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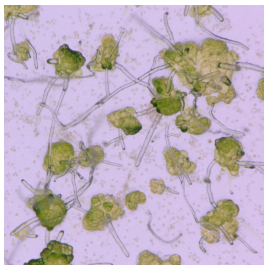
Marchantia spores sterilisation



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Protocol status: Working
We use this protocol and it's working

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Guidelines

Use 2-4 sporangia per transformation planned

1 Add one Milton tablet into 25 mL of sterile water to prepare the "sterilization solution".

2 Add the sporangia into a 1.5 mL centrifuge tube and add 0.5 mL of sterilization solution into the tube (B in Figure)

3 Use sterile metal tweezers to crush the sporangia (C in Figure).

4 Place a 40 µM cell strainer into a 50 mL Falcon tube and pour the 0.5 mL suspension of crushed spore heads onto the filter and then wash the strainer with another 1 mL of sterilization solution (D in Figure).

5 Once filtered, aliquot the spore solution in 1.5 mL Eppendorf tubes (1.5 mL per tube) and incubate at room temperature for 10 min (the exposure to the sterilization solution should not exceed 25 min) (E in Figure). 10m

6 Centrifuge for 2 min at 15800 xg at room temperature, discard the supernatant and re-suspend the spores in sterilization solution (150 µl per transformation planned) (F in Figure). 2m

7 Spread 150 µl of re-suspended spores on a plate containing growth media (G in Figure), seal the lid with micropore tape and place under continuous light at 21 °C 150 µmol/m^2/s for 7 days (H in Figure). Incubate with the bottom side up, which will prevent rhizoids from growing into the agar, making the removal of sporelings from the plate easier by simply using a sterile scalpel.

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