Seed C/N protocol

Note: because of the large influence of environment on seed C and N content, for seed samples to be compared, they must be from plants grown together under as similar conditions as possible. Plants must be grown in the same soil mix, and watered/fertilized using the same protocol. All flats must be rotated frequently in the growth chamber to provide approximately equal amounts of light throughout the seed maturation process, and all pots within a flat must be kept equally moist at all times during growth.

We analyze cohort plants from the same flat of approx. 32 plants, and derive statistics based upon the median values from this flat.

Preparation of seed samples for absolute %C and %N elemental analysis at Duke Environmental Stable Isotope Laboratory:

Materials:
Tin capsules for solid samples (CE Elantech #240-064-40)

1. Seed aliquots (~12 mg) are transferred to decapped 2 ml microfuge tubes and placed in a vacuum dryer for 3 days to equilibrate all the samples to equal dryness.
2. 10 mg samples are quickly weighed on an analytical balance (accurate to 0.1 mg) and prepared and packaged according to the facility requirements (see http://www.biology.duke.edu/jackson/devil/sampleprep.html).
3. Waiting samples are kept dry under vacuum until all the samples are packaged.
4. An identical “gold standard” pooled wild-type seed sample is prepared and placed in every plate of seeds submitted to track run-to-run differences.

Reference:

Li, Y.H., Beisson, F., Pollard, M., and Ohlrogge, J. 2006. Oil content of Arabidopsis seeds: the influence of seed anatomy, light, and plant-to-plant variation. Phytochemistry 67 (9): 904-915.