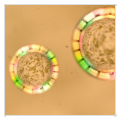

Sep 24, 2019

Marchantia agrobacterium transformation of sporelings in multi-well plates

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1 *Works for me* dx.doi.org/10.17504/protocols.io.48tgzwn

OpenPlant Project

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ABSTRACT

A modification of the Ishizaki et al 2008 *Agrobacterium* mediated Marchantia sporeling transformation protocol is used.

Sterilised spores are grown for 5-7 days in ½ strength Gamborg plates prior to co-cultivation for 2 days with agrobacterium in liquid media in multiwell plates. Sporelings are then spread on media with the appropriate selective antibiotic. In about 5 days, positive transformants start to emerge.

1 **Day -7**
Spore preparation:

Marchantia spores are grown for 7 days in ½ strength Gamborg agar plates (A in Figure).

2 **Day -2**
Agrobacterium preparation:

- Inoculate a single colony of agrobacterium, transformed with the plasmid of interest, into 5mL LB media plus antibiotics, and then incubate at 28°C with shaking at 150 rpm for two days.

Day 1

- After two days, centrifuge the 5mL agrobacterium culture for 10 minutes at 2000g
- Re-suspend in 5ml of ½ strength Gamborg's B5 plus vitamins media with 1% sucrose.
- Add acetosyringone to a final concentration of 100 µM
- Incubate with shaking for 6h at 28°C at 150 rpm.

3 **Day 1**

Using a sterile scalpel transfer the 5-7 days old Marchantia sporelings in 6 mL of of liquid ½ Gamborg media in a 50 mL Falcon tube (B and C in Figure) and mix well.

4 Aliquote 3 mL of liquid ½ Gamborg B5 vitamins plus supplements media in each well of a 6-well plate.

5 Aliquote 1 mL of spores in each well (D in Figure).

6 Add acetosyringone to each well to a final concenration of 100 µM.

7 Place the 6- well plate on a shaker at 120 rpm for 2 days at 21^{o}C under continuous light (E in Figure).

8 Using a pipette tranfer the sporeling in a 70 or 100 µm cell strainer placed in a 50 mL Falcon tube (F and G in Figure).

9 Wash the sporelings with 50 mL of sterile water supplemented with 100 µg/ml cefotaxime to remove excessive agrobacterium.

10 Plate on ½ strength Gamborg B5 plus vitamins 1.2% agar plates with cefotaxime and antibiotics (H and I in Figure)

11 **Day 7**

After 5-7 days succesful transformants start to be visible on the plate (J in Figure)

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