**Phenol Chloroform DNA extraction protocol**

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**Before starting:**

* Cool centrifuge to 4°C
* Prepare all solutions, *i.e.* make sure extraction buffer is correct temperature (37°C) by placing in 55⁰C oven to warm up (will go clear when correct temperature)
* Sterilise equipment ie scalpel, scissors etc using a flame and then wipe using a tissue and ethanol
* Pick which samples you will use and label tubes to minimise the amount of time that the samples are out of the freezer.
* 3.5mm stainless steel beads are acid washed and rinsed in RO water then baked at 200⁰C for four hours before use.

**Dissection**

* Take sample out of freezer and place in clean petri dish on dry ice. Carefully remove the head and two legs. Place wings/legs etc back into the envelope that I am sure is from the sample .

**Between each sample, clean scalpel and scissors with tissue and ethanol and flame.**

* Place the dissected sample into a 1.5ml Lobind Eppendorf microfuge tube.
* Keep tubes in -80⁰C until ready to use.

**DIGESTION**

* Place one sterile 3.5mm stainless steel bead into the sample tube.
* Put 650µl of insect extraction buffer (50 mM Tris HCL pH 8.0, 25mM NaCL, 25mM EDTA pH 8.0, 0.1% SDS) into sample tube.
* Vortex proteinase K. Put 5.2µl (2 units) of proteinase K (10mg/ml) into tube.
* Place in tissuelyserII for 2minutes @ 25Hz.
* Incubate in 55°C oven for 2-3 hours, mixing every 30 minutes by inverting them.

**PHASE SEPARATION**

* Add 500µl of phenol/chloroform (this is toxic so work in fume cabinet) to the lysed sample. Mix thoroughly by inverting until the solution is homogeneous.
* Spin at 13,000 rpm for 5 minutes or more.
* Prepare tubes with 850µl of 100% ethanol (from brown glass bottle) plus 30µl 3 M NaoAc pH 5.2 while the samples are in the centrifuge. At the point, the tubes need to be labelled in great detail.
* After centrifugation, carefully decant with a wide orifice tip ~450µl supernatant into a fresh clean tube and add 450µl chloroform isoamylalcohol mix.
* Spin at 13,000 rpm for 5 minutes or more.
* Carefully decant ~370µl supernatant with a pipette (use a wide orifice tip) and add to the ethanol/ NaoAc tubes that were prepared in the previous step. Do not remove all of the supernatant as this will increase the chance of contamination from the intermediate layer.
* Store in freezer (-20°C) for at least one hour (although can leave it over night).

**PRECIPITATION**

* Spin at 13,000 rpm (or max speed) at 4°C for 5 minutes (can leave it upto an hour).
* Pipette off the ethanol without disrupting the pellet.
* Add 500µl of room temperature 70% ethanol to pellet (do this carefully as squirting it in can disrupt the pellet).
* Centrifuge at 13,000 rpm at 4°C for 1 minute and pipette off ethanol (can be left on for more than one minute).
* Add 500µl of room temperature 70% ethanol to pellet (do this carefully as squirting it in can disrupt the pellet).
* Centrifuge at 13,000 rpm at 4°C for 1 minute and pipette off ethanol (can be left on for more than one minute).
* Air dry the pellet with the lid open.
* Add 200µl Low TE and resuspend the pellet by gently flicking the tube and placing in over at 60°C for 10-15 minutes (or leave at 37°C overnight).