

## Induction of sporophyte development

### Overview

Beside the vegetative propagation of *Physcomitrella*, sexual reproduction can be induced under particular cell culture conditions. Engel (1968) had already reported the influence of temperature on sexual reproduction of *Physcomitrella*. Sporophyte formation was found to be induced at 15-19°C whereas no sporophytes developed at 20-23°C. Based on these results the influence of the light intensity, light/dark period and temperature on the development of gametangia and sporophytes was further investigated (Hohe et al., 2002). The highest numbers of spores were obtained upon culture of *Physcomitrella* plants at 15°C, 8 h light with an intensity of 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The spores can be harvested and kept for long-term storage or they can immediately be plated on solidified medium to initiate a new line. The initiation of new *Physcomitrella* lines may be necessary when decreasing qualities of used lines are observed (e.g. reduced protoplast yield).

### Method

To induce sporophyte development gametophores are transferred onto Knop medium supplemented with 200 mg/l glucose and grown in 15°C, a light intensity of 20  $\mu\text{mol/m}^2$  per second and a 8/16 h (light/dark) regime for gametangia induction. After four to six weeks the dishes are flooded with 10 ml autoclaved tap water and cultivated for another six to eight weeks under the same growth conditions for development of mature spore capsules. These short day conditions were found to be most effective to promote the sporophyte development. The sporophytes are harvested and the spores can be released from the spore capsules by mechanical disruption of the capsules. For this, transfer the sporophytes into liquid Knop medium and disrupt the spore capsules using tweezers or a scalpel. To induce germination of the spores aliquots of the suspension are transferred onto solidified Knop medium and grown under normal growth conditions. For long-term storage up to several years the spores are dried under sterile conditions and kept dry at 4°C in the dark. To induce germination they are resuspended in liquid Knop medium and plated as described above.

## Reference

Hohe, A., Rensing, S. A., Mildner, M., Lang, D., and Reski, R. (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.