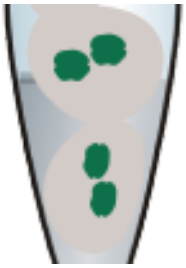
Nov 28, 2019

Marchantia Cryopreservation of Gemmae



Forked from Marchantia cryopreservation of gemmae

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Protocol status: Working
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

MATERIALS

Agar **Melford** **Catalog #**A20021

Abscisic acid **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A1049

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

Calcium chloride **VWR International (Avantor)** **Catalog #**22328.262

Sucrose **Fisher Scientific** **Catalog #**10634932

Glycerol **Merck MilliporeSigma (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 (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 $CaCl_2$

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

Thawing solution
1/2 strength Gamborg B5 + 1M sucrose

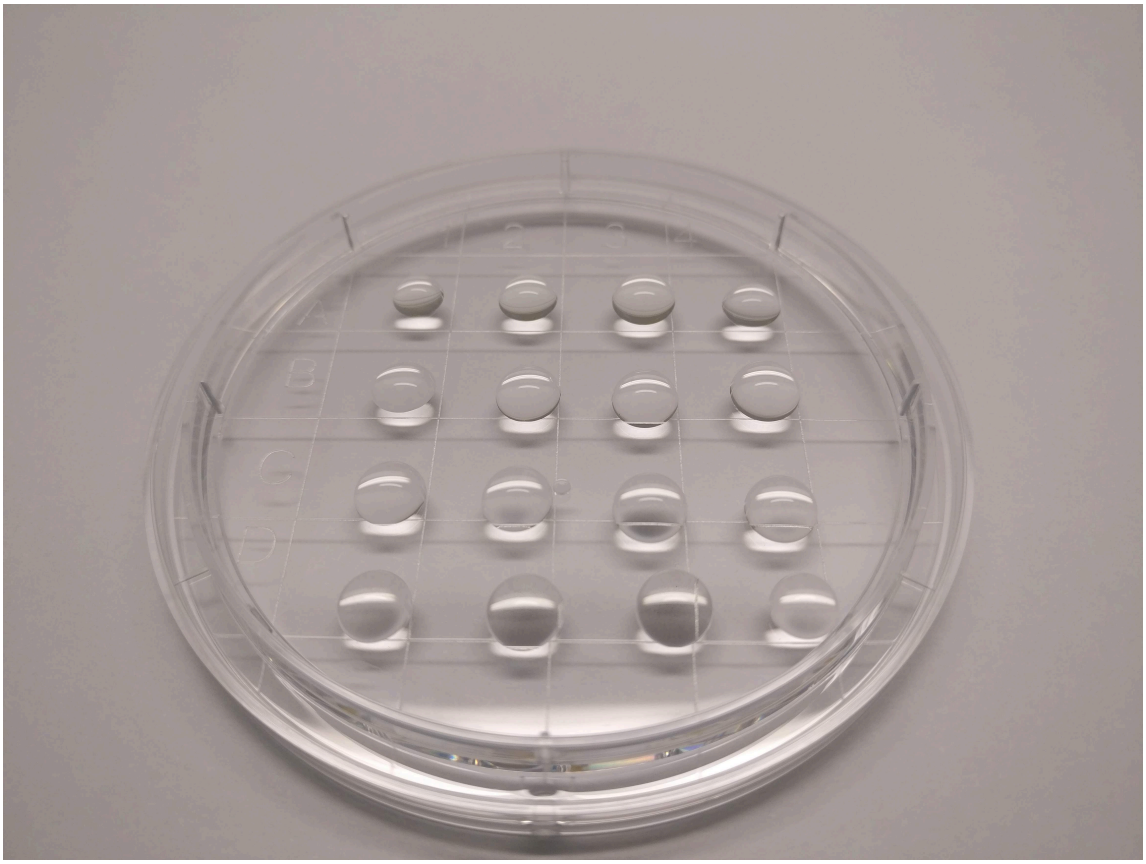
Rinse solution
1/2 strength Gamborg B5 + 1% sucrose

