Please cite this article as: Xiaoqin and Yikun, (2015). Tissue Culturing and Harvesting of Protonemata from the Moss *Physcomitrella patens*, *Bio-protocol* 5 (15): e1556. DOI: 10.21769/BioProtoc.1556.

Tissue Culturing and Harvesting of Protonemata from the Moss *Physcomitrella patens*

Xiaoqin Wang[1, 2*] and Yikun He[1*]

[1]Beijing University of Agriculture, Beijing, China; [2]College of Life Sciences, Capital Normal University, Beijing, China

For correspondence: wxqtycn@gmail.com; yhe@cnu.edu.cn

[Abstract] Moss spores germinate to form an alga-like filamentous structure called the protonemata. Protonemata are the earliest stage (the haploid phase) of a bryophyte life cycle and eventually give rise to a mature gametophyte. Protonemata of the moss *Physcomitrella patens* (*P. patens*) are important not only in their life cycle, but also for research. Protonemata are used for various things such as RNA/DNA extractions and protoplast isolation. We can obtain high yield of intact protoplasts from protonemata. Protoplasts can be used to study a variety of cellular processes, such as subcellular localization of proteins, isolation and analyses of intact organelles and DNA transformation. In addition, the completed sequence of the *P. patens* genome facilitates the use of genetic and molecular approaches to identify genes and the ability of the moss to undergo homologous recombination at appreciable frequency offers a powerful way to determine gene function. Therefore, culture of *P. patens* protonemata is critical.

Materials and Reagents

1. A vigorously growing tissue which is about 10 d old (the earliest stage of gametophyte; *Physcomitrella patens* subspecies patens (Gransden) was used as the tissue and it was obtained from Ralph S. Quatrano (Department of Biology, Washington University in St. Louis, MO 63130, USA)
2. 200 ml sterile distilled water
3. 70% alcohol in a spray bottle (for surface sterilization)
4. Growth medium (see Recipes)

Equipment

1. 1 L flask in which to prepare the growth medium
2. Sterile petri dishes (90-mm)
3. Sterile cellophane discs
4. Sterile tweezers
5. Sterile test tubes (25 x 150 mm)
6. Micropore surgical tape
7. Sterile pipettes (1 ml)

Please cite this article as: Xiaoqin and Yikun, (2015). Tissue Culturing and Harvesting of Protonemata from the Moss *Physcomitrella patens*, *Bio-protocol* 5 (15): e1556. DOI: 10.21769/BioProtoc.1556.

8. Sterile tips (1 ml)
9. Dispensing instrument (*e.g.*, IKA T 10 basic ULTRA-TURRAX®)
10. Laminar flow cabinet
11. Autoclave (*e.g.,* Sanyo, model: MLS-3780)

Procedure

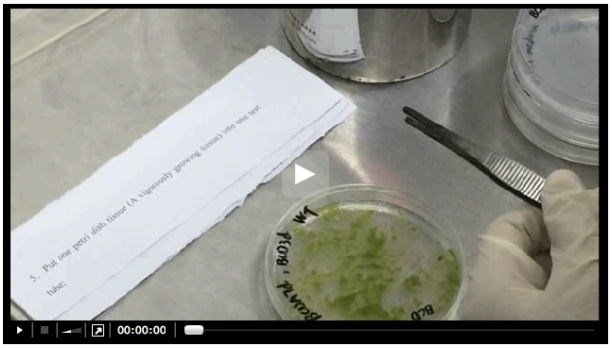
Note: Steps 2-12 should be carried out under sterile conditions.

