Dry Ash Digestion Protocol
(Cardelús lab, update 02/17/2011)

Digestion procedure for the determination of P, Ca, Mg, K, Na, Mn, Cu, Fe, Zn, and N in plant tissue and organic soils (>8% SOM—this hasn't been tested for lower organic matter content)

P detection limit: Colorimetry: It is very sensitive. Essentially the Malachite green protocol which we use gets precipitation above 1.37 mg/L of P in the solution. You can use the table below to choose sample weight and total volume. Remember: the ratio of acid to water must be 1:10 so that the final carrier solution is 0.6M HCl. We got beautiful replication as low as 0.01 g which makes me very confident that we can go below that. If you do go below 0.025 g, speak to me first. We will want to put extra peaches at the weight in to make sure our methods are analytically correct.

g Peach	ml acid	Total Volume	mg/L	%P
1	5	50	27.4	0.137
0.5	**5**	**50**	**13.7**	0.137
0.2	**5**	**50**	**5.48**	0.137
0.1	**5**	**50**	**2.74**	0.137
0.05	**5**	**50**	**1.37**	0.137
0.025	**5**	**50**	**0.685**	0.137
0.01	**5**	**50**	**0.0274**	0.137

Calculating %P: (((Pmg/L/1000)*(Total Volume of solution/1000))/(Weight of sample))*100

Standard Curve: You must make a standard curve for the run. We want lots of values close together between 0.1 – 1.5 mg/L. Follow the guidelines below. REMEMBER: the carrier solution is 0.6M HCl for ASH DIGESTION and 0.5M for Soil Available P. For soils, cluster the curve between 0.05 and 0.5, for plants you often end up having to dilute, but cluster the curve higher.

Calibration Curve			
mg/L Standard	ml of Standard 1000ppm	uL of Standard	Volume of flask
0.05	0.0025	2.5	50
0.075	0.00375	3.75	50
0.1	0.005	5	50
0.25	0.0125	12.5	50
0.5	0.025	25	50
0.75	0.0375	37.5	50
1	0.05	50	50

For solution (mg/L)	Add (ml)	of mg/L solution	To 25 ml Flask and bring to volume
1	16.7	**1.5**	25
0.75	18.8	1	25
0.5	16.7	0.75	25
0.1	5.0	0.5	25
0.075	18.8	0.1	25
0.05	16.7	0.075	25
0.01	5.0	0.05	25
To make 1.5 mg/L Solution, take 0.075ml of 1000ppm standard and add to 50ml flask, bring to volume.			

Standard: NIST Peach (1547), P= 0.137%

Supplies
Repipettor (calibrated to 2.5ml) and reservoir or pipette
Stir bar
Stir plate
3-place balance
crucibles (acid washed)
sharpie marker and lab tape for labeling
Muffle furnace (in Ted's lab)
Pasteur pipette and bulb
Squirt bottle with nanopure DI water (from Ted's lab)
Plastic funnels
Volumetric flasks (volume dependant on sample mass)
Whatman #42 quantitative filter papers to fit funnels
1 20 ml vial per sample for storage (ARL lab for analysis via ICP)
OR
2 4 ml vials per sample for storage (MM's lab for P analysis only via colorimetry)

Solutions
6 M HCl: Calibrate 1 L repipette reservoir and suit up in protective clothing and face mask. In the fume hood, add stir bar and 500 ml deionized nanopure H2O to the reservoir and SLOWLY bring up to 1 L volume with 12.1 M TraceMetal HCl. Place on stir plate in hood and stir slowly for 5-10 minutes.

Procedure
Note: Before beginning procedure, samples must be ground to a fine powder, and re-dried at 60 degrees C overnight. Peach must also be dried at 60 degrees C overnight. It works well to run 30 samples (including duplicates and peach) at a time.

Day 1

1. Let samples cool to room temperature in dessicator (~20 min).
2. Weigh out your sample into each crucible. (takes 3-4 h for 30 samples)
    a. **Duplicate every 10th sample to measure the precision of the test (4 duplicate per batch of 40)**
    b. **Include at least 3 peach standards in each sample batch.**
    c. **Include an EMPTY crucible that will be the analytical blank.**
3. Place samples in muffle furnace. Set temperature controls to 500 degrees C and turn furnace on.
4. Once oven reaches 500 degrees C, allow samples to ash for a minimum of 5 hours. Shut oven off (or set to 30 degrees C) and allow to cool. To expedite cooling, the furnace door can be opened, only after the furnace has cooled below 200 degrees C. If you open the furnace door at a temperature higher than 200 degrees C, samples may ignite or be disturbed by the rapid influx of air.
5. Label vials to be used on day 2.

Day 2

6. Once samples reach room temperature, remove them from oven, place 15 samples in the hood, and moisten the ash by adding approximately 5 drops of pure water from a Pasteur pipette, then use the volume of 6M HCl that corresponds you're your sample volume in the chart on page 1 from the repipettor or pipette. Let mixture stand for at least 30 minutes.
7. Put funnel on top of the volumetric flask, and place filter in funnel. Quantitatively transfer ash mixture into funnel. Rinse the crucible twice with pure water and add to flask. Rinse funnel with pure water. Bring flask up to volume with pure water using squirt bottle and Pasteur pipette.
8. Cap flask and mix solution by vigorous shaking (can use mixer if necessary). If a lot of ash gets into the volumetric flask, it can be refiltered.
9. Transfer aliquot of sample to labeled vial for storage.
10. Repeat procedure on the other 15 samples. On average, it takes 3 hours per set of 15 samples.

Repeat days 1 and 2 as necessary. Typically, it is possible to run 2 sets per week.

*If running many samples, it is important to get the volumetric flasks and other dishes into the acid bath at the end of day 2. They can be rinsed and dried on day 1 (the oven can speed drying so they will be ready to be used the next day 2).

Calculations

Results will be in units of solution concentration (ug ml^{-1} or ppm). You must then calculate % plant dry weight:

$$\frac{(x \text{ mg P}/1000) * y \text{ l extractant}}{z \text{ g plant material}} = \%P$$

To check standards:

Known P content of NIST 1547 peach leaves:

0.137% Total P= 0.69 mg P per 0.5 g peach.
Weight extracted: 0.5 g=500 mg
Extraction volume: 25 ml

Thus the P concentration in extract acid is:

0.5 g plant material in 25 ml extract

$$=0.69 \text{ mg P in 25 ml extract} = \frac{0.69 \text{ mg P}}{25 \text{ ml extract}} = 0.026 \text{ mg P ml}^{-1}$$

To convert units to mg/l (or ug/ml or ppm):

= 0.026 mg P x 1000 ml extract
= 26 mg P per 1 extract

Since 25 ml of extract should contain all the P from 0.5 g of plant material, a direct substitution is possible:

0.69 mg P in 25 ml undiluted extract = 0.69 mg P in 0.5 g plant material.

Weight of analyte (P) divided by actual weight (i.e. slightly more or less than 0.5 g) of dry material extracted gives proportion (weight to weight) of analyte in the dry material:

$$\frac{A \text{ mg P}}{B \text{ g plant}} = \frac{X \text{ mg P}}{1.0 \text{ g plant}}$$

$$\frac{A}{B} \text{ mg per g} = X \text{ mg P per g plant}$$

=1.37 mg P in 1.0 g plant material

=0.137 % Total P in NIST 1547 peach leaves.

Prepare two replicates at each of the following sample masses using peach standard (NIST 1547):

Include two blanks (DI H_2O + HCl) and 5 unknowns from sample set (use 0.5 g sample mass and appropriate acid/water dilution).