Preculture

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

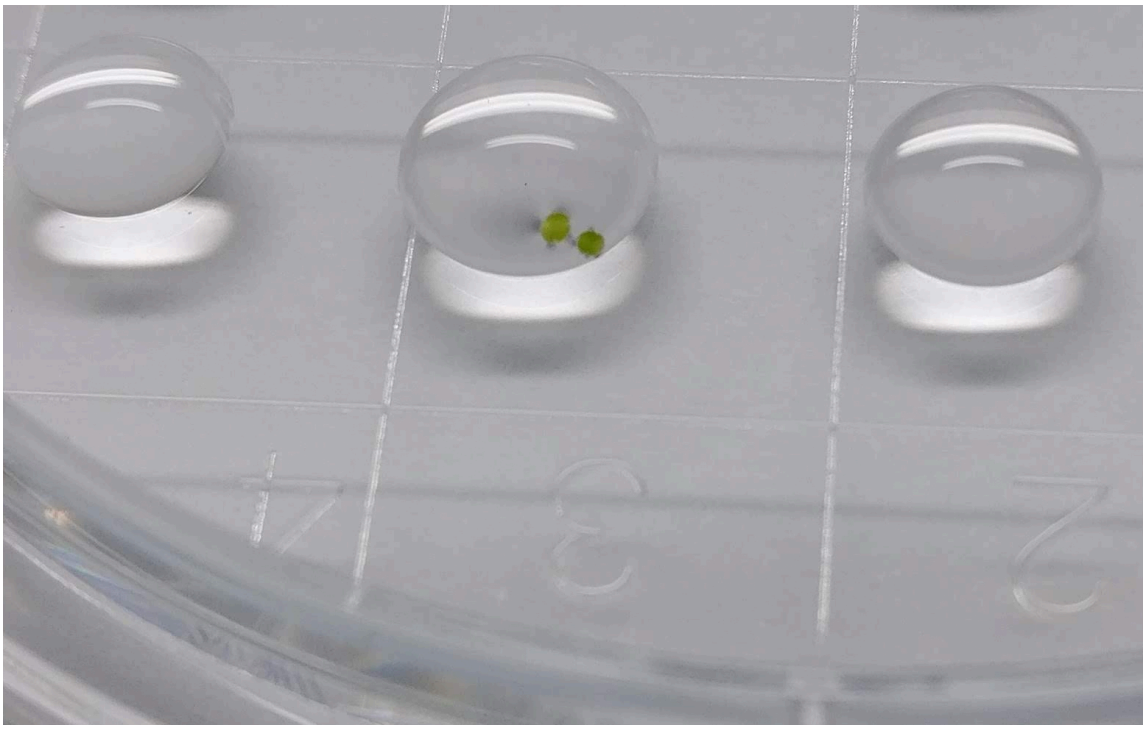
Encapsulation

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



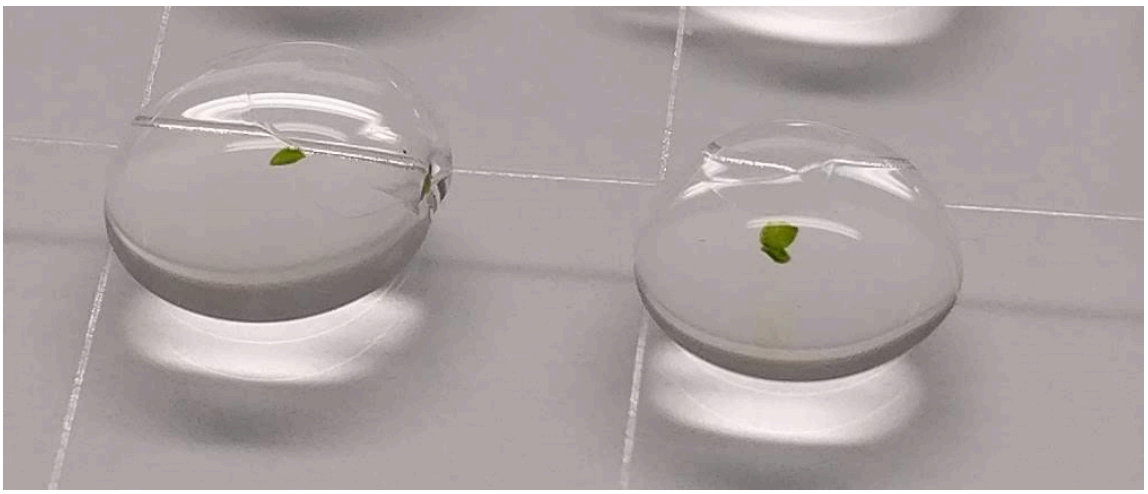
3 Use forceps to transfer gemmae from preculture plates, place 1-5 gemmae inside each bead.

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.

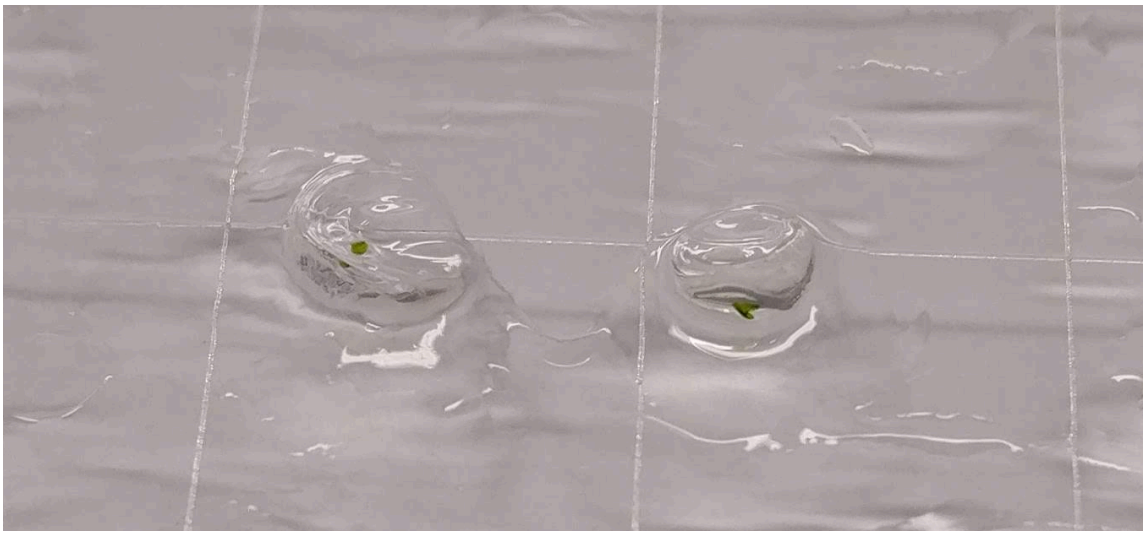
4.1 Allow beads to solidify for 10min.



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 rinse solution, transfer beads on 1/2 Gamborg plates, incubate under normal growth conditions (e.g. 21°C constant light) until thalli grow