DNA Preparation from Blood

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Reagents

Ammonium chloride (NH_4Cl)
Chloroform
Mallinckrodt, Cat. 4440
Ethanol, absolute
HCl
Isoamyl alcohol
Sigma, Cat. I-3643
Isopropanol (2-Propanol)
Phenol
Potassium carbonate ($KHCO_3$)
Proteinase K
EM Science, Gibbstown, WV, Cat. 24568-2 (100 mg)
Sodium acetate, 3M, ph 5.2
Quality Biological Inc., Cat. 351-035-060
Sodium chloride
Sodium EDTA (Na_2EDTA)
Sodium dodecyl sulfate (SDS 10%)
EM Science, Cat. 24568-2
Tris EDTA buffer, pH 8.0

Preparation

Lysis Buffer

NH_4Cl	8.29 g	f.c. [155 mM]
$KHCO_3$