1M sucrose

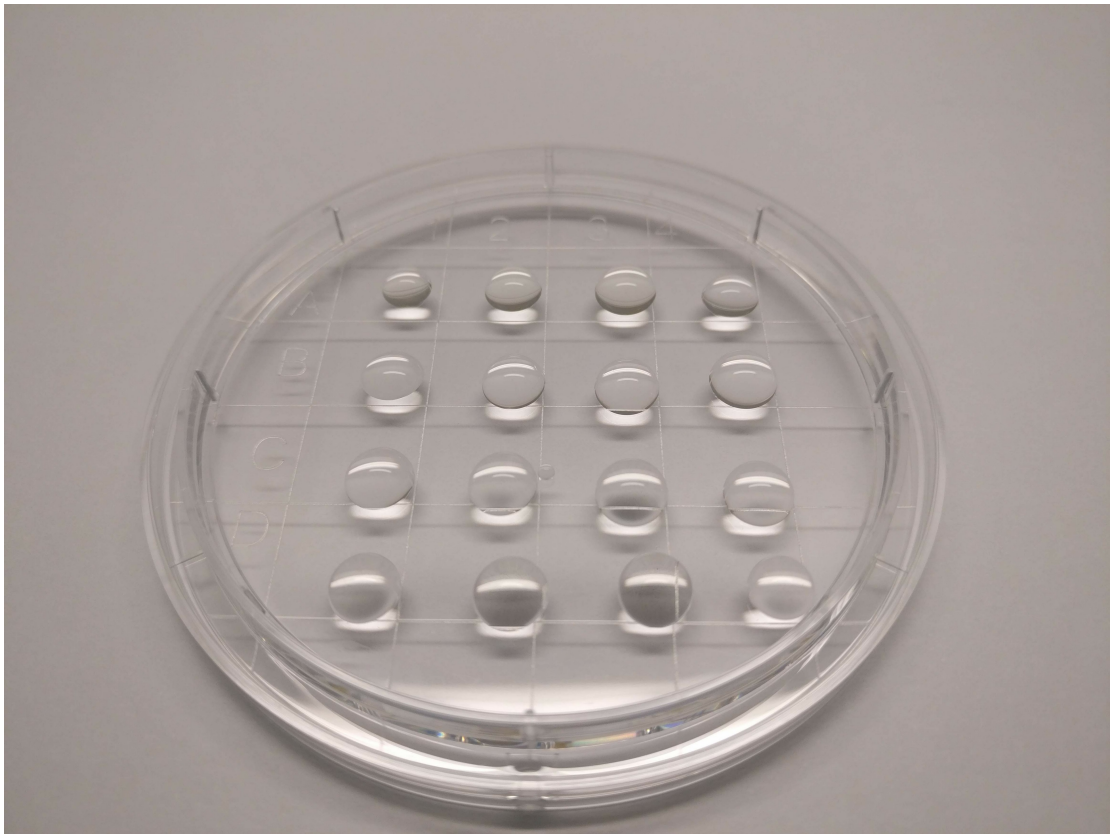
Rinse solution
1/2 strength Gamborg B5 + 1% sucrose

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

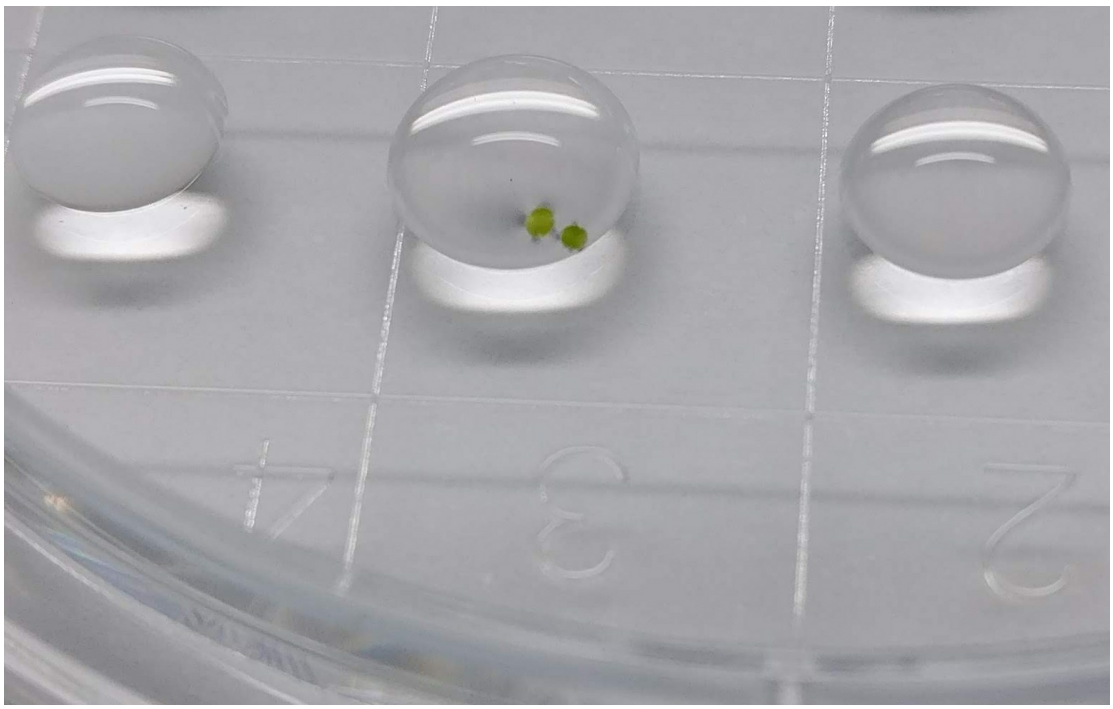
2 Place small drops (approx. 40µL) of **alginate solution** on an empty 4.5cm petridish/multi-well plate to form beads.



