Protocol

Transformation of the Moss *Physcomitrella patens* Using Direct DNA Uptake by Protoplasts

David J. Cove, Pierre-François Perroud, Audra J. Charron, Stuart F. McDaniel, Abha Khandelwal, and Ralph S. Quatrano[1]

Department of Biology, Washington University, St. Louis, MO 63130, USA

INTRODUCTION

This protocol describes how to transform moss (*Physcomitrella patens*) protoplasts using polyethylene glycol (PEG)-mediated DNA uptake. The transformation rates for direct uptake by protoplasts of DNA with and without genomic sequence (a targeting construct) are typically 10^{-5} and 10^{-3}, respectively. (These are the frequencies of stable transformants among regenerants surviving the transformation procedure.)

RELATED INFORMATION

For more information about *P. patens* as a model organism, see **The Moss *Physcomitrella patens*: A Novel Model System for Plant Development and Genomic Studies** (Cove et al. 2009a). A method for isolating protoplasts can be found in **Isolation and Regeneration of Protoplasts of the Moss *Physcomitrella patens*** (Cove et al. 2009b). For details on the use of a hemocytometer to estimate protoplast density, see **Estimation of Cell Number by Hemocytometry Counting** (Sambrook and Russell 2006).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <**!**>, and recipes for reagents marked with <**R**>.

Reagents

<**R**>BCD medium (solid) containing common moss media supplements and antibiotics as necessary

D-Mannitol (8.5%, w/v)

DNA to be used in transformation

Use 15-30 µg of DNA in a volume no greater than 30 µL.

<**R**>MMM solution

Prepare 10 mL of MMM solution; this is sufficient for 10-15 transformations.

<**R**>PEG solution for transformation (PEG/T)

[1]Corresponding author (rsq@wustl.edu)

This article is also available in *Emerging Model Organisms: A Laboratory Manual*, Vol. 1. CSHL Press, Cold Spring Harbor, NY, USA, 2009.

Cite as: Cold Spring Harb. Protoc.; 2009; doi:10.1101/pdb.prot5143

<R>Protoplast regeneration medium for the bottom layer (PRMB)

For each transformation, prepare three 90-mm Petri plates containing PRMB and overlay with sterile cellophane.

<R>Protoplast regeneration medium for the top layer (PRMT)

This medium should be melted and kept at 45°C in a water bath.

Protoplasts, isolated as described in Steps 1-8 of **Isolation and Regeneration of Protoplasts of the Moss *Physcomitrella patens*** (Cove et al. 2009b)

Equipment

Centrifuge
Hemocytometer
Incubator preset to 25°C, with white light at intensities between 5 and 20 W/m^2 (e.g., Percival Scientific Model CU-36L5)
Pipettes
Tubes (10 mL, sterile)
Water bath preset to 45°C

METHOD

Except where stated otherwise, perform all procedures at room temperature, providing this is between 20°C and 25°C. Otherwise, use a water bath. The ten-step dilution described in Steps 8-11 maximizes the recovery rate; however, the dilution can be made in fewer steps if maximum recovery is not required.

1. Resuspend the protoplasts in 8.5% (w/v) D-mannitol solution. Use 2.5 mL for each plate of tissue digested.
2. Use a hemocytometer to estimate protoplast density (see **Estimation of Cell Number by Hemocytometry Counting** [Sambrook and Russell 2006]).
3. Centrifuge the protoplasts at 300*g* for 5 min at room temperature and discard the supernatant. Resuspend the protoplasts in MMM solution at a concentration of 1.6×10^6 protoplasts/mL.
4. Prepare the DNA to be used in the transformation by dispensing 15-30 µg of DNA into a sterile 14-mL tube. Centrifuge briefly to bring the DNA to the bottom of the tube.
5. Add 300 µL of protoplast suspension from Step 3 and 300 µL of PEG/T solution to the DNA from Step 4. Mix by tapping the tube gently.
6. Heat the mixture in a water bath for 5 min at 45°C.
7. Transfer the tube to room temperature (20°C) and leave it for 5 min.
8. Add 300 µL of 8.5% D-mannitol solution. Gently invert the tube to mix. Wait at least 1 min.
9. Repeat Step 8 four times.
10. Add 1 mL of 8.5% D-mannitol solution. Invert gently to mix. Wait at least 1 min.
11. Repeat Step 10 four times.
12. Centrifuge at 100-200*g* for 5 min at room temperature.
13. Discard the supernatant and resuspend the pellet in 500 µL of 8.5% D-mannitol solution.
14. Add 2.5 mL of molten PRMT. Dispense 1 mL of the mixture to each of three 90-mm plates containing PRMB and overlaid with cellophane.
15. Incubate the plates in continuous white light for 5 d at 25°C.
16. Transfer cellophane and regenerating protoplasts to BCD medium containing the appropriate antibiotic to select for transformants.

REFERENCES

Cove, D.J., Perroud, P.-F., Charron, A.J., McDaniel, S.F., Khandelwal, A., and Quatrano, R.S. 2009a. The moss *Physcomitrella patens*: A novel model system for plant development and genomic studies. *Cold Spring Harb. Protoc.* (this issue). doi: 10.1101/pdb.emo115.

Cove, D.J., Perroud, P.-F., Charron, A.J., McDaniel, S.F., Khandelwal, A., and Quatrano, R.S. 2009b. Isolation and regeneration of protoplasts of the moss *Physcomitrella patens*. *Cold Spring Harb. Protoc.* (this issue). doi: 10.1101/pdb.prot5140.

Sambrook, J. and Russell, D.W. 2006. Estimation of cell number by hemocytometry counting. *Cold Spring Harb. Protoc.* doi: 10.1101/pdb.prot4454.