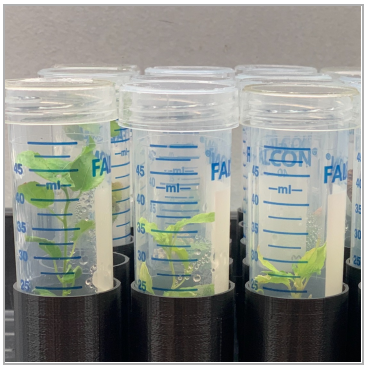

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We use this protocol and it's working

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Populus Microcuttings

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ABSTRACT

This is the protocol I use for micropropagation of trees in the genus Populus to generate germ-free plant material for microbiome/mycobiome studies. I have successfully used this protocol to generate clones of P. trichocarpa (Black cottonwood), P. deltoides (Eastern cottonwood), P. tremuloides (Quaking aspen), and 717 hybrids (P. tremolo L. × P. Alba L).

I generally use parent-plant material from semi-clean stock plants kept in the greenhouse, but have also used shoot material directly from sown seed, coppicing each shoot after they reach 1cm in height, in order to increase the genetic diversity of the resultant plants. Rooting efficiency varies by species (highest for 717 at ~60%, and lowest for P. tremuloides at ~30%), and the hormone balance could probably be optimized further for each species. Using wild plant material is also possible, but more difficult to clear (you will get more contaminated isolates that need to be discarded).

This typically generates ample roots, but if you need more fine root biomass, you can increase the number of fine roots by transplanting the rooted cuttings into liquid N1 media for an additional 2-3 weeks (shaken every 3 days to oxygenate the root systems).

MATERIALS

- **Parent material (donor plant)**
- **50ml falcon tubes (1 per replicate + 4 for clearing)**:
- **MS (gel) media (20ml per replicate)**
- **IBA (Stocks for NAA and IBA are made by mixing 8mg of NAA or IBA with 40ml of ddi water, which will yield 0.2 mg / L when you add 1 ml / L of working soln.).**
- **NAA**
- **Antibiotics - Ampicillin and Chloramphenicol**
- **Tween 20**
- **0.5L sterile ddi H2O (autoclave 20 min on wet cycle)**
- **2 500ml beaker (for waste - but autoclave it to keep the hood sterile).**
- **Sterile long tweezers (autoclaved)**
- **500 ml of sterile ddi water (autoclave)**
- **small petri dishes (3mm)**
- **3d printed falcon tube racks** 50ml_FalconTube_rack v5.stl

Overview

1 This is the protocol I use for micropropagation of trees in the genus *Populus* to generate germ-free plant material for microbiome/mycobiome studies. I have successfully used this protocol to generate clones of *P. trichocarpa* (Black cottonwood), *P. deltoides* (Eastern cottonwood), *P. tremuloides* (Quaking aspen), and 717 hybrids (*P.* tremolo L. × P. Alba L).

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Make media tubes

2

- Make .5 strength MS media. If pre-mixed (often this is the case because we go through a lot of MS) supplement with agar to increase the osmolarity to a gel, since our MS comes with agar already in it and we're making .5 strength - I supplement with 6g agar/L. Autoclave for 45 mins.

- After autoclaving, allow media to cool slightly and add 0.2 mg/L IBA and 0.2mg/L NAA (1 ml of working stock/L media)

- Add antibiotics to media: Ampicillin (0.5 ml of 50ml/ml soln.) and Chloramphenicol (0.5ml of 25mg/ml soln.).

- Add 20ml of media per falcon tube using an autopipetter, cap falcon tubes, and allow media to cool completely - note, gel takes longer to stabilize then agar plates do. I typically make this the day before and let them sit overnight.

Harvest and clear cuttings

3

- Harvest: Using a sterile razor blade, harvest terminal nodes from the parent plant- use small pieces with no more than 2 nascent leaves per cutting, ~.5-1cm. Note- the more tissue you take, the higher the chance of contamination, and the more difficult it is to clear endophytes. NOTE- you can also take lateral stem cuttings, but rooting efficiency is decreased. Harvest into 50ml falcon tube- no more than 25 per tube.

- Clear: for each set of 25, pre-fill 50ml falcon tubes with reagents and place in order: Tween, EtoH, NaOCl, H_2O:

    Tween 1% v/v (4ml in 40ml sterile ddi H2O)
    EtoH 70% v/v (28ml in 40ml sterile ddi H2O)
    NaOCl 10% v/v (4 in 40 sterile ddi H2O)

In germfree biosafety cabinet:

Pour Tween soln. into falcon tube containing microcuttings, cap, shake gently, and set timer for 1 min. After 1 min. Pour off tween soln. into waste container. NOTE: pour slowly to prevent pouring off microcuttings.

Pour EtOH soln. into falcon tube containing microcuttings, cap, shake gently, and set timer for 1 min. After 1 min. Pour off EtOH soln. into waste container 1.

Pour NaOCl soln. into falcon tube containing microcuttings, cap, shake gently, and set timer for 10 mins. After 10 mins. Pour off NaOCl soln. into waste container 2. **NOTE: USE WASTE CONTAINER 2 - mixing EtOH and NaOCl will make chloroform.**

Pour sterile ddi H2O into falcon tube containing microcuttings, cap, shake gently, and pour into waste container. **Repeat this rinse step 5 times.**

Transplant and grow

4

- Using long sterile tweezers (pre-autoclaved or flame/bead sterilized - remember to cool completely first), transfer cuttings into 50ml falcon tubes containing amended gel media. Place cut side into media at least .3cm below the surface. Place 1 microcutting in each tube. **NOTE: placing more than one will increase the build up to toxic secondary root products and inhibit callous formation.**

- Cap tubes with small (3cm) inverted petri dishes to allow light to reach microcuttings. Place cuttings into 3d printed falcon tube rack (see 50ml_FalconTube_rack v5.stl) to shade developing root systems.

- Place racks under grow lights at ambient room temp on a 16/16 day/night cycle.

Callus formation should proceed in ~4-6 weeks, and rooting will occur in 8-10 weeks, depending on the species. As cuttings grow, discard any replicates with signs of contamination.
NOTE: some *Populus* species (like the infamous 717 cross) can undergo shoot development faster than root development at this hormone concentration- if they get tall and start pushing up the caps, weigh them down with an inverted small glass beaker, or coppice with a small sterile scissors.

day 1

day 49

capped, in tube rack