1. BCDA medium is prepared according to Table 1 and sterilized by an autoclave for 20 min at 121 °C;
2. Surface sterilization is performed with 70% alcohol in the Laminar flow hood;
3. Media was cooled to 60 °C and approximately 30 ml BCDA medium was poured into sterile petri dishes (90 mm). This was allowed to cool until the media solidified; Place a piece of sterile cellophane discs onto the BCDA medium (Video 1);

Video 1. Place a piece of sterile cellophane discs onto the BCDA medium

4. Place one petri dish tissue (vigorously growing tissue) into one test tube (Video 2);

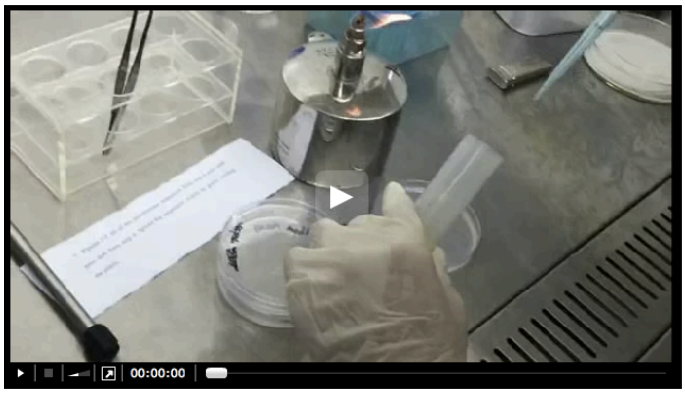
Video 2. Place one petri dish tissue into one test tube



5. Add 6-8 ml of H_2O to one sterile test tube (containing one petri dish tissue), and cut it into fragments using dispensing instrument for 1-2 min at a speed of about 15,000 rpm;
6. Pipette 1-2 ml of the protonemata suspension from step 6 onto each petri dish from step 4. Spread the suspension evenly by gently swirling the plates (Video 3);

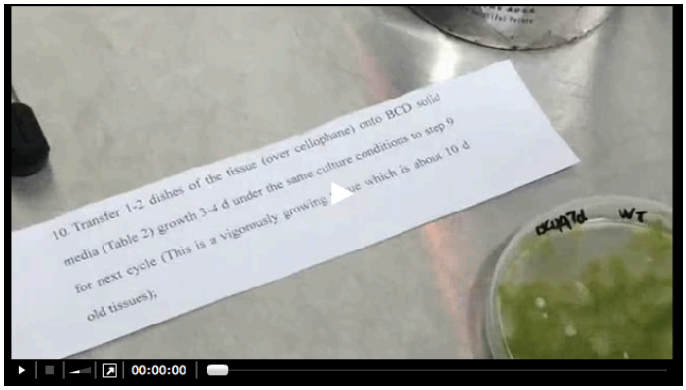
Please cite this article as: Xiaoqin and Yikun, (2015). Tissue Culturing and Harvesting of Protonemata from the Moss *Physcomitrella patens*, *Bio-protocol* 5 (15): e1556. DOI: 10.21769/BioProtoc.1556.

Video 3. Pipette 1-2 ml of the protonemata suspension from step 6 onto each petri dish from step 4. Spread the suspension evenly by gently swirling the plates



7. Seal the Petri dishes with micropore surgical tape;
8. Incubate these fragments for 6-7 d under standard conditions (25 °C) with a light cycle of 16 h of light/8 h of darkness and a light intensity of 70-80 μmol/s/m^2;
9. Transfer 1-2 dishes of the tissue (over cellophane) onto BCD solid media (Table 2) growth 3-4 d under the same culture conditions to step 9 for next cycle (this is a vigorously growing tissue which is about 10 d old tissues; Video 4);

Video 4. Transfer 1-2 dishes of the tissue onto BCD solid media growth 3-4 d under the same culture conditions to step 9 for next cycle



10. Harvest the tissue (protonemata of *P. patens* from step 9) for downstream analysis by scraping them from the cellophane using sterile tweezers.
11. For tissue stock, take a tiny amount (about 2 mm^2; using sterile tweezers) of 10 d old tissues into sterile test tubes with BCD medium. Wrap the tubes in foil and store them at 4 °C. They remain viable for about six months.

Please cite this article as: Xiaoqin and Yikun, (2015). Tissue Culturing and Harvesting of Protonemata from the Moss *Physcomitrella patens*, *Bio-protocol* 5 (15): e1556. DOI: 10.21769/BioProtoc.1556.

