Protocol: HMW-DNA extraction

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HMW-DNA extraction from Animal tissue using PureLink buffers and Homemade magnetic beads

Proteinase K (20 mg/ml) (OMNILAB, PanReac AppliChem, A4392,0010)
RNAse A (DNAse Free) (10 mg/ml)
PureLink™ Genomic Digestion Buffer (Thermofisher ,K182301)
PureLink™ Genomic Lysis/Binding Buffer (Thermofisher, K182302)
200 µl wide orifice pipet tips
SpeedBeads™ (GE65152105050250 from Sigma)
PEG8000 (Promega, V3011)

1. Transfer small piece of tissue (thin slice of thorax of a butterfly, or 3x3mm piece of mouse ear/ or similar) into 12 x 8-tube pcr strips (with separate strip-lids) on dry ice, or into a 96-well plate (I prefer using strip-lids not sealing foils, unless one has an efficient plate sealer to prevent heated sample leakage, during mixing in steps below.)
   (Note: Also, check that your tubes/wells can take around 240 µl of liquid in total)

2. At RT, prepare per sample: **90 µl PureLink Digestion buffer + 20 µl ProtK (20mg/ml).** Then pipette 110 µl of the mix to each tissue sample (96-samples => 9 ml + 2 ml ProtK).

3. Close tightly with the strip-lids, incubate at 58°C for 2-3 hours in thermo-shaker, **no lid heating**, and with shaking (950 rpm) if possible, and manually mix by inverting the tubes more vigorously at least once every 15 min.
   (Note: carefully press the lids every time before manually mixing the strips to prevent sample loss!).

4. Short-spin the tubes, then bring to RT, and add with repeater **pipette 4 µl of RNAseA (DNAse free)** in each sample (stock 10 mg/ml).

5. Close tightly with the strip-lids, mix by inverting, and incubate 10 min at RT.

6. Short-spin the tubes, then add **90 µl Purelink Lysis buffer** into each sample *(this will form white precipitate in some samples, which will disappear during incubation).*
   Note: to increase DNA recovery/purity, add the Lysis buffer with multichannel pipette and with wide orifice tips and mix up and down 5-10 times. This should not affect the size of DNA much…(not compared).

7. Close tightly with the strip-lids, mix and incubate at 58°C for 30 min, with shaking (950 rpm).

8. Spin 10 min at RT, 3000-6000x g to pellet any undigested/solids.

Magnetic-bead-DNA extraction from the lysate

9. Into a new 96-well plate: pipette 75 µl of magnetic beads (recipe below) into each well.
10. Transfer 150 µl of lysate to the 96-well plate containing beads, use **wide orifice** pipet tips.
11. Gently but thoroughly mix 10-15x by pipetting.
12. Incubate 10-15 min at RT.
13. Put the strips on 96-well magnetic stand (“96 Dynamag, side” or other) for 5-10 min.
14. Remove supernatant and wash the beads 2 x with 200 µl 80% EtOH, 2 minutes each.
15. Remove all the EtOH (pipette out from wells 3-5 times with same tips), then let evaporate on magnet for 2-4 min at RT, but do not dry completely.
16. Away from the magnet, add 100 µl of 10mM Tris, pH=8 to the beads to elute the DNA, close tubes but do not mix yet.
17. Let stand at 45C for 15 min and only then invert/mix the tubes 5-10x to gently re-suspend the beads.
18. Incubate another 20 min at RT, and then spin the plate to collect all the liquids.
19. On magnet, with wide orifice pipet tips , transfer DNA to a new plate / or leave DNA in the same tubes with beads.

Preparation of Magnetic-BEADS:

(*15ml, SpeedBeads™(GE65152105050250 from Sigma /or/ GElifesciences Sera-Mag SpeedBead, 65152105050250)*

For 50 ml of bead buffer (recipe below) in a 50 ml Falcon Tube, use 1 ml of STOCK beads.

1. On magnetic stand, transfer 1 ml of the Stock Beads into a 1.5 ml Eppendorf tube.
2. After 1 min remove the solution and wash beads with 1 ml of 10mM Tris-HCl, pH=8
3. On magnet, remove Tris buffer
4. Use some of the Bead Buffer from the Falcon Tube to transfer and re-suspend the beads into the 50 ml of Bead Buffer.

Beads are ready to use, store at 4°C, use at RT.

Magnetic-BEAD BUFFER recipe:

To make 50 ml of Bead-buffer, dissolve in 35 ml of 'molecular biology grade' H_2O:

9 g	*PEG8000 (Promega, V3011)*	*(final = 18% PEG8000)*
7.3 g	*NaCl*	*(final = 2.5 M NaCl)*
500 µl	*1M Tris-HCl, pH=8.0*	*(final = 10mM Tris)*
100 µl	*0.5M EDTA, pH=8.0*	*(final = 1mM EDTA)*

➔ then *add H_2O to 50 ml*

1 : 1 ratio (50 µl sample + 50 µl beads) => binds all DNA above 200 bp
1 : 0.5 ration (50 µl sample + 25 µl beads) => binds all DNA above 600 bp
1 : 0.74 ratio (50 µl sample + 37 µl beads) => binds all DNA above 300 pb