

Sep 24, 2019

## Marchantia spores production in Microboxes

DOI

[dx.doi.org/10.17504/protocols.io.4v8gw9w](https://dx.doi.org/10.17504/protocols.io.4v8gw9w)



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**Protocol Citation:** Eftychis Frangedakis 2019. Marchantia spores production in Microboxes. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.4v8gw9w>



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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** June 28, 2019

**Last Modified:** September 24, 2019

**Protocol Integer ID:** 25248

**Keywords:** marchantia spore, microboxes spore sterilisation, production in microboxes spore sterilisation, using microbox, lid of microbox, marchantia, microbox, fungi, such as fungi, entry of contaminant, contaminant

## Abstract

Spore sterilisation

Marchantia spores can be obtained usually in three months using Microboxes. The lid of Microboxes has a specially designed filter that allows high gas exchange, limited dehydration and blocks the entry of contaminants such as fungi into the culture. We are using the TP4000+TPD4000 Microboxes with a green XXL+ filter.

## Materials

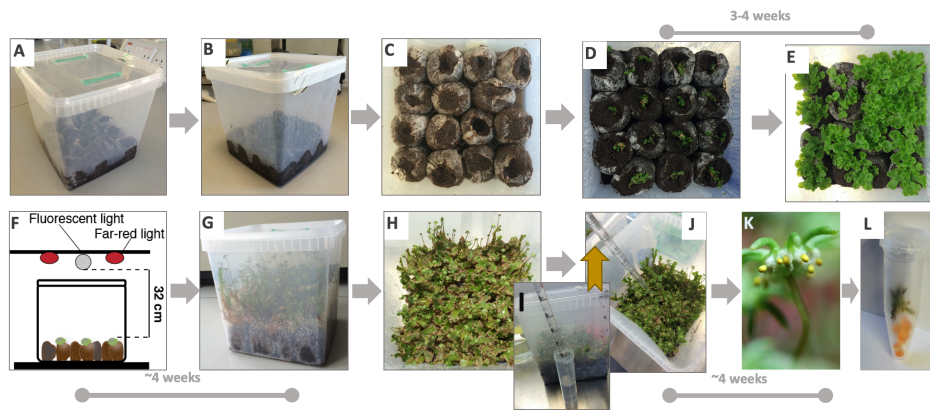
44 mm Jiffy-7 peat pellets (#32170142, Jiffy)

Microboxes with green XXL+ filters (TP4000-TPD4000, SACO2, Belgium)

## Troubleshooting

- 1 Put 15 "Jiffy 7" pellets in a Microbox, add 800 mL of water, close the lid and autoclave (A and B in Figure).
- 2 After autoclaving the Microbox with the "Jiffy 7" pellets, work in a flow hood.
- 3 Put at least one 10 mm x 10 mm thallus fragment or 3 gemmae per pellet using sterile tweezers (C and D in Figure).
- 4 Add another 150-200 mL of sterile water, close the lid and place at 21 °C under continuous light with light intensity of 150  $\mu\text{mol}/\text{m}^2/\text{s}$  (E in Figure).
- 5 After one month transfer plants under a 16 h light / 8 h dark regime, with light intensity of 150  $\mu\text{mol}/\text{m}^2/\text{s}$  supplemented with far red light (peak emission: 700-750 nm, spectral photon flux density: 2400  $\mu\text{mol}/\text{m}^2/\text{s}$ , distance from plants: 32 cm) (F in Figure).
- 6 After approximately 4 weeks mature male and female reproductive organs are produced (G and H in Figure).
- 7 In a flow hood, open the lid of the Microbox, and using a pipette add 5-10 mL of sterile water on top of the male and female reproductive organ (I and J in Figure). After a few seconds you will be able to see the sperm as a cloudy exudate in the water drop. For fertilisation, use the pipette to "move" the reproductive organs in order to touch each other.
- 8 Alternatively, for fertilisation, transfer the water drop from the male gametophore with a disposable sterile plastic pipette on the top of the female reproductive organ (pic in step 11).
- 9 After one 4 weeks mature sporangia are ready for collection (K in Figure). Place 1-3 mature sporophytes in a 1.5 mL Eppendorf tube with 5 silica gel beads (L in Figure) and store at 4 °C if the spores are to be used within 1-2 months. For longer storage, allow the sporophyte to desiccate for a week at 4 °C and then store at -80 °C.

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