Assessing Circadian Rhythms Using Delayed Fluorescence

Stacey Harmer, 11/3/2010

Protocol derived from Gould et al (2009) Plant Journal, 58:893-901
(doi: 10.1111/j.1365-313X.2009.03819.x)

1. Sterilize seeds using your favorite method and plate onto 0.5x MS media, 1.5% agar (no sucrose). For *Arabidopsis*, sow seeds in dense clusters of 15 – 20 seeds. For canola, plate seeds individually. (For *Arabidopsis*, the Col-0 accession works better than Ler or WS).
2. Stratify seeds in the dark at 4 degrees for 2 - 4 days.
3. Transfer plates to a growth chamber set to light/dark cycles (typically, 12 hr light (approximately 80 μmol m^{-2} s^{-1})/12 hr dark). Grow for 16 days.
4. *Arabidopsis* plants can be imaged as is. For larger plants, excise leaves in sterile hood and move to fresh plates (same media as above).
5. Move plates to an imaging chamber equipped with a highly sensitive CCD camera and LED lights. Set chamber lights to deliver either 20 μmol m^{-2} s^{-1} red or 20 μmol m^{-2} s^{-1} red + 20 μmol m^{-2} s^{-1} blue light.
6. Using software to drive the camera and lights, program the following imaging schedule:
    a. Lights on for 58 minutes
    b. Lights off
    c. Immediately capture image using 2 minute exposure time
    d. Loop above sequence for 5 days
7. Images are saved as TIFF files using MetaMorph software.
8. After experiment is finished, use MetaMorph to extract pixel values from each group of plants (Arabisopsis) or each leaf (canola) at each time point.
9. Analyze using BRASS (available from http:// www.amillar.org).
    a. Normalize data
    b. De-trend data
    c. Determine periodicity using fast Fourier transformed non-linear least-square analysis.