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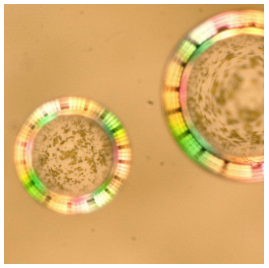
Marchantia agrobacterium transformation of sporelings in multi-well plates (plus materials info) V.1



Forked from Marchantia agrobacterium transformation of sporelings in multi-well plates

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We use this protocol and it's working

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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.

Materials

Samples required:

Fertilised spore heads from Cam2 archegonia stored at -80ºC or 4 ºC (dried using self-indicating silica gel – Fisher Scientific S/0761/53)
GV2260 Agrobacterium Electro-Competent Cells - included in these cells is the pSoup helper plasmid (Hellens et al, 2000)
Plasmid of interest to insert T-DNA into Marchantia cells

Overview of Antibiotics Required for selection of Agrobacterium transformants and Marchantia transformants

Agrobacterium strains:
GV2260 strain is resistant to Rifampicin 10µg/ml + Carbenicillin 50µg/ml
GV3103 strain is resistant to Rifampicin 10µg/ml + Gentamycin 25µg/ml (currently not using this strain in the lab).

Plasmid of interest: Loop pCsA plasmid that confers Spec resistance to Agrobacterium and Hyg resistance to Marchantia plants
- GV2260 transformed with a Loop pCsA plasmid: will be screened in LB plates with Spec 100µg/ml + Rif 10µg/ml + Carb 50µg/ml
- Marchantia sporelings infected by Agrobacterium with a Loop pCsA plasmidwill be screened on Gamborg plates with Hyg 20µg/ml + Cefo 100µg/ml

Overview Chemicals and Media required:
- Milton Tablets purchased from Boots (1 tablet in 25ml sterile water = 0.05%) or Sodium dichloroisocyanurate from Sigma
- Sterile Water
- Acetosyringone (3',5'-Dimethoxy-4'-hydroxyacetophenone – Sigma D134406) - 100mM stock solution - dissolved in DMSO (x1000, added to 1/2 GB media plus supplements)
- Liquid **LB media** (LB pH7)
- Antibiotics stocks
- Cefotaxime 100mg/ml stock solution, dissolved in water (1000x, added to sterile water)

- LB plates (LB ph7 agar 1.5%) + **antibiotics** (see ex above)
- Gamborg plates (½ Gamborg B5 with vitamins pH5.8 agar 1.2%) for plating spores
- Liquid Gamborg media plus supplements = ½ Gamborg B5 with vitamins pH5.8 (Duchefa Biochemie Cat. G0210) + 0.1% N-Z amino A (Sigma C7290) + 0.03% L-Gultamine + 2% sucrose
- Gamborg plates (½ Gamborg B5 with vitamins pH 5.8 agar 1.2%) **+ antibiotics** (see example above)

Consumables required:

40µm cell strainer,
2mm electroporation cuvettes,
falcon tubes,
empty 1L/500ml bottles,
6-well and/or 12-well Cell Culture Plates (Corning Costar 3516 and 3513),
Eppendorfs

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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