3 ⚠

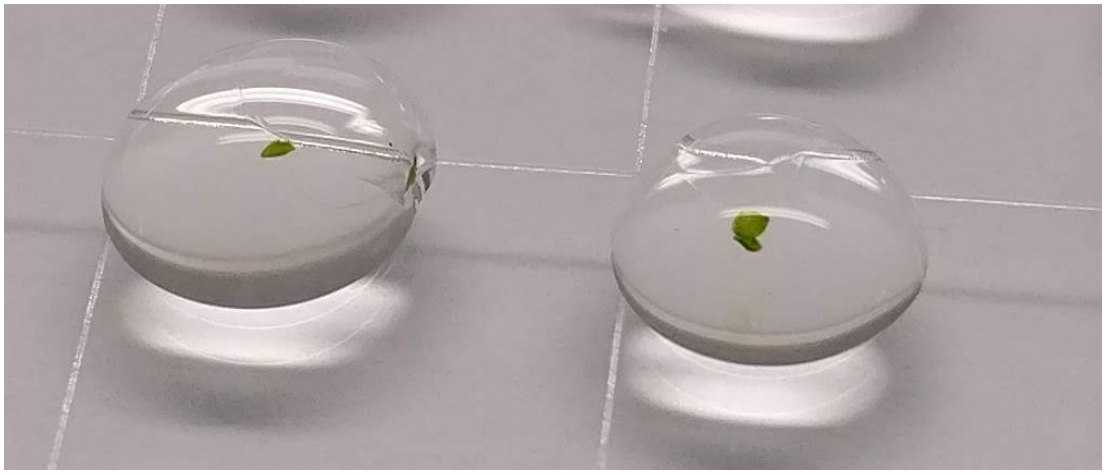
Use forceps to transfer gemmae from preculture plates, place 1-5 gemmae inside each bead.

Proceed quickly to step 4 to avoid gemmae sinking to the bottom or edge of the bead.



4 Add a drop of **$CaCl_2$ solution** to each bead, make sure to not displace gemmae from the bead.

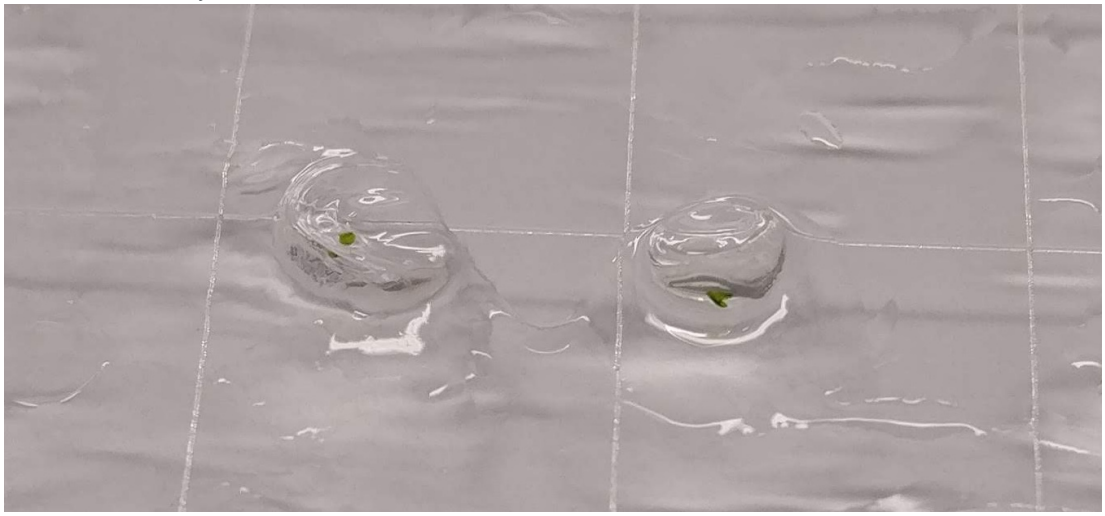
4.1 Allow beads to solidify for 10min.



5 Use a serological pipette to fill the plate with **loading solution**, submerging the beads. Saok 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 37ºC 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 beads and place on 1/2 Gamborg plates, incubate under normal growth conditions (e.g. 21°C constant light) until thalli grow