

## 2. Cultivation

### 2.3 Induction of gametangia (archegonia and antheridia) and sporophytes (originally written by Tomoaki Nishiyama, and edited by Mitsuyasu Hasebe: last updated 25 Nov., 2004. Photos of sporophyte development were took by Keiko Sakakibara)

Gametangia are induced at high frequency at 15C under short day conditions (8 hours light and 16 hours dark) as described in Hohe et al. (2002).

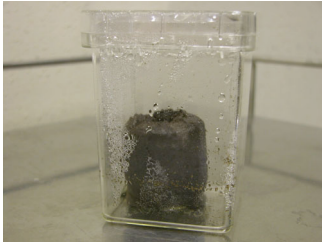
Hohe, A., S. A. Rensing, M. Mildner, D. Lang, and R. Reski. 2002. Day length and temperature strongly influence sexual reproduction and expression of a novel MADS-box gene in the moss *Physcomitrella patens*. *Plant Biology* 4:595-602.

0. Use Jiffy7 (peat moss pot: Jiffy Products International AS, Kristansand, Norway) to grow healthy gametophores. There are 42 and 30 mm diameter peat pellets. Use 42 mm for regular culture. For crossing, put two 30 mm peat pots in a plastic box.



1. Put dry Jiffy in a plastic box, and add water to immense dry Jiffy. Keep about 2 hours.

2. Remove excess water (otherwise, peat pellet will be collapsed by autoclaving), then autoclave the wet Jiffy in a plastic box.



Wet Jiffy in a plastic box after autoclaving

3. Add distilled water until 1 cm below the upper surface of the pot.
4. Inoculate protonemata blended by polytron or motor and pestil.
5. Cultivate 1 to 2 months (until gametophores with more than 10 leaves) at 25C under long day conditions (16 hours light and 8 hours dark) or under continuous light.



about a month cultivated at 25C with continuous light after inoculation.

6. Move to 15C under short day conditions (8hours light and 16 hours dark). After two weeks, start to observe the gametophore apex, where matured archegonia with opening are formed. If the canal turns brown, it is likely fertilized. Nothing to do is necessary for fertilization in this method, and just wait. Usually a single sporophyte grows at each apex. After a month, you can see bunch of sporophytes.

Development of sporophytes (took by Keiko Sakakibara)

