**Root tip DNA extractions from bulk root samples**

Nhu Nguyen, with modifications by Ying Han, Noah Strom, and Peter Kennedy, 12/15

*Preparing roots*

1. Cleanly wash the soil away from the root system. Wash soil off over a sieve to prevent soils from filling drain (empty in bucket and dump at greenhouse).
2. In a tray of clean water, carefully to make sure roots are totally clean.
3. Dry the roots in a weighing dish or on weighing paper at 40**°**C for 24 hours (or until dry and crisp).
4. Transfer root sample into white office paper and fold so that roots are sealed into the paper.
5. Crush the dried root samples in the white paper, so that all the smaller secondary roots separate from the larger primary roots.
6. Open and remove the larger primary roots. If desired, the smaller roots can be further homogenized with additional rolling.
7. Fold white paper to keep roots inside, add label and store at room temperature (have kept for months with success).

*Weigh roots for DNA extraction*

1. Measure 20 mg and put in a screw-cap tube. Flame sterilize tools between samples.
2. Add sterile glass beads of two sizes. Add ~10 beads small white beads and 3 of the 2 mm clear glass beads.
3. Homogenize the samples in the bead beater in the Bushley lab. Pulverize for ~30 seconds. To use Bushley lab equipment, get permission from Kathryn first.

*CTAB DNA extraction*

1. Add 1 ml of 2% CTAB buffer + 2% PVP (w/v) (*can pre-warm to the buffer mix to 65****°****C ahead of addition*). Shake well and incubate at 65**°**C for 30 minutes. Every 5 minutes, take the samples out and gently shake by hand inversion for 1 min. Once incubation is done, spin at 13,500 rpm for 1 minute.
2. Transfer 100 ul of supernatant to a new 1.7 ml tube (*the leftover material can be frozen for other extractions*). Add 200 ul of fresh 2% CTAB + 2% PVP and 300 ul of chloroform. Gently shake for 1 minute by hand inversion. Then spin for 1 min at 15,000 rpm to separate the layers.
3. Transfer 200 ul of the supernatant to a fresh tube, add 15 ul of 4M ammonium (or sodium) acetate and 300 ul ice-cold isopropanol. (Keep all the alcohols at -20**°**C and keep on ice when adding). *Carefully draw the supernatant from the top layer near the phase line, but avoid drawing up material from the phase line (i.e. cellular debris).*
4. Shake gently for 10 minutes. Can use orbital gyrator or vortexer (for the latter, use lowest speed). Can also mix in hand inversion. *This step can also be done by putting samples in the -20****°****C freezer for 10 minutes or longer (could stay overnight).*
5. Spin for 1 minute at 15,000 rpm. *Because the pellet may not be visible, orient the tubes in the centrifuge with the hinge facing up, so the pellet will be stuck on the outer wall.*
6. Discard the isopropanol (*carefully pulling the liquid out and avoiding touching the area where the pellet is located*) and wash the pellet with 500 ul of ice-cold 70% ethanol. Gently shake by hand inversion for 1 min.
7. Spin for 1 minute at 15,000 rpm and discard 70% ethanol (*carefully pulling the liquid out and avoiding touching the area where the pellet is located*). Add 500 ul of 100% ice-cold ethanol shake by gentle inversion for 1 min.
8. Spin for 1 minute at 15,000 rpm. Discard 100% ethanol and let pellet dry (*can dry by puting on the block incubator at 30****°****C with the tube cap open for 10-15 min or you can blot dry the tubes on a kim wipe and leave them upside to dry*).
9. Resuspend with 50 ul of TE buffer. *Gently finger-flick samples to get the DNA back into solution.*

*PCR*

1. Dilute 1:9 with TE buffer before PCR.
2. Check which barcodes to use, read Nhu’s NGS guide, and calculate reagents with PCR spreadsheet.