DNA extraction protocol: Genomic DNA extraction using the Salting Out Method with a TNS buffer

Remember: Always include a -ve extraction blank sample alongside each run of extractions

Equipment:

- Heat-block / water bath
- Vortex
- Centrifuge
- Freezer
- Fume hood
- Micro pestles

Consumables:

- Proteinase K (20 mg/mL)
- TNS lysis buffer:
  - Recipe to make 100 mL buffer: 1.2 g Tris-base (1 mM), 0.58 g NaCl (1 mM), 2 g SDS (2%). Make to 100 mL with ultrapure water. Sterilise by filtration
- 5M NaCl
- 70% EtOh (made fresh each week)
- 96-100% EtOH (ice-cold, place in freezer prior to extraction)
- TE buffer: 10 mM Tris–HCl pH 7.5, 1 mM EDTA pH 8.0
- Phenol:chloroform:isoprononal solution (25:24:1; pH *c.* 8)
- Chloroform
- RNAse A (10 mg/mL)

Protocol: Total time: *c. 6.5-7* h

Preparation:

- Switch on the heatblock to 56°C
- Pre-heat and mix the lysis buffer at 50-70°C on a magnetic hot plate
- Place some 96% Ethanol in the freezer

Sample preparation:

In-buffer homogenisation (homogenisation in liquid nitrogen works just as well)

- Collect individual insects (or separated legs and rest of beetle) in 1.5 mL eppendorf tubes
- Add 500 uL lysis buffer and 12.5 uL Proteinase K (if processing multiple samples pre-mix these into a master mix to reduce pipetting time and error)
  - It is often best to add 100 uL of lysis solution for the initial homogenisation and add the remaining 400 uL after the sample has been disrupted, using this to rinse the end of the micro pestle
  - If processing separated insect legs, reduce total lysis buffer volume to 300 uL
- Crush insects using a micro pestle until the sample is disrupted

Sample lysis:

- Incubate samples at 56°C for 3.5 hours. Mix the samples regularly (e.g., every 15-20 minutes) by vortexing for 5 s
- Vortex for 5 s and heat the solution at 96°C for 10 minutes to deactivate Proteinase K
- Let the solution cool and add 20 uL RNAse A, mix by vortex for 5 s and incubate at 56°C for 10 minutes

Phenol-Chloroform step (optional but greatly increases sample quality) – this step should be completed in a fume hood

- Add 500 uL Phenol:Chloroform:Isoproponal solution (25:24:1), mix by vortex for 5 s, and leave to incubate at room temperature for 5 mins
- Add 500 uL chloroform, mix by vortex, and leave to incubate at room temperature for 5 mins. Centrifuge at full speed for 15 minutes
  - Note: If processing separated insect legs, reduce volume of P:C:I and Chlorofotm to 300 uL
- Remove the upper aqueous layer (usually 250-300 uL) and add this to a new Eppendorf tube

DNA precipitation

- Add 2.5 x ice cold (-20°C) 96% ethanol to the sample
- Add sufficient NaCl (5M stock) to give a final NaCl concentration of 0.2 M. For example, if there is a 700 uL solution (200 uL aqueous extract from the chloroform step + 2.5 x EtOH) add 28 uL NaCl and mix vigorously (vortex)
- Incubate the sample at -20°C for 1 h; overnight incubation can also be used.
- Centrifuge at full speed for 20 minutes to pellet the DNA

Sample cleaning

- Discard supernatant and add 70% EtOH (prepared fresh weekly), centrifuge at full speed for 20 minutes
- Repeat this step

Pellet drying

- Discard supernatant and air-dry pellets (if desired use laminar flow to reduce contamination). Usually 15 mins is sufficient to air-dry pellets

DNA suspension

- Resuspend DNA in 85 uL TE buffer (if extracting legs and rest of beetle separately, use a smaller volume, e.g., 55 uL, for resuspension of leg DNA)
  - Buffer can be heated to 56°C to aid with pellet suspension
- Measure DNA concentration using standard protocol
- Store at -20°C until analysis