**Switchgrass Node SHOOT-INDUCING Medium USING BENOMYL**

**MS-M with 10uM 6BAP & 5mgL Benomyl**

MS Basal Medium with Gamborg's Vitamins (Sigma)

D-(+) Maltose monohydrate powder, suitable for plant culture (Sigma)

dd H2O

Phytoblend plant agar (Caisson Labs)

6 Benzylaminopurine (stock 15mM) (Caisson Labs)

Benomyl (Methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate) - Sigma

DMSO

1 liter beaker

1 liter graduated cylinder

1 liter bottles with screw caps

15ml sterile centrifuge tube (Corning)

microfuge tubes (~1.5ml)

pH meter and calibration standards

1 M KOH, 0.1M KOH

Stirring platform and stir bars

Heated 57 deg.C water bath

Autoclave/autoclave tape

Sterile Petri Plates 100x25mm

PhytaTray II, Sigma

100ml Filter Flask, Sigma

Aluminum Foil

**6-Benzylaminopurine:** MW = 225.255 g/mol (soluble in H2O 60mg/L) Dissolve in few drops of 1M NaOH initially: Stock solution: 15 mM, 100mls. Add 337.88 mg to a small tube, and add a several drops of 1M NaOH to dissolve. Add ddH2O to tube and transfer all to 100 ml graduated cylinder. Continue adding ddH2O to small tube and then pouring it into cylinder until certain all 6-benzylaminopurine has been transferred to 100 ml cylinder. Fill to mark with ddH2O, and then filter sterilize. Wrap bottle with aluminum foil - light sensitive. Store at 4 deg C.

**Benomyl**: 5 mg/ml stock. Add 50mg Benomyl to 10ml DMSO in a 15ml sterile plastic centrifuge tube. Tighten cap and vortex until dissolved. This is a 1000x preparation. In hood, aliquot into 1.5ml microfuge tubes. Do not add more than 1 ml per tube. Store at -20deg. C.

**To prepare 1 liter medium:** weigh 4.4g MS medium and add to 1 liter beaker containing ~800ml ddH2O with stir bar. Add 30 g D+ maltose, and dissolve. Adjust pH to 5.5 with 1M (and 0.1M) KOH. Add medium to 1 liter cylinder and bring volume to 1000ml.

Add 4.0 Phytoblend agar to each of two 1000 ml bottles. Add 500ml MS-M medium to each bottle. Autoclave 45 minutes on liquid cycle. Cool to ~57 deg. C in heated water bath.

**Additions**: (in hood)

-10uM 6-Benzylaminopurine: add 333ul stock solution to each 500ml cooled medium bottle

-5mg/L Benomyl, add 500ul 5mg/ml stock Benomyl (well vortexed after freezing), to each 500ml cooled medium bottle.

For plates: pour plates <= 1/2 full and let cool completely before packaging. Store at 4 deg C.

For PhytaTrays: In hood, assemble PhytaTrays carefully ahead of time. The larger piece is the "bottom" into which the medium is poured. Crack lids, and pour ~100-125ml medium PhytaTray and cover with shorter lid piece, snapping in place. Store at 4 deg C.