Recipes

1. Growth medium

Table 1. BCDA medium recipe

Reagent	Quantity (for 1 L)	Final concentration
Solution B	10 ml	1 mM $MgSO_4$
Solution C	10 ml	1.84 mM KH_2PO_4
Solution D	10 ml	10 mM KNO_3
$CaCl_2$	111 mg	1 mM
$FeSO_4 \cdot 7H_2O$	12.5 mg	45 μM
$(NH_4)_2C_4H_4O_6$	0.92 g	5 mM
Agar	7.5 g	0.75% (w/v)
Glucose	5 g	0.5% (w/v)
Hoagland's A-Z trace	1 ml	Trace element solution
H_2O	To 1 L	

Table 2. BCD medium recipe

Reagent	Quantity (for 1 L)	Final concentration
Solution B	10 ml	1 mM $MgSO_4$
Solution C	10 ml	1.84 mM KH_2PO_4
Solution D	10 ml	10 mM KNO_3
$CaCl_2$	111 mg	1 mM
$FeSO_4 \cdot 7H_2O$	12.5 mg	45 μM
Agar	7.5 g	0.75% (w/v)
Glucose	5 g	0.5% (w/v)
Hoagland's A-Z trace	1 ml	Trace element solution
H_2O	To 1 L	

Please cite this article as: Xiaoqin and Yikun, (2015). Tissue Culturing and Harvesting of Protonemata from the Moss *Physcomitrella patens*, *Bio-protocol* 5 (15): e1556. DOI: 10.21769/BioProtoc.1556.

Table 3. Recipe of Solution B, C, D and Hoagland's A-Z trace

	Reagent	Quantity (for 1 L)	Final concentration
Solution B	$MgSO_4 \cdot 7H_2O$	25 g	0.1 M
	H_2O	To 1 L	
Solution C	KH_2PO_4	25 g	184 mM
	H_2O	To 1 L	Adjust the pH to 6.5 using KOH
Solution D	KNO_3	101 g	1 M
	H_2O	To 1 L	
Hoagland's A-Z trace	$Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$	55 mg	0.006% (w/v)
	$CoCl_2 \cdot 6H_2O$	55 mg	0.006% (w/v)
	$CuSO_4 \cdot 5H_2O$	55 mg	0.006% (w/v)
	H_3BO_3	614 mg	0.061% (w/v)
	KBr	28 mg	0.003% (w/v)
	KI	28 mg	0.003% (w/v)
	LiCl	28 mg	0.003% (w/v)
	$MnCl_2 \cdot 4H_2O$	389 mg	0.039% (w/v)
	$SnCl_2 \cdot 2H_2O$	28 mg	0.003% (w/v)
	$ZnSO_4 \cdot 7H_2O$	55 mg	0.006% (w/v)
	H_2O	To 1 L	

Solution B, C, D and Hoagland's A-Z trace are sterilized and then stored at 4 °C or room temperature.

Acknowledgments

This work was supported by grants from Beijing Natural Science Foundation (No. 5132004), China Postdoctoral Science Foundation and State Education Ministry Scientific Research Foundation for the Returned Overseas Chinese Scholars to Dr. Wang.

References

1. Ashton, N. W., and Cove, D. J. (1977). The isolation and preliminary characterisation of auxotrophic and analogue resistant mutants in the moss *Physcomitrella patens*. *Mol Gen Genet* 154, 87-95.
2. Boyd, P. J., Hall, J., and Cove, D. J. (1988). An airlift fermenter for the culture of the moss *Physcomitrella patens*. In: Glime, J. M. (ed). *Methods in bryology.* Hattori Botany Laboratory, 41-45.
3. Cove, D. J., Perroud, P. F., Charron, A. J., McDaniel, S. F., Khandelwal, A. and Quatrano, R. S. (2009). The moss *Physcomitrella patens*: a novel model system for

Please cite this article as: Xiaoqin and Yikun, (2015). Tissue Culturing and Harvesting of Protonemata from the Moss *Physcomitrella patens*, *Bio-protocol* 5 (15): e1556. DOI: 10.21769/BioProtoc.1556.

plant development and genomic studies. *Cold Spring Harb Protoc* 2009(2): pdb emo115.