**Sarah and Thanapat’s Single Larva DNA Extraction Protocol 2012**

Reagents:

* DNA Digest Buffer: 100mM NaCl, 10mM Tris-Cl pH 8.0, 25mM EDTA pH 8.0, 0.5% SDS. This buffer can be pre-made and stored for years.
* RNase A (1000 U/ul) dilution prepared and stored on undergrad shelf.
* Proteinase K (900U/ml)
* PCA: 25:24:1 buffer-saturated phenol, chloroform, and isoamyl alcohol stored 4C fridge
* 10mM EDTA
* 3M NaOAc
* 100% EtOH
* 80% EtOH
* Fresh milliQ water

Steps (Time estimate: ~3.5 hrs.):

* Fill out sample sheet and collect samples from -20C freezer
* Add 50µl of DNA Digest Buffer with 1uL proteinase K and 1uL RNase to sterile, labeled 1.5ml tube and put on ice.
* Transfer coral larva using sterile pipette tip and place into digest solution. Another option is to remove all ethanol from the sample tube so only the larva is left and then add digest buffer into the tube. Ensure cap is on tight.
* Using the same pipette tip back pipette to immerse the coral tissue. You want to do this until you cannot see chunks of larva anymore. You may also want to try the blue pestles if there are large chunks. Avoid getting too many bubbles when back-pipetting.
* Repeat steps 2-4 for all samples. Vortex all samples 1-2 seconds.
* Incubate for 30 mins in 42C water bath. Back-pipette changing tip between samples and then incubate at 42C for another 30 mins.

\*\*\*\* Note: While samples are lysing, prepare centrifuge for 4C spin.

* Vortex all samples 1-2 seconds.
* In the fume hood add 50uL of PCA (from below the buffer line) and ensure cap is on tight. Careful PCA will burn your skin!
* Vortex samples several seconds or invert tube and place on ice for 1 min.
* Vortex samples several seconds again.
* Spin at max speed for 10 minutes at 4C.
* Remove 40uL of the upper aqueous phase (No interphase!) and put in a sterile, labeled tube (Sample number, Date, Initials). **Write clearly.**
* Add 1uL 10mM EDTA, 4uL 3M NaOAc and 100uL 100% EtOH (These can be added to labeled tubes beforehand if you desire).
* Vortex samples several seconds.
* Spin at max speed for 30 minutes at 4C.
* Pour off supernatant into liquid waste.
* Add 500uL 80% EtOH and ensure lid is on tight. **GENTLY** wash EtOH around tube.
* Spin at max speed for 5 minutes at RT.
* Pour off ethanol supernatant and place upside down on Kimwipe. Tap off excess ethanol.
* Dry tubes for ~15 mins until **NO** liquid can be seen in tube.
* Leave tubes upright in tube holder covered in kimwipe for 5 additional minutes to ensure all EtOH is evaporated.
* Re-suspend pellet in 25µl milliQ water, vortex no more than ~10 sec.
* Nanodrop the sample and record in both your notebook and the spreadsheet.
* Store in -20 fridge in your personal box.