Morning

1. *Plot harvest (1-2 people)*
   1. Locate logs on the [plot diagram](http://drive.google.com/open?id=0B7SVdP4r0KlBUGtzUm9ybGpERWM). There should be two logs per species remaining in each plot in 2014. Make general notes about the logs - are they present and in the correct places as determined by the plot map? is there animal/insect damage?
   2. If both logs for a species are intact, randomly choose one of them to harvest. If a log has been gnawed or is otherwise too damaged to harvest, the other log of the same species and year should be selected. Note on the plot map which log you take.
   3. Harvest logs in groups of sixteen. If top and bottom are distinguishable, push a thumbtack into each side of the top of each log. Place each log with its wire and tag in a separate plastic bag. Collect large pieces of unattached bark into the plastic bags. If you’re not sure that a piece of bark came from a particular log, leave the bark behind.
2. *Lab preparation (1 person)*
   1. Prepare 70% EtOH and 0.5% bleach for surface sterilization.
   2. Clean bench tops with 70% EtOH.
   3. Can make dry mass measurements of harvested logs in the drying oven (see section following afternoon protocol).
3. *AM Station 1 (1 person)*
   1. Label paper bags with species, symbol, plot, and harvest date.
   2. Cut the ID tag wire.Scrape any bark and soil that is loose on the log into the paper bags. Pass this material through a piece of 0.5cm2 mesh. Remove insects, snakes, and fruiting bodies. Any other material that did not pass through the mesh should be collected in the labeled paper bags. Carry the ID tag with the log through processing.
4. *AM Station 2 (1 person)*
   1. Enter log info (**Species**, **Symbol**, **Plot**, **Pull Date**) in the [spreadsheet](http://drive.google.com/open?id=1MQX3okKdmnaA7YrBLxaAxGQ50HUtD1DJCpSebErGibg).
   2. Take a picture of the log using the tripod and record **Picture ID**. Make general **Notes** about the condition of the log.
   3. Note obvious **Insect Damage (y/n)**.
   4. Assess for presence of **Fruiting bodies (y/n)** and, if present, make notes in **Fungal ID**. Collect the fruiting bodies in coin envelopes if they are relatively fresh. Describe mycelium/rhizomorphs in **Notes** if they are extensive.
5. *AM Station 3 (1 person, w/gloves)*
   1. Surface sterilize log in a plastic shoe box by rotating continuously in:
      1. 95% EtOH for 5 seconds
      2. 0.5% sodium hypochlorite (bleach) for 2 minutes
      3. 70% EtOH for 2 minutes
6. *AM Station 4 (1 person, w/gloves)*
   1. Place the log in a dry plastic shoe box labeled with log ID, plot ID, and time of removal from 70% EtOH. Cover the box loosely and place in an air-conditioned room along with the tag and labeled paper bag(s) for the log.
   2. Leave the log to air dry for approximately 1-2 hours.
   3. Turn the Germinator on.
   4. This person can work on tube/envelope/box labeling during down time (see *Sawdust Allocation* in PM protocol).

Afternoon

1. *Wet Weights (1 person - can also do Volume Displacement)*
   1. Remove sieved material from bags, record mass as “**Wet weight excess**” and discard.
   2. Determine log dryness by the disappearance of EtOH odor.
   3. In order of dryness, record log wet weights as “**Wet weight log**”.
   4. After recording wet weight, wrap the log twice in Saran Wrap to maintain structural integrity during processing and to contain bark.
   5. Turn the drying oven on to 100-105 C.
2. *Drilling (2 people - 1 drills, 1 sterilizes, both w/gloves)*
   1. Sterilize two ¼” drill bits, two metal scoops, and metal scissors - wipe each with a KimWipe soaked in 70% EtOH and then put in the Germinator for at least 15 seconds.
   2. Clamp the log to the drill rig with the top side facing out.
   3. Cut eight small holes in the Saran Wrap with sterilized scissors.
   4. Drill sawdust from each hole in the Saran Wrap with a Germinator- sterilized ¼” drill bit and collect in a UV-sterilized weigh boat. Use the drill bumper to determine the 2.5 cm sampling depth. Place a weigh boat labeled “t” on top of the weigh boat containing sawdust to cover it. Transfer the weigh boats to the sawdust allocation bench.
   5. Flip the log over in the drill rig and repeat the previous 2 steps on the bottom of the log. Collect bottom sawdust into a separate weigh boat with a cover labeled “b”.
3. *Volume Displacement (1 person)*
   1. Prepare volume rig - container, 1.5L water, large balance. You may want to prepare a piece of paper to write down volume measurements.
   2. Unwrap the drilled log and record log mass as “**Post-drill wet weight**”.
   3. Pin one dissecting needle into each end of the log.
   4. Tare the weight of the rig on the balance.
   5. Push the log underwater and shake out bubbles if they appear.
   6. Record the mass on the scale as “**Volume**”.
   7. Remove the log from the water and let drip for ~10 seconds.
   8. Put log aside and record negative value on balance as “**Mass after volume**”.
   9. Wrap the log in its labeled paper bag and place in the drying oven (100-105C).
4. *Sawdust Allocation (1 person w/gloves)*
   1. For each log, label six tubes and two coin envelopes (See [3rd Harvest Decoder](http://drive.google.com/open?id=1Zl0TpCA_DSpeGpXZotT98sfafkPqcRqjTTaFUSEVxyA)).
      1. four 2 ml tubes for DNA: SPECIES (A-X) PLOT (1-8) TOP/BOTTOM (t/b) REPLICATE (+/-)
      2. two 2 ml cryovials for enzymes: SPECIES (A-X) PLOT (1-8) TOP/BOTTOM (t/b)
      3. two coin envelopes: SPECIES (A-X) PLOT (1-8) TOP/BOTTOM (t/b).
   2. Wipe down bench with 70% EtOH.
   3. Using a sterilized scoop, stir the top sawdust. Fill DNA tubes (+/- t replicates) to 100-200 ul and the t cryovial to the top. Put the rest of the sawdust in the t coin envelope. Wipe excess sawdust off of the scoop with a KimWipe soaked in 70% EtOH and put the scoop in the Germinator.
   4. Repeat the previous step for the b tubes and envelope with a sterilized scoop.
   5. Pipette 600 ul of 1% CTAB into each DNA tube.
   6. Allocate tubes into appropriate plot boxes: DNA(+), DNA(-), ENZ
   7. Put all boxes in the -20C freezer as they are finished. Transfer the ENZ boxes on dry ice to a -80C freezer ASAP.
   8. Put all coin envelopes in a box in the drying oven if there is room for them.
   9. Wipe bench with 70% EtOH between logs.
5. *Cleanup (everybody, everywhere)*
   1. UV-sterilize the weigh boats.
   2. Return bleach and ethanol to gallon jugs for re-use.
   3. Rinse shoe boxes and leave upside-down to dry overnight.
   4. Turn off the Germinator.
   5. Back up the data sheet onto a flash drive.

Dry mass (can be combined with the AM tasks of the lab preparation person)

1. Weigh logs in every day until the change in mass from one day to the next is <5%. You can do this in the paper bags.
2. On the day that the change in mass is <5%, remove the logs from their bags, remove tacks, and record mass as “**Dry weight**”. Record date of removal from oven as “**Date removed from oven**”.