SOP: DNA Extraction From Whole Blood and Liver

Whole Blood: Pipet 2mL of blood into a 15ml conical tube. Centrifuge at room temperature for 20 minutes at 2200 RPM. Pour off and discard the supernatant.

Liver: Place approximately 1g of liver on a microscope slide. Using a different razor blade for each sample, mince the sample thoroughly and transfer to the appropriate 15ml conical tube.

Add 2.26ml of digestion buffer to each sample:

200ul	10% SDS
60ul	RnaseA (10mg/ml)
2ml	NFB

Shake at 37C for 1-3 hours

Add 25ul Proteinase K (20mg/ml)

Shake at 37C overnight.

Transfer lysed solution to a spun down phase lock tube.

Extract twice by adding 2.5 ml of Tris-saturated phenol:chloroform:isoamyl alcohol (25:24:1) pH 8.0

After the addition, vortex each tube vigorously for *at least* 30 seconds

Spin 10 min in the swinging bucket Beckman (GH3.8) rotor at 2200 rpm at 23^{O}C.

Extract once with 2.5 ml of chloroform.

Vortex thoroughly.

Spin 10 min in the swinging bucket (GH3.8) rotor at 2200 rpm at 23^{O}C.

Add 5.5 ml of 100% ethanol and 250uL of 3M Sodium Acetate to a 15 ml conical tube

Precipitate the DNA by gently inverting until complete mixing of the ethanol is achieved.

If a large amount of DNA is present, spool the DNA and wash with 5mL of cold 70% ethanol.

Decant the ethanol, and allow the DNA to dry for 15-30 minutes.

Once the residual ethanol has evaporated, elute DNA 250uL-1mL of 1X TE.

If there are no visible strands, or if there is not enough DNA to spool

Set tube in -20^{O}C freezer for 60 minutes.

Centrifuge for 30 minutes at 3200RPM, 4C

Decant the ethanol and wash the pellet with 5mL of cold 70% ethanol.

Centrifuge for 15 minutes at 3200RPM, 4C

Decant the ethanol, and allow the pellet to dry for no more than 10 minutes.

Elute DNA with 100uL of 1X TE.

Nuclei freezing buffer (NFB): 10 mM Tris-HCl, 400 mM NaCl, 2 mM EDTA, pH 8.0)

10 ml of 1 M Tris-Cl pH 8.0 stock solution
400 ml of 1 M NaCl stock solution
4 ml 500 mM EDTA pH 8.0 stock solution
586 ml Milli-Q water

No adjustment in pH is needed, autoclave and cool to 4^{O}C before use.

RNase A (Dnase-free, 10mg/ml, 100mM Tris-Cl, pH 7.4)

0.5g RNase A (Sigma R4875)

22.5 ml of 10mM NaOAc pH 5.2

Add 2.5ml of 1M Tris-Cl pH 7.4 once RNase A is dissolved. Dispense in 1 ml aliquots and store at -20C.

Proteinase K (20 mg/ml in Milli-Q water)

500 mg proteinase K (Sigma P6556)

25 ml sterile Milli-Q water

Dissolve and dispense in 1 ml aliquots. Store at -20C. Re-freeze immediately after use.

Phenol:chloroform:isoamyl alcohol (25:24:1) saturated with TE (pH 8.0)

Order pre-made molecular biology grade solution from Sigma (cat. # P-2069, 400 ml, ~$105)

Chloroform

Use from stock bottle in hood.

100 % ethanol (absolute)

Fill a 500 ml bottle with 100% ethanol and store at -20^{O}C.

70 % ethanol

350 ml of 100% ethanol

150 ml of Milli-Q water

Combine in a 500 ml bottle and store at -20^{O}C.