Protocol for Fatty Acid Methyl Ester (FAME) analysis of Arabidopsis leaves

1. Wash sample tubes: add 1 ml. dry methanol to 196 screw-cap 13 x 100 mm disposable glass round-bottom test tubes. Cap each tube with a screw cap in which an extra white PFTE liner has been inserted, vortex for a few seconds, and discard the methanol as hazardous waste. Invert the tubes in a rack; air dry caps and tubes.
2. Prepare methylation reagent:
   70 ml 3N methanolic HCl (Sigma)
   140 ml dry methanol
   1.05 ml pentadecanoic acid (1 mg/ml in toluene)
   Add BHT to10 ug/ml*
   Mix thoroughly and use the same day.
   **For harvests prior to 03-29-07, no BHT was added. For the 3-29-07 harvest, BHT was added to a final concentration of 5µg/ml.*
3. Add 1.0 ml. aliquots of methylation reagent to the washed sample tubes and cap tightly.
4. Place an adhesive bar code sticker to the outside of each sample tube containing methylation reagent.
5. Preheat 3 dry heat blocks, 80°C.
6. Remove one flat of Arabidopsis plants from the growth chamber and place them on the bench beneath a fluorescent light fixture (~100 uE) during sampling.
7. Scan sample tube barcode and pot barcode into the database. Harvest leaf number 5 or 6 (counting from the newest visible leaf), omitting the petiole. Weigh the leaf sample, send the weight to the database, place the sample into a tube of methylation reagent, and cap tightly. Be sure the sample is completely immersed. *For harvests prior to 04-12-07, leaf disks (size no. 4 cork borer) were harvested instead of whole leaves.*
8. Place the tightly capped tube into the heat block.
9. Repeat steps 7-8, taking a similar sample from the same plant.
10. Repeat steps 7-9 for each pot until all plants scheduled for harvest have been sampled in duplicate.
11. After the samples have been incubated in the heat block for at least 30 minutes, remove from the heater and place into a test tube rack, allowing them to cool to RT.
12. In a fume hood, remove the cap from each tube and add 1.0 ml. of 0.9% NaCl and 0.15 ml. of heptane. *For harvests prior to 03-29-07, hexane was used instead of heptane.*
13. Replace the caps and vortex each tube for 16 seconds.
14. Centrifuge sample tubes for 10 minutes, 1000 rpm, Eppendorf 5810R tabletop centrifuge.
15. Transfer the upper hexane layer (~0.075 ml) containing the fatty acid methyl esters to a barcoded GC vial containing a micro-insert, and cap the vial.
16. Store the GC vials at 4 ºC short-term, or -20 ºC long-term.

<u>GC method</u>:

Column: J+W DB-23 (50% cyanopropyl) methylpolysiloxane

FID: Temp: 270 ºC

Injection:

- mode: split ratio 30:1
- temperature: 270 ºC
- injection volume: 1 µL

Oven:

- intial temp: 140 ºC
- temp ramp: 10 ºC / min
- final temp: 260 º
- Hold at final temperature for 3 minutes. For sequences run prior to 03/30/07, the final temperature was held for 5 minutes.

Reference: Browse, J., McCourt, P.J. and Somerville, C.R. 1986. Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. Analytical Biochemistry 152: 141-145.