Chemical and UV Mutagenesis of Spores and Protonemal Tissue from the Moss *Physcomitrella patens*

David J. Cove, Pierre-François Perroud, Audra J. Charron, Stuart F. McDaniels, Abha Khandelwal, and Ralph S. Quatrano

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

**INTRODUCTION**

This protocol describes how to mutagenize spores and protonemal tissue from moss (*Physcomitrella patens*) using chemicals or ultraviolet (UV) light. Spores are mutagenized using the alkylating agents *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) and ethyl methanesulfonate (EMS), and protonemal tissue is mutagenized with NTG and UV light. Compared to alkylating agents, UV is less effective as a mutagen, but it may be advantageous because it is less hazardous and may not lead to clustered lesions.

**RELATED INFORMATION**

The chemical mutagenesis protocols were adapted from those described by Ashton and Cove (1977) and Boyd et al. (1988). 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). Standard procedures for cultivating *P. patens* are described in *Culturing the Moss Physcomitrella patens* (Cove et al. 2009b), and a method for isolating protoplasts after mutagenesis can be found in *Isolation and Regeneration of Protoplasts of the Moss Physcomitrella patens* (Cove et al. 2009c).

**MATERIALS**

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

**Reagents**

- <!> BCD medium (solid) containing common moss media supplements as necessary in 90-mm Petri dishes
- <!> EMS (ethyl methane sulfonate) (for Procedure I, “Using EMS”)
- Moss (*P. patens*) spores (see Steps 26-41 of *Culturing the Moss Physcomitrella patens* [Cove et al. 2009b]) or protonemal tissue on a cellophane overlay plate (see Steps 4-9 of *Culturing the Moss Physcomitrella patens* [Cove et al. 2009b])
- <!> NTG (*N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine) (for Procedures I and II, “Using NTG”)
- <!> Orthophosphoric acid (H₃PO₄; 100 mM, pH 7.0; sterile) (for Procedure I, “Using EMS”)
- Sodium thiosulfate (Na₂S₂O₅; 400 mM, sterile) (for Procedure I, “Using EMS”)

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Perform all chemical mutagenesis procedures in safe handling facilities for mutagens.

**Procedure I. Mutagenesis of Spores**

**Using NTG**

1. Prepare 10 mL of a spore suspension containing ~10^6 spores in sterile Tris-maleate buffer. Incubate the mixture for 30 min at 25°C.

2. Observing strict safety procedures, carefully add 1.2 mg of NTG to a separate tube containing 10 mL of Tris-maleate buffer. Incubate the tube for 30 min at 25°C.

3. Mix the spore suspension with the NTG solution, and shake it gently for an additional 30 min at 25°C.

4. Terminate the treatment by sedimenting the spores by centrifugation (100g for 4 min at room temperature), and dispose of the supernatant safely.

   *Usually ~10% of the originally viable spores survive this treatment.*

5. Wash the spores three times as follows:
   
   i. Add 10 mL of H_2O to the tube.
   
   ii. Centrifuge the tube at 100g for 4 min at room temperature.
   
   iii. Discard the supernatant.

6. After the final wash, resuspend the spores in H_2O to give ~10^4 spores/mL. Spread 1 mL of spore solution onto the surface of appropriately supplemented BCD medium in 90-mm Petri dishes.

7. Incubate the plate under the desired conditions. For standard cultivating procedures and conditions, see *Culturing the Moss Physcomitrella patens* (Cove et al. 2009b).

   *The mutagenic treatment results in an initial delay in development, but once germinated, the sporelings grow as usual. This should result in ~100 developing sporelings per dish.*

**Using EMS**

8. Prepare 9.5 mL of a spore suspension containing ~10^4 spores in sterile 100 mM orthophosphoric acid (pH 7.0).

9. Observing strict safety procedures, add 500 µL of EMS.

10. Incubate the mixture for 45 min at 25°C.

11. Terminate the treatment by adding 10 mL of sterile 400 mM sodium thiosulfate.
12. Sediment the spores by centrifugation (100g for 4 min at room temperature), and dispose of the supernatant safely. Usually 35%-40% of the originally viable spores survive this treatment.

13. Wash the spores three times as follows:
   i. Add 10 mL of H2O to the tube.
   ii. Centrifuge the tube at 100g for 4 min at room temperature.
   iii. Discard the supernatant.

14. After the final wash, resuspend the spores in H2O to give ~3 × 10^3 spores/mL. Spread 1 mL of spore solution onto the surface of appropriately supplemented BCD medium in 90-mm Petri dishes.

15. Incubate the plate under the desired conditions. For standard cultivating procedures and conditions, see Culturing the Moss Physcomitrella patens (Cove et al. 2009b). The mutagenic treatment results in an initial delay in development, but once germinated, the sporelings grow as usual. This should result in ~100 developing sporelings per dish. The mutagenized spore suspension may be stored for at least 1 yr at 4°C.

Procedure II. Mutagenesis of Protonemal Tissue

Using NTG

16. Add 1 g (fresh weight) of young protonemal tissue from cellophane-overlay plates to 10 mL of sterile Tris-maleate buffer. Incubate the mixture for 30 min at 25°C.

17. Observing strict safety procedures, carefully add 1.2 mg of NTG to a separate tube containing 10 mL of Tris-maleate buffer. Incubate the tube for 30 min at 25°C.

18. Mix the tissue with the NTG solution and shake it gently for an additional 30 min at 25°C.

19. Terminate the treatment by filtering the mixture through a sterile filter with pore size 100 µm. The filter retains the protonemal tissue. This procedure results in a cell survival rate of ~10%.

20. Wash the tissue thoroughly, with at least 100 mL of H2O. If individual cells are required, these may be obtained by protoplasting the tissue as described in Steps 1-8 of Isolation and Regeneration of Protoplasts of the Moss Physcomitrella patens (Cove et al. 2009c).

Using UV Light

21. Remove the lid from a cellophane-overlay plate, and irradiate the protonemal tissue with UV light. The kill level is difficult to determine, but a dose of 2000 J/m^2 should give ~10% survival. It is best to perform several experiments to calibrate the UV source in order to get a feel for the correct exposure. Petri dishes are opaque to UV light, so make sure to remove the lid.

22. Immediately after irradiation, transfer the plate to darkness for at least 24 h to prevent photoreactivation. Following incubation in the dark, the tissue can be protoplasted (see Steps 1-8 of Isolation and Regeneration of Protoplasts of the Moss Physcomitrella patens [Cove et al. 2009c]), but very few protoplasts regenerate following UV irradiation. Yields are better if the tissue is allowed to grow following incubation (see Steps 4-9 of Culturing the Moss Physcomitrella patens [Cove et al. 2009b]), but this may yield mutant clones.

REFERENCES


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*Cold Spring Harb Protoc*; doi: 10.1101/pdb.prot5142

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