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 µl sterile dH₂O.

2. Add 100 µl sodium hypochlorite, and incubate for 5 min. Invert to mix at ~1 min intervals.

3. Wash the sporogons with 1 ml sterile dH₂O 3-4 times. (At each wash add fresh dH₂O 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 µl sterile dH₂O. Squash it against the side with the same tip to burst it.

5. Add 800 µl sterile dH₂O and mix well by pipetting up and down.

6. Add 200 µl suspension to each plate, and spread it out by adding 400-1000 µl dH₂O to it.


Growth media for spore germination
5 ml solution B
5 ml solution C
5 ml solution D
5 ml 500mM ammonium tartrate
4g agar
dH₂O to make up to 490 ml
Autoclave
Add 10 ml sterile 500mM CaCl₂
Mix and pour plates.

Stock solution B:
MgSO₄.7H₂O (magnesium sulphate 7-hydrate) 2.5 g
(or 1.2 g of anhydrous MgSO₄)
dH₂O           to 100 ml
Make several 2.5 ml aliquots and store these and any remaining solution at -20°C.

Stock solution C:
KH₂PO₄ (potassium phosphate)  2.5 g
dH₂O           to 50 ml
Adjust pH to 6.5 with minimal volume of 4 M KOH, then make up to 100 ml with
additional dH₂O. Make 2.5 ml aliquots (as above) and store at -20°C.

Stock solution D:
KNO₃ (potassium nitrate)  10.1 g
FeSO₄.7H₂O (iron sulphate 7-hydrate)  0.125 g
dH₂O           to 100 ml
Make aliquots and store at -20°C (as above).