Culturing *Physcomitrella patens*

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**GUIDELINES**

All solutions and materials must be sterile. MgSO₄·7H₂O, FeSO₄·7H₂O and antibiotics stock solutions must be sterilized only via filtration. All other solutions can be autoclaved. Cellophane disks should be divided by simple paper, e.g. printing paper, for autoclaving to avoid them sticking together. The whole culturing operation must be carried out under a horizontal laminar flow hood to assure sterile conditions. Petri dishes should be sealed off in a way that still allows gas exchange.
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Prepare in advance

1 Liquid PPNH4 medium with 300 mg/L Cefotaxim or 250 mg/L Vancomycin.

   1.1 Add 10 mL of a macro element-solution, 1 mL of a micro element solution, 1 mL of the potassium phosphate solution and 2.7 mL of the ammonium tartrate solution for each liter of medium.

2 Solid PPNH4 medium on sterile petri dishes

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2.1 Add 7.2 g agar per liter of PPNH4-liquid medium. Add further 300 mg cefotaxime or 250 mg Vancomycin per liter of medium to suppress bacterial growth.

3 Cellophane disks with the appropriate size for the petri dishes. Wet the cellophane disks with sterile ultrapure water to avoid creases.

3.1 Overlay the solid medium on the petri dishes with fitting cellophane disks to prevent the mosses from growing into the medium.

Execution

4 Scrape 7 – 10 days old moss protonemal tissue with spatula.

5 Put 4 mL of water into a (conical tube). Put in the protonemal tissue. Cut it with a Homogenizer until no big chunk is visible and the water is uniformly green.

6 Spread 2-3 mL of freshly fragmented protonema on the cellophane on the petri dish.

7 Seal off with micropore tape to allow gas exchange.

8 Incubate plates at 24°C with a light regime of 16 h light : 8 h darkness at 80 $\mu$mol×m$^{-2}$×s$^{-1}$

9 For next harvest gently scrape surface of cellophane disks after 7 – 10 days (before culture starts to turn brown). Repeat cycle. PPNH4 liquid medium with 300 mg/L Cefotaxim or 250 mg/L Vancomycin is recommended for storage as the moss stays available for culture for >6 months.

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