

Tatum Norrell

McDaniel Lab, University of Florida

EDEN Undergraduate Internship June–August 2011

## **Spore Germination and Sex Ratio Determination Protocol**

This protocol describes how to take field collected moss sporophytes and estimate spore number, percent germination, and ratio of male to female spores from the individual sporophyte.

### **Materials**

---

#### **Reagents**

Field collected sporophytes, operculum still attached  
20% Bleach solution  
BCDa Medium (Solid) <R>

#### **Equipment**

Forceps  
Microcentrifuge tubes (1.5 mL)  
50 mL beaker  
Lab timer  
Hemocytometer  
Plant growth chamber  
Microspore surgical tape

### **Methods**

---

#### **I. Sporophyte sterilization and spore number approximation**

1. Prepare 25 mL of 20% bleach solution and place in a 50 mL beaker.
2. Prepare three microcentrifuge tubes with 1 mL of water for each sporophyte to be sterilized.
3. Isolate a single sporophyte with a lengthy amount of the seta still attached.

4. Using flame-sterilized forceps, submerge the sporophyte in the bleach solution for 30 seconds.
5. Transfer the sporophyte to the first microcentrifuge tube and submerge it under the water for 30 seconds to rinse off bleach.
6. Repeat step 5 in second tube.
7. Transfer the sporophyte to the third microcentrifuge tube; disrupt the capsule using flame-sterilized forceps to crush the capsule against the microcentrifuge tube wall, releasing the spores into the water.
8. Vortex the suspended spore solution and pipette 10uL onto each side of a hemocytometer.
9. Count the number of spores seen in each side of the 3X3 grid and calculate the average number of spores (total spores counted divided by 18).
10. Multiply the average by 10,000 to get an estimate of the total number of spores in the capsule.

## **II. Germination Percent**

1. Using the spore number estimation and suspended spore solution, create a dilution of the stock solution in a new microcentrifuge tube that has a spore density of approximately 300 spores in a total volume of 100uL
2. Mark the bottom of a BCDa media Perti dish with 9 evenly spaced dots (3x3 grid).
3. Vortex the diluted solution and pipette 10 uL of the solution onto each dot.
4. Allow the plates to grow in an incubator at 25C under continuous light for approximately 3 days.
5. After germination, count the germinated and ungerminated spores from each spot under a stereomicroscope at 100X.

## **III. Sex Ratio Determination**

1. Using the suspended spore solution (part I, step 8), create a dilution using water in a new microcentrifuge tube that has a

spore density of approximately 1000 spores in a total volume of 200uL.

2. Spread this 200uL solution across the surface of a BCDA media Petri dish.
3. Allow plates to grow in an incubator at 25C and continuous light for 4 days.
4. Isolate single germinating spores under a stereomicroscope and transfer to a new BCDA plate. Place in a 4x4 grid, a total of 6 plates (96 individual spores).
5. Allow plants to grow for 3 weeks at 25C and continuous light.
6. Extract genomic DNA from the 96 individuals
7. Amplify sex-linked region AW098442.loc:86 with primers: AGCTCTGCACTGGGCTGG, CCCGTGGAACCGTGAAAG .
8. Digest PCR product using SbfI restriction enzyme.
9. Visualize banding pattern using gel electrophoresis to determine sex - males have one cut site, females have none.

## Recipes

### BCDa Medium

Reagent	Quantity (for 1L)	Final Concentration
Agar	7 g	0.7% (w/v)
CaCl <sub>2</sub>	111mg	1 mM
FeSO <sub>4</sub> *7H <sub>2</sub> O	12.5 mg	45 uM
Solution B	10 mL	1mM MgSO <sub>4</sub>
Solution C	10 mL	1.84 mM KH <sub>2</sub> PO <sub>4</sub>
Solution D	10 mL	10 mM KNO <sub>3</sub>
Diammonium tartrate	92 g	5mM
TES	1 mL	trace
H <sub>2</sub> O	to 1 liter	

### Hoagland's A-Z Trace Element Solution (TES)

Reagent	Quantity (for 1L)	Final Concentration
Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> *K <sub>2</sub> SO <sub>4</sub> *24H <sub>2</sub> O	55mg	0.006% (w/v)
CoCl <sub>2</sub> *6H <sub>2</sub> O	55mg	0.006% (w/v)
CuSO <sub>4</sub> *5H <sub>2</sub> O	55mg	0.006% (w/v)
H <sub>3</sub> BO <sub>3</sub>	614mg	0.061% (w/v)
KBr	28mg	0.003% (w/v)
KI	28mg	0.003% (w/v)
LiCl	28mg	0.003% (w/v)
MnCl <sub>2</sub> *2H <sub>2</sub> O	389mg	0.039% (w/v)
SnCl <sub>2</sub> *2H <sub>2</sub> O	28mg	0.003% (w/v)
ZnSO <sub>4</sub> *7H <sub>2</sub> O	55mg	0.006% (w/v)
H <sub>2</sub> O	to 1 liter	

### Solution B

Reagent	Quantity (for 1 L)	Final Concentration
MgSO <sub>4</sub> *7H <sub>2</sub> O	25 g	0.1 M
H <sub>2</sub> O	to 1 liter	