Hornwort sporophyte induction - Bonn

Eftychis Frangedakis
1University of Cambridge

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Eftychis Frangedakis
University of Cambridge, Plant Sciences

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MATERIALS TEXT

**G1910 - Sigma**
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**Gelzan™**
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**CM**
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**Synonym: Agar substitute gelling agent, Gellan Gum**

**BCD medium**

**Stock B (1L)**
25g MgSO$_4$ x 7H$_2$O

**Stock C (1L)**
25g KH$_2$PO$_4$
pH to 6.5 with KOH

**Stock D (1L)**
101g KNO$_3$
1.25g FeSO$_4$ x 7H$_2$O

**Stock CaCl$_2$ (1L)**
14.7g CaCl$_2$ x 2H$_2$O

**Trace element solution (1L)**
55mg CuSO$_4$ x 5H$_2$O
614mg H$_3$BO$_3$
55mg CoCl x 6H$_2$O
25mg NaMoO$_4$ x 2H$_2$O
55mg ZnSO$_4$ x 7H$_2$O
389mg MnCl$_2$ x 4H$_2$O
28mg KI

**BCD working solution (1L)**
10ml Stock B
10ml Stock C
10ml Stock D
10ml Stock CaCl$_2$
1ml Trace element solution
[for plate: 8g agar]

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1. Grow small thallus fragments for two weeks in petri dishes on Knop or BCD media at pH 5.7 and containing 0.7% (w/v) Gelzan (A in figure).

2. Transfer plants to Magenta pots on Knop medium or BCD medium (B and C in figure) at pH 5.7, containing 0.7% (w/v) Gelzan. Add 2mL of sterile water into the pot using a pipette.

3. Place pots in a Panasonic MLR-352 Versatile Environmental Test Chamber (or similar growth chamber / tissue culture room) at 21°C, 12 h of light and 12 h of dark, 1500 lux light intensity.

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4 After approximately one month, antheridia start to appear. Add another 1-2 mL of sterile water into the pot using a pipette (D in figure).

5 After one more month sporophytes emerge (E in figure).