**SWITCHGRASS NODE PROPAGATION PROCEDURE**

**Materials:**

Scalpel and #10 blades

Forceps

Petri dishes 100x25mm and 100x15mm

PhytaTray II plant culture containers (Sigma)

Clorox Bleach (6% sodium hypochlorite)

TritonX-100

Sterile ddH2O

Sterile 50ml centrifuge tubes

Graduated cylinder - 100ml

Timer

Sharpie marker

Nitrile gloves

10ml individually wrapped sterile pipettes

Pipette bulb/pipettor

Laminar flow hood, EtOH, & flame source for sterilizing instruments

70% ethanol in spray bottle

Kimwipes, large

Variable speed rocking platform

Switchgrass strain of choice

Micropore tape, 1" wide

Time Tape

**Propagation Medium (without Benomyl)**

**For 1 liter medium**: = 24 100x25mm petri plates = 2 sleeves

MS Basal Medium: weigh amount on bottle per liter, add to 800 ml ddH2O with stir bar

Add 30 g D+Maltose, and dissolve.

Adjust pH to 5.5 with 1M KOH.

Add MS medium to 1000ml cylinder and bring to 1000ml.

Add 4 g PhytoBlend agar to each of two 1L bottles. Add 500ml MS medium to each bottle. Loosely cap, add autoclave tape and autoclave 45 minutes. Place in water bath set to 57 deg C. until cool enough to handle.

In hood, add 333ul filter-sterilized 15mM 6-benzylaminopurine stock to each 500ml of medium and swirl bottle, final conc. = 10uM 6-benzylaminopurine. Pour medium into 100 x 25mm petri plates ~1/2 full. Store at 4 deg. C.

**Procedure:**

1. Prepare 75% bleach/1% Triton X-100 solution fresh each time. Use deionized H2O.
2. Spray 70% EtOH on all metal surfaces of laminar flow hood, and wipe down well with Kimwipe. Place MS-M culture medium plates in hood and tilt lids slightly to dry moisture on medium.
3. If switchgrass has visible dirt, rinse with ddH2O using a pipette or whatever is effective. Using scalpel or sharp scissors, cut switchgrass internode tissue ~1 cm above and below the culm node. Do not take the node closest to the root.
4. (If unable to start work immediately, place node+tissue in a petri dish with a bit of sterile ddH2O to keep them moist. Cover dish.)
5. In hood, place node tissue in 50ml centrifuge tube(s), 10-15 per tube, depending on size.
6. Add 25 ml 75% bleach/Triton X-100 solution to each tube. Screw on cap TIGHTLY. Place on rocking platform set to speed 4.5, and rock for 15 minutes. (Place tubes in the bottom half of a 150mm x 15mm petri plate to prevent them from rolling off platform.)
7. At the end of the bleaching time, remove tubes from rocker, and work in the laminar flow hood. Using a sterile 10ml pipette, remove bleach solution and add to waste bottle. Add ~40ml sterile ddH2O to each tube. Cap tightly and place on rocker for 5 minutes. Remove wash water with a pipette. Repeat this wash step for a total of 6 sterile ddH2O washes using a fresh pipette each time, and remove all water after the last wash.
8. Dump nodes carefully from one tube into a 100 or 150 mm diameter sterile petri dish. Transfer one node to the lid of the petri dish and, using a sterile scalpel and forceps, cut node in half lengthwise (**if** nodes are large enough to easily cut). Place node halves, cut side down, on propagation medium, 4-6 nodes per plate, depending on size.
9. Mark identifier/date, etc., on top of plates. Seal plate with 1 round of Micropore tape. Place plates in Room 290 Growth Room (16/8 photoperiod, ~80-90 uE/M2/sec, ~78 deg. F room temperature.
10. Monitor often for mold contamination and shoot formation and record. If a node becomes contaminated, carefully remove uncontaminated nodes and plate on fresh medium.

Shoots should begin to form within 1 week. Check often, and note the condition of medium. When it begins to crack and recede, nodes must be transferred to fresh propagation medium prepared in PhytaTrays (~100-125 ml medium per PhytaTray). Label PhytaTray using a piece of Time Tape; Sharpie markers do not write well on the PhytaTray plastic. In hood, in a steril petri dish, aseptically trim any necrotic/brown edges from the tissue, and transfers nodes with shoots to the PhytaTrays. Do not transfer any nodes that have not produced shoots - discard these.

After 2-3 weeks, transfer shoots to Rooting Medium prepared in PhytaTrays. Return PhytaTrays to Room 290 Growth Room. Monitor often for root formation and contamination.

When >=1 cm long roots have formed, rooted shoots may be planted in pots containing "clean" or sterile soil mix (Redi-Earth Propagation Mix or other suitable mixture).