CULTURING MOSS

(Based on Essential Moss Methods, from the University of Leeds).
The following should all be carried out under sterile conditions.

Cellophane overlay plates:
These are 90 mm plates of moss media, covered with a cellophane disc to stop the moss growing down into the media. They are a source of protonemal tissue, used for various things including DNA and RNA extractions, and making protoplasts to use in transformations. The cellophane discs used are 80 mm diameter and are made from Type 325P cellophane, obtainable from Cannings Packaging Ltd., Unit A, Nova Distribution Centre, Nova Way, Avonmouth, Bristol BS11 9DZ, U.K. Email: canningspackaging@compuserve.com ; Phone: 0117 982 4861; Fax: 0117 982 3810. Unfortunately, the minimum order that Cannings will do is 25,000 discs, costing about £8.80 per 1000. So find out if there are any other options before ordering them!

1. Interleave cellophane discs (80 mm; see below) with filter paper discs (the filter paper can be reused). Place in a glass petri dish, wrap in foil and autoclave.

2. Using sterilised tweezers, place a cellophane disc on each 90 mm plate of moss media that will be used. They will crinkle up as soon as they come into contact with the agar, but be patient, they should flatten out a bit eventually. Try not to handle them too much however, as this increases the likelihood of contamination. The technicians in Leeds recommended using freshly poured plates and freshly autoclaved cellophane discs, and said to leave the discs on the plates for 5-10 minutes, to give them a chance to flatten out a bit.

3. Pipette 2 ml of sterile dH_2O into a blending tube (we use cut-off 50 ml Corning tubes).

4. Using a sterile scalpel or metal spatula, scrape the moss from a 7 day old cellophane overlay plate and place in the blending tube with the water. Blend the moss for about 1 minute (we use an electric drill with a modified mini-whisk instead of the drill bit). It may be necessary to add another 1 ml of water if you have a lot of tissue.

5. Add more water to the blended moss to make the volume enough for about 2 ml per plate to be used. This will vary depending on how much moss was on your original overlay plate. A good culture (with nearly the whole plate covered) should be enough to make 4-5 new cultures.

6. Pipette about 2 ml of the blended moss onto each plate + cellophane disc. Spread the moss by gently swirling the plate. To reduce the chance of contamination, put Micropore tape around the plate.

7. Place the cultures in the 25°C growth room. (According to the Leeds protocol, the lighting conditions are not critical – they suggest continuous light from fluorescent tubes at an intensity of 5-20 Wm^{-2}, with a light cycle of 16 hours light and 8 hours dark).

Bottle stocks:
These are a way of storing cultures from cellophane overlay plates, if they are not needed for immediate use. Leeds say that they remain viable for at least 6 months, but ours do not seem to keep for longer than a month.

1. Autoclave small glass bottles containing 5 ml of dH_2O.

2. Scrape the moss from a 7 day old cellophane overlay plate (using a sterile scalpel or metal spatula) and place it in one of the small bottles of water.

3. Wrap the bottle in foil, so that the moss is kept in the dark. Store at 4°C.

Small stock plates:
These are a simple way of maintaining a healthy stock of moss, which can be used as back up if all the other cultures go wrong. It should also be possible embed samples from these plates, to use in histological studies.

1. Use small deep petri dishes, that are about 2/3 full with moss media.

2. Using sterile tweezers, take a tiny amount (about 2 mm^2 is sufficient) of protonemal tissue (e.g. from a 7 day old cellophane overlay plate, or from a mature small stock plate) and place this on the media. This can be repeated 4 or 5 times for each small plate of media (i.e. 4 or 5 moss cultures on one plate).

3. Bind the petri dish with Micropore tape and place in the 25°C growth room (as for the cellophane overlay plates).

4. Once the cultures have matured (i.e. have produced numerous gametophytes), replace the Micropore tape with parafilm and transfer the cultures to the 19°C growth room. They should then stay viable for several months.