Observation of mitosis in a protonemal cell of Physcomitorella

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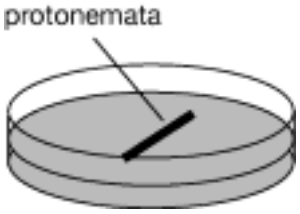
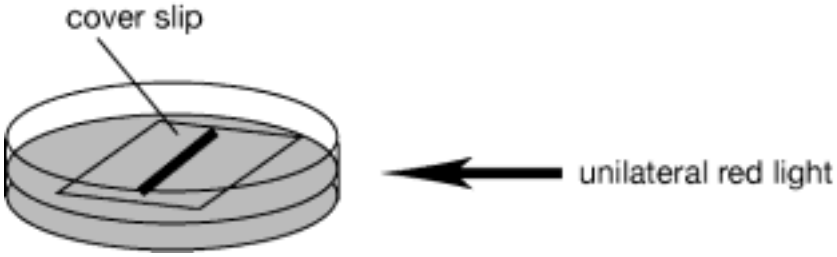
This protocol gives you the opportunity to see cell divisions of protonemal apical cells of Physcomitrella.

Pre-culture

1. Sub-culture protonemal cells on cellophane-overlaid BCDATG* plates under continuous white light (30-40 µmol m-2 sec-1) at 25˚C every 5~7 days.
2. Inoculate ~7-day-old protonemal cells into BCDATG plates (3 cm-diameter dish) and cover protonemal cells with a sterile cover slip (18 x18 mm).



3. Incubate the plates under unilateral red light (15-20 µmol m-2 sec-1) at 25˚C for 7 days. Red light can be obtained from fluorescent tube filtered through a red plastic sheet (We use acrylite 102; Mitsubishi Rayon, Japan, http://www.mrc.co.jp/acrylite/acryindex.html)



***BCDATG**

1 stock solutions

solution A (x 100)

Ca(NO3)2·4H2O	118 g
FeSO4·7H2O	1.25 g

H2O	fill up to 1000 ml

SolutionB (x 100)

MgSO4·7H2O	25 g
H2O	fill up to 1000 ml

Autoclaved

SolutionC (x 100)

KH2PO4	25 g
	adjust pH to 6.5 with 4 M KOH
H2O	fill up to 1000 ml

Autoclaved

Solution D (x 100)

KNO3	101 g
FeSO4·7H2O	1.25 g
H2O	fill up to 1000 ml

Alternative TES (x 1000)

CuSO4·5H2O	55 mg
H3BO3	614 mg
CoCl2·6H2O	55 mg
Na2MoO4·2H2O	25 mg
ZnSO4·7H2O	55 mg
MnCl2·4H2O	389 mg
KI	28 mg
H2O	fill up to 1000 ml

Autoclaved

500mM Ammonium Tartrate (x 100)

Ammonium Tartrate	92.05 g

H2O	fill up to 1000 ml

Autoclaved

50mM CaCl2 (x 50)

CaCl2·2H2O	7.35 g
H2O	fill up to 1000 ml

Autoclaved

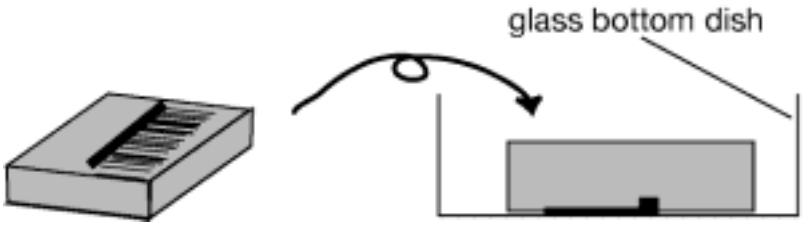
2.BCDATG

H2O	900 ml
Stock B	10 ml
Stock C	10 ml
Stock D	10 ml
Alternative TES	1 ml
500mM Ammonium Tartrate	10ml (= 5 mM)
50mM CaCl2・2H2O (powder)	20 ml (= 1 mM) (0.15 g)
Glucose	5 g
Agar (Sigma, A6924)	8 g (= 0.8%)
	Fill up to 1000 mL with H2O

Autoclaved

Time-lapse observation

1. Remove a cover slip from the plate and cut an agar block containing protonemal cell from the solid medium with a scalpel.
2. Turn the block up-side down and place it into 35 mm glass-bottom dish (IWAKI 3910-039: a 27 mm diameter opening in the center of a dish, http://www.atgc.co.jp/div/rika/hbine/index_e.html) as protonemal cell are touched on the bottom of the dish. Seal the dish with parafilm.



3. Place the dish on a stage of an inverted microscope. While you observe, room temperature should be set at 25˚C.
4. Seek protonemal apical cell just before cell division and focus a nucleus. An apical cell before division is highly elongated and its cytoplasm is localized to apical side of a cell. One of the signs of mitosis is transition of a nuclear shape. Just before entering prophase, a nucleus will be a spherical shape rather than an oval shape.
5. Carry out the time-lapse observation. Mitosis will finish ~30 min in this condition. I usually acquire an image every 60 sec. If you examine the dynamics of GFP-fusion protein, you should not illuminate strong emission light to the cell because strong light not only fades GFP fluorescence but also inhibit cell division.