**DNA Extraction with PCA Protocol 2014**

Modified Jan. 2018 (Brooke Benson & Nicola Kriefall)

Theory:

First, the steps including proteinase K + buffer, incubating at 42˚C, and vortexing help break up the coral skeleton and lyse the coral, Symbiodiniaceae, and microbial cells in the tissue sample open, which forms the cell lysate. We take the cell lysate & buffer away from the skeleton debris and add it to an equal volume of PCA and mix everything up. The molecules in solution then separate by their solubility. The PCA layer & interphase contain the heavier proteins and lipids and are denser than the top aqueous layer, which contains the cell lysate & buffer & the nucleic acids (our DNA). The aqueous layer is added to sodium acetate (NaOAc) which binds and precipitates the DNA out of the solution, down to the bottom of the tube. We add fresh ethanol in the next steps to “wash” the DNA of the salts and other contaminants, leaving pure DNA behind. We re-suspend the DNA in pure MilliQ water (water that is pure & ultra-filtered).

Reagents:

1. **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; aliquots kept at bench at RT
2. **Proteinase K** (900U/ml; stored in Enzymes box in -20˚C freezer)
3. **PCA:** 25:24:1 buffer-saturated phenol, chloroform, and isoamyl alcohol (stored in 4°C fridge)
4. **3M NaOAc** (aliquots kept at bench at RT)
5. **100% EtOH** (aliquots kept at bench at RT)
6. **80% EtOH** (aliquots kept at bench at RT)
7. **Fresh milliQ** **water** (aliquots kept at bench at RT)
8. **Tissue samples** (stored in -20˚C freezer in >80% ethanol)

Steps (Time estimate: ~3.5 hrs.):

1. Turn on the machines: 4˚C centrifuge and 42˚C water bath or heat block.
2. Add 600µl of DNA Digest Buffer and 6µL Proteinase K to labeled 1.5mL tubes and put on ice.
3. Sterilize forceps and workspace using 50-70% EtOH squirt bottle & Kimwipes. Use the forceps to transfer coral fragment into the digest solution tubes created in Step 2.
   * Always use half or less than half of the sample only, in case of contamination.
   * If skeleton fragment is too big to fit into the tube: cut in half using sterilized razorblade.
   * Very much recommended to use a very small piece or there will be too much slime during later steps.
4. Vortex all samples 1-2 seconds & spin down.
5. Incubate for 30 minutes in 42°C water bath or heat block. Vortex for several seconds, spin down, then incubate again for 30 minutes.
6. Vortex all samples 1-2 seconds, spin down.
7. In the fume hood: add 600µL (SAME VOLUME as Digest Buffer in Step 2) of PCA and ensure cap is on tight. PCA will burn skin so be sure to wear gloves and if PCA gets on them, switch them!
8. Invert the tubes several times and place on ice for 1 min.
9. Vortex samples several seconds again.
10. Spin at max speed for 5 minutes at 4°C.
11. While samples are spinning, add 30µL 3M NaOAc and 750µL (2.5X AQUEOUS SUPERNATANT, next step) 100% EtOH to a sterile, labeled tube. This tube will be the final tube so label clearly.
12. Pipette 300µL of the upper aqueous phase from samples (take care to avoid the interphase (white middle layer)! If you touch the interphase, re-spin for 5 mins and aim for less) and put in the labeled tube from step 12. Gently invert tubes several times. Discard PCA waste in PCA waste container.
13. Spin at max speed for 20 minutes at 4°C (this will pellet the DNA).
14. Pour off supernatant into liquid waste.
15. Add 1000µL 80% EtOH and ensure lid is on tight. **Gently** invert tubes.
16. Spin at max speed for 5 minutes at 4°C.
17. Pour off ethanol supernatant, gently tap off excess ethanol, and place upside down on Kimwipe.
18. Dry tubes for ~15 mins until no liquid can be seen in tube.
19. Leave tubes upright, covered by a Kimwipe, in your tube rack for 10 additional minutes to ensure all EtOH is evaporated.
20. Re-suspend pellet in **50µl** milliQ water, vortex no more than ~10 sec.  (You can also re-suspend in 30µl if your sample was small or the species is low-yielding and you want to increase your DNA concentration.)
21. Nanodrop the sample and record the DNA concentration in your notebook.
22. Additional check (optional): Load 100ng of each sample onto a 1% agarose gel to check integrity. Genomic band with/without smear should be detected, if not DNA isolation was not successful.