DNA EXTRACTIONS- Phenol/Chloroform

(Note: see document DNA EXTRACTIONS- Arthropod Preparation for additional instructions)

Warning: Phenol and choloroform are toxic, wear appropriate protective gear (goggles, gloves, lab coat) and handle phenol and choloroform in a fume hood.

1. Place macerated tissue in a sterile 1.5ml centrifuge tube.
2. Digest tissue overnight at 56ºC in 180 µl Buffer ATL and 20 µl Proteinase K (Qiagen DNeasy kit reagents).
3. Centrifuge tube for 20 sec.
4. Optional: Add RNAse to degrade RNA (for high purity DNA extractions). Pipet to mix and maintain tubes at 37°C for 15 min.
5. Add 50 µl phenol and flick tubes to mix solution. Place tubes at 56°C for 10 - 15 min (flick tubes every 2-3 min).
6. Add 50 µl chloroform / isoprophyl alcohol [CHCl/IAA] (24:1) to each tube. Vigorously mix tubes.
7. Centrifuge tubes for 5 min, and during this time, label a new set of tubes. Transfer all of the aqueous phase to the new tubes.
8. 8.Add 100 ul CHCl3/IAA to each tube, flick to mix as in step 6.
9. Centrifuge tubes for 5 min, during this, labeling another set of tubes. Transfer aqueous phase to new tubes.
10. Add 4 µl of 5 M NaCl to facilitate DNA precipitation.
11. Add 200 µl of 100% ethanol (stored at -20°C), and invert tube to mix. Store tubes at -20°C for at least 1 hour.
12. Centrifuge tubes for 10 min. Remove ethanol with pipettor.
13. Add 250 µl 70% ethanol (stored at -20°C) into each tube. Centrifuge tubes for 7-10 min.
14. Remove the ethanol with a pipettor. Place open tubes in hood for 15-30 min.
15. Resuspend DNA pellet in 30-100 µl Elution Buffer (Qiagen), tap the tube repeatedly so the buffer washes completely the side walls. Briefly centrifuge tubes and store at -20°C until use.