Chlorophyll a/b extraction notes and procedure

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It's best to do extractions as soon as possible after collecting leaves from the field. Keep leaves on ice or in the fridge and in the dark if you're going to be storing them for more than a few hours. I have had luck doing extractions 1-2 days after collecting leaves by storing them on ice in the field and then in the fridge, but I don't recommend this unless absolutely necessary.

Supplies needed:

Glass test tubes
Blue or clear size pipette
Dimethylformamide (DMF) – usually in the fume hood or flammable cabinet
Parafilm
Polypropylene microplates; only needed if using microplate reader – (DMF quickly dissolves polystyrene plates and your results will be off if you use them)

DMF will dissolve lots of things – It's a strong solvent! **WEAR APPROPRIATE GLOVES AND USE THE FUME HOOD.

Day 1

1. Use the orange/white leaf disk punches in the lab to punch out leaf disks, avoiding large veins if possible. The area of this punch is approximately 1.99 cm^2.
2. Label enough test tubes for the number of leaves you are analyzing. Note that DMF will easily dissolve sharpie, so I recommend labeling tubes in two places, or using lab tape.
3. For a 2 cm^2 leaf disk, I recommend using 2mL of DMF to extract. If you're using smaller disks, you can probably get away with using 1mL. Pipette 2mL DMF into each test tube.
4. Your results may vary, but many of our study species have super waxy cuticles, and DMF sometimes has a hard time penetrating through this. I recommend using a small razor or Exacto knife to score the leaf disk 4-5 times before placing into DMF-filled test tubes. You can do a few trials with your species to see if this is necessary.
5. Place your leaf disks into test tubes, making sure they are fully submerged.
6. Place parafilm over all the tubes; you will be storing them in the fridge, and since the fridge often gets filled with dirt samples, you don't want any contaminants falling into the tubes.
7. Store tubes in fridge for at least 24 hours, or up to one week.

Day 2

1. After 24 hours, check the leaf disks to see the extraction progress. You want to see that most of the green color has been removed from the leaf disk and absorbed into the DMF. If the disks still look really green or are streaked with areas of green, let them sit in the fridge for another day or two before running them on the spec. You can leave them for about a week in the fridge. Don't leave them for too long, as the chlorophyll will break down over time.
2. Decide if you're using a microplate reader (Hoagland lab; consult with Jason or Doug) or a single read spectrophotometer (e.g., Raina Lab; consult with Jason or Doug).

a. If using microplate reader, pipette ~100µL of each extraction into a separate well.
b. If using the Raina Lab spec, you can keep the samples in their vials and pour them one by one into a glass or quartz cuvette (don't use the plastic ones!)

3. Determine absorbance of each sample at 663.8, 646.8 and 750 nm. Be sure to read a blank DMF sample. (If you're running many samples in the Raina lab, try to keep groups of them in the dark as you work. Chlorophyll degrades very quickly when it's exposed to light.)
4. Use the equation found in Porra et al. (1989) to calculate chlorophyll content for each sample. Note that to determine total mg/cm^2, you will need to divide by your leaf disk area. Be careful if you're using 2mL of DMF to extract, as you will need to take this into account when doing area/mass based conversions from concentration in your extractions.

TABLE III

Corrected equations for the determination of chlorophylls in N,N′-dimethylformamide, methanol and buffered 80% aqueous acetone

These equations (1–18) are derived from the difference extinction coefficients presented in Table II; therefore all absorbance measurements at the indicated wavelengths must have the absorbance at 750 nm subtracted.

Equations for chlorophyll concentrations in nmol/ml		Equations for chlorophyll concentrations in µg/ml	
In DMF			
Chl a $= 13.43\ A^{663.8} - 3.47\ A^{646.8}$	(1)	Chl a $= 12.00\ A^{663.8} - 3.11\ A^{646.8}$	(10)
Chl b $= 22.90\ A^{646.8} - 5.38\ A^{663.8}$	(2)	Chl b $= 20.78\ A^{646.8} - 4.88\ A^{663.8}$	(11)
Chls a + b $= 19.43\ A^{646.8} + 8.05\ A^{663.8}$	(3)	Chls $a + b = 17.67\ A^{646.8} + 7.12\ A^{663.8}$	(12)
In methanol			
Chl a $= 18.22\ A^{665.2} - 9.55\ A^{652.0}$	(4)	Chl a $= 16.29\ A^{665.2} - 8.54\ A^{652.0}$	(13)
Chl b $= 33.78\ A^{652.0} - 14.96\ A^{665.2}$	(5)	Chl b $= 30.66\ A^{652.0} - 13.58\ A^{665.2}$	(14)
Chls $a + b = 24.23\ A^{652.0} + 3.26\ A^{665.2}$	(6)	Chls $a + b = 22.12\ A^{652.0} + 2.71\ A^{665.2}$	(15)
In buffered 80% aqueous acetone			
Chl a $= 13.71\ A^{663.6} - 2.85\ A^{646.6}$	(7)	Chl a $= 12.25\ A^{663.6} - 2.55\ A^{646.6}$	(16)
Chl b $= 22.39\ A^{646.6} - 5.42\ A^{663.6}$	(8)	Chl b $= 20.31\ A^{646.6} - 4.91\ A^{663.6}$	(17)
Chls $a + b = 19.54\ A^{646.6} + 8.29\ A^{663.6}$	(9)	Chls $a + b = 17.76\ A^{646.6} + 7.34\ A^{663.6}$	(18)

(Taken from Porra et al. 1989)

5. DMF must be disposed of by EHS, don't pour it down the drain!

References

Porra, R. J., W. A. Thompson, and P. E. Kriedemann. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents : verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta:384–394.