**Junior PAM Protocol**

Author: Hannah Aichelman

Modified by: Brooke Benson

Last Updated: November 30, 2017

1. Plug Junior PAM into the computer and insert the fiber into the black port gently until the plastic guide has been reached. Do not force further. Hand-tighten the screw (hard tightening will break the fiber). This step will already be done for you.
2. Place the black guide on the other end of the fiber, and gently hand-tighten (this is what keeps the fiber optic cable a standard distance from the surface of the coral).
3. Start the PAM software, WinControl-3
4. Check the settings:
   * Saturation pulse width: 0.6
   * Saturation light intensity: 12
   * Electronic signal damping: 2
   * Electronic signal gain: 4
   * Measuring light intensity: 2
5. Select ‘record online’ at the top of the ‘Chart’ tab (may already be checked).
6. Click Autoscale button on the ‘Chart’ tab if you can’t see the fluorescence trace. Sometimes not necessary.
7. When you are ready to make a measurement, hold the black guide on the surface of the coral for at ~5 seconds. This should be done GENTLY. You want to make contact, but don’t apply pressure.
8. Press the <Fo,Fm> button. Now you have your measurement. Check the yield area to make sure that you have a measurement and that it makes sense (not super low or high).

Notes

* DO NOT adjust the black guide at all during the course of the experiment, or you will no longer have a standard distance between the fiber and the coral surfaces.
* If you choose, you can create new records to separate measurements in different tanks, treatments, etc. This makes it easier to assign nubbin ID’s to values later on.
  + You can do this on the ‘Chart’ tab, under Val. click to the Rec. box. You should see buttons for New Record and Delete Record. Click New Record, which will separate your measurements by record under the ‘Report’ tab.
* The published literature for *Astrangia poculata* Fv/Fm values is slightly less than that of tropical corals. For example, Burmester et al. (2017) reported values of Time 0 symbiotic *A. poculata* fragments between 0.4 and 0.5. I observed similar values, but still have more data to analyze. Note: you will still see an Fv/Fm signal in aposymbiotic *A. poculata,* but you are likely measuring mostly endolithic algae. Tropical corals often range from ~550-700 when healthy.
* I did not need to make a map of my fragments because I was measuring Fv/Fm of corals in respirometry chambers, but maps are an efficient way to keep track of the order of fragments measured.
* I dark-adapted *Astrangia poculata* for at least 30 minutes before making a measurement.
* We place the fluorometer on a box or stand that holds it above the water level of the tank. We move the fluorometer to the different tanks instead of bringing corals to the instrument**. DO NOT let water drip down the fiber optic cable and into the fluorometer while moving between tanks.**
* I save the data in 2 different ways. Under File, I Save Data. Additionally, under the ‘Report’ tab click on Options and then Export All. This way you have your data in both the WinControl software format and as an Excel spreadsheet.