HMW DNA extraction protocol for individual ant pupae

Modified from 10x Genomics’ protocol available at https://assets.ctfassets.net/an68im79xiti/3oGwQ5kl6UyCocGgmoWQie/768ae48be4f99b1f984e21e409e801fd/CG000145\_SamplePrepDemonstratedProtocol\_-DNAExtractionSingleInsects.pdf

Required:

-Wide-bore pipette tips.

-Lo-Bind 2 ml tubes.

-Liquid nitrogen.

Notes:

-If possible, do this work in a 4 C room (especially the spinning steps, or use a chilled centrifuge). If you do this, avoid chilling anything with SDS – it will freeze. Keep all reagents containing SDS at room temperature.

-Never vortex tubes containing HMW DNA.

-Use wide-bore pipette tips for mixing HMW DNA.

-Pipette slowly at all times to avoid shearing (3 seconds down stroke, 3 seconds up

stroke for wide-bore tips. If using narrow-bore tips, increase the time for each

stroke to 5 seconds.

-For mixing, slowly draw >80% of the solution into the pipette tip then gently

discharge at the solution surface 10 times.

-Centrifuge speeds are calculated for an Eppendorf Centrifuge 5415 C.

Preparation – Buffers:

-Prepare Lysis Buffer containing 10 mM Tris-HCl, 400 mM NaCl, and 100 mM EDTA pH 8.0. **600 ul Lysis Buffer is needed for each sample.**

-Prepare Proteinase K Solution containing 1 mg/ml Proteinase K, 1% SDS, and 4 mM EDTA pH 8.0. **100 ul Proteinase K Solution is needed for each sample.**

Homogenisation:

1. In a 1.5 ml Eppendorf tube snap-freeze a pupa in liquid nitrogen and grind against the tube. Add **600 ul Lysis Buffer**, **40 ul 10% SDS**, and **100 ul Proteinase K Solution**. **Do not allow thawing until reagents have been added!**
2. Vortex for 5 seconds and spin down.
3. **Incubate overnight** (12 – 18 hours) at 37 C. This can be done in a water bath, but is best done on a stirring plate at 37 C (120 rpm is a good speed).

Extraction:

1. Add **4 ul RNase A** and incubate at room temperature for 2 minutes.
2. Add **240 ul 5M NaCl** and mix by inverting 5 times.
3. Centrifuge at 4 C for 15 minutes at 4,500 rpm.
4. Pipette **1.2 ml 100% EtOH** into a 2 ml Lo-Bind tube.
5. Using a wide-bore pipette tip, slowly transfer the supernatant containing the DNA into the EtOH in the Lo-Bind tube. When pipetting into the EtOH, place the tip below the surface of the EtOH. Pipette slowly and avoid picking up any precipitate (the precipitate is protein). *If necessary, spin a second time after removing whatever you can get without picking up any protein and take more supernatant. It’s more important to not get any protein in the EtOH than it is to get all the supernatant out.*
6. Invert the tube a few times to mix.
7. Centrifuge at 4 C for 5 minutes at 10,000 rpm. Make sure the tubes are all oriented the same way in the centrifuge, so that you know where the DNA pellet is.
8. Carefully remove the supernatant, retaining the DNA pellet in the tube. The pellet may be extremely hard to see.
9. Add **500 ul ice cold 70% EtOH** and flick the tube.
10. Centrifuge at 4 C for 5 minutes at 10,000 rpm.
11. Carefully remove the supernatant, retaining the DNA pellet in the tube. Remove all the EtOH you can get.
12. Air dry for 5 minutes. After 5 minutes, if there is any EtOH on the side of the tube, remove with a pipette tip.
13. Using a wide-bore pipette tip, add **35 ul TE** buffer and resuspend the DNA pellet with gentle pipette mixing.
14. Allow the solution to homogenise at room temperature for 1 hour.
15. Store DNA in 4 C fridge for up to 2 weeks, then put in -20 C freezer. **Avoid freezing and thawing at all costs.**