

# GROWING MOSS FROM SPORES

Prepare

- Sterile pairs of forceps
  - Growth media plates (see below) with cellophane discs on top.
1. Harvest sporogons under sterile conditions with a pair of forceps. They should look swollen and brown, and should come off easily as you pull. Handle with care as they may be ready to burst. Transfer to 900  $\mu$ l sterile dH<sub>2</sub>O.
  2. Add 100  $\mu$ l sodium hypochlorite, and incubate for 5 min. Invert to mix at ~1 min intervals.
  3. Wash the sporogons with 1 ml sterile dH<sub>2</sub>O 3-4 times. (At each wash add fresh dH<sub>2</sub>O and invert the tube several times. As the sporogons sink to the bottom, remove the liquid).
  4. Take out one sporogon by sucking it gently in a blue pipette tip and put it in a tube containing 200  $\mu$ l sterile dH<sub>2</sub>O. Squash it against the side with the same tip to burst it.
  5. Add 800  $\mu$ l sterile dH<sub>2</sub>O and mix well by pipetting up and down.
  6. Add 200  $\mu$ l suspension to each plate, and spread it out by adding 400-1000  $\mu$ l dH<sub>2</sub>O to it.
  7. Incubate at 25 °C in continuous light.

## Growth media for spore germination

5 ml solution B  
5 ml solution C  
5 ml solution D  
5 ml 500mM ammonium tartrate  
4g agar  
dH<sub>2</sub>O to make up to 490 ml  
Autoclave  
Add 10 ml sterile 500mM CaCl<sub>2</sub>  
Mix and pour plates.

## Stock solution B:

MgSO<sub>4</sub>.7H<sub>2</sub>O (magnesium sulphate 7-hydrate)      2.5 g  
(or 1.2 g of anhydrous MgSO<sub>4</sub>)

dH<sub>2</sub>O to 100 ml

Make several 2.5 ml aliquots and store these and any remaining solution at -20°C.

**Stock solution C:**

KH<sub>2</sub>PO<sub>4</sub> (potassium phosphate) 2.5 g

dH<sub>2</sub>O to 50 ml

Adjust pH to 6.5 with minimal volume of 4 M KOH, then make up to 100 ml with additional dH<sub>2</sub>O. Make 2.5 ml aliquots (as above) and store at -20°C.

**Stock solution D:**

KNO<sub>3</sub> (potassium nitrate) 10.1 g

FeSO<sub>4</sub>.7H<sub>2</sub>O (iron sulphate 7-hydrate) 0.125 g

dH<sub>2</sub>O to 100 ml

Make aliquots and store at -20°C (as above).