**Tweaked Phenol Chloroform DNA extraction for Discovar *de novo* genome sequencing:**

*Protocol written by Carl Yung*

*Preparation prior to extraction:*

 Dissect on dry ice and store in -80⁰C in Eppendorf low-bind tubes until needed.

 Pestle and mortar or Tenbroeck tissue grinder should be soaked in bleach rinsed in RO water and oven dried. Prior to use could be baked @200⁰C four hours so is DNase and RNase free.

 Insect Extraction Buffer (50 mM Tris HCL pH 8.0, 25mM NaCL, 25mM EDTA pH 8.0, 0.1% SDS) should be solution so 20-30 minutes in 55⁰C oven should dissolve the precipitated SDS back into solution.

**!!! USE FILTER TIPS !!!**

* Grind tissue in Tenbroeck tissue grinder in liquid nitrogen and add 200 µl insect extraction buffer
* Move the liquid to a low-bind tube (1.5ml) using a wide bore filter tip (Axygen™ TF-205-WB-R-S)
* Wash the mortar and pestle with 100 µl extraction buffer and add this to the low-bind tube
* Repeat previous step twice (i.e. initial 200 µl + 3X100µl = **500 µl**)
* Add **10** µl proteinase K (20mg/ml) and **1**µl RNAse’A’ (10mg/ml) into the tube with IEB sample.
* Digest 60°C for 3 hours.
* Add **500** µl phenol. Hand mix.
* Centrifuge for 10 minutes 15,000RPM@ RT
* Remove **360** µl upper phase and combine with **300** µl Chloroform
* Mix the chloroform tube and centrifuge 10 minutes 15,000RPM@ RT
* Prepare precipitation tube:

**850** μl 100 % ethanol

**30** μl 3 M NaoAc pH 5.2

* Add **340** µl upper phase of chloroform tube into the precipitation tube. Hand mix.
* Incubate at -20°C O/N

*next day:*

* Cool centrifuge to 4⁰C
* Spin down pellet 4°C max speed 15-30 minutes keep supernatant and freeze further to ppt. more DNA.
* Wash 2 times in 1.5ml freshly made 70%ethanol, centrifuge 10 minutes 15,000RPM@ 4°C.
* air-dry and dilute in **110** µl lowTE pH 8.0 (~100 left after gel and qubit)

*Some results for references:*

Run 2µl sample with 6µl orange G in a 1% agarose gel 100V 60minutes.



