MANUAL DNA EXTRACTION FROM BLOOD or LYMPHOCYTES with PHENOL/CHLOROFORM

The cell and nuclear membranes are destroyed by the combined action of SDS and proteinase K. The cell debris from lysis, mainly protein in nature, is captured by organic solvents such as phenol and chloroform.
The DNA, which is insoluble in the organic phase, is present in the non-miscible aqueous phase.
The DNA is precipitated, washed, dried and redissolved in solution, in an aqueous buffer.

Equipment and Materials

- 50 ml SARSTEDT tubes
- Nunc tubes 4.5 ml
- An aspiration system including : a pump, a vacuum trap, a bottle.
- 2-3 DONASET tubules and 1-2 needles (lumbar puncture type)
- Centrifuge
- Disposable gloves

1. REAGENTS, MEDIA & SOLUTIONS

To be kept at room temperature :
TRIS Base PM 121.1g (1kg)
Magnesium Chloride PM 203.3 g (1kg)
NaCl PM 58.44 g (1kg)
EDTA PM 372.4 g (1kg)
HCl 37% (1L)
NaOH (PASTILLE) (500 g)
SDS 20% (500 ml)
Absolute ethanol
Phenol
Chloroform (1l)
Propanol (1l)

To be stored at 4°C :
Proteinase K (500 mg)

2. PREPARATION OF STOCK SOLUTIONS

TRIS 2M pH 7.6
- Weigh 242.2g of Tris Base
- 800ml distilled water
- Adjust to pH 7.6 with HCl (≅ 95 ml)
- Distilled water to 1000 ml

MgCl2 1M
- Weigh 203.3g of $MgCl_2$
- Distilled water to 1000 ml

NaCl 3M
- Weigh 175.32g of NaCl
- Distilled water to 1000 ml

EDTA 0.4 M PH 8
- Weigh 148.96 g of EDTA
- Weigh 18 g of NaOH
- Water 800 ml

- After dissolving, adjust to pH 8 by adding Na OH, pastille by pastille
- Distilled water to 1000 ml

These stock solutions should be stored at +4°C and are used for the preparation of working solutions.
Working solutions are prepared in sterile graduate cylinders using sterile distilled water (cover with parafilm to mix) and stored at +4°C.

3. PREPARATION OF WORKING SOLUTIONS

3.1 SLR

- TRIS 2M pH 7.6	10 ml
- MgCl2 1M	10 ml
- NaCl 3M	6.6 ml
- Distilled water to	2000 ml

3.2 SLB

- TRIS 2M pH 7.6	10 ml
- EDTA 0.4 M pH 8	50 ml
- NaCl 3M	34 ml
- Add 500 ml water, shake (parafilm)	
- SDS 20%	20 ml
- Distilled water to	2000 ml

3.3 SLB Solution / Proteinase K

Δ Gloves must be worn

Dissolve 500 mg of proteinase K powder (stored at +4°C) in 10 ml SLB. Pour this solution in a 2 L graduated cylinder containing 500 ml of SLB, rinse the flask with 10 ml of this mixture, and adjust the volume of SLB to 1250 ml.
Freeze this solution at –20°C in small aliquots.

3.4 TE 10^{-1}

- TRIS 2M pH7.6	5ml
- EDTA 0.4 M pH8	2.5 ml
- Distilled water to 1000 ml	

3.5 Saline

- NaCl 3M	50 ml
- Distilled water to 1000 ml	

3.6 Ethanol Solution 70 %

- TE 10^{-1}	600 ml
- Absolute ethanol to 2000 ml	

Procedure

1. LYSIS OF RED BLOOD CELLS

1.1 Discarding plasma

- Pool the tubes of blood in a 50 ml GREINER tube
- Centrifuge for 10 min at 2000 rpm
- Aspirate the plasma without touching the leukocyte layer (buffy coat)
- Mix the red blood cells and leukocytes

1.2 Lysis of red blood cells

- Lyse in a 50 ml final volume with a solution of SLR
- Centrifuge for 10 min at 2000 rpm
- Aspirate about 45 ml of lysed red blood cells -Resuspend the pellet using a sterile pipette, and make up to 50 ml with SLR
- Centrifuge 5 min at 2000 rpm
- Remove the supernatent
- If red blood cells still remain, resuspend the cellular pellet in 5 ml of SLR
- Proceed to a third centrifugation and remove the supernatant.
- Freeze the pellet at -80°C

2- LYSIS OF LEUKOCYTES

- Defrost the pellet in a water bath at 50°C for one minute
- Lyse the leukocytes in one volume of SLB + proteinase K (see 3.3); the volume to use depends on the size of the pellet:
  -very small pellet: 5 ml
  -small pellet: 10 ml
  -nice pellet: 15 ml
- Homogenize the tube with gentle rotation
- Incubate overnight at 42°C with agitation

3- EXTRACTION OF PROTEINS

Step 1:

- Add one volume of phenol-chloroform equal to the volume of SLB-Proteinase K-
- Stir for 10 min
- Aspirate the lower phase after stabilization or after centrifugation for one minute at 2000 rpm
- Repeat Step 1, with 5 minute-agitation
- Depending on Step 1, proceed to the extraction with chloroform, with 5 minute-agitation at each step.

4- DNA PRECIPITATION & WASH

- Precipitate the DNA with 1 to 1.5 volumes of absolute isopropanol (propanol 2) in the presence of NaCl, 60 mM final concentration
- Homogenize with gentle rotation until the DNA precipitate (medusa) appears
- Discard the liquid by transferring the medusa to a 5 ml Nunc tube
  Wash the medusa twice with 3 ml of isopropanol.
- Wash the medusa three times with 3 ml of 70% ethanol in TE 10^{-1}
- Remove the liquid between each wash
- Dry the medusa at room temperature

5- RESUSPENSION OF DNA

- When the medusa is translucid and therefore dry, resuspend it in TE 10^{-1} (depending on the size of the medusa, suspend the DNA in 0.5 to 2 ml of TE 10^{-1}).
- Incubate overnight at 37°C with rotation
- Place it at 4°C for one week
- Then you can store the tube at –20°C for a long period of time

6- CHECK THE QUANTITY/QUALITY OF DNA (see QUALITY CONTROL OF DNA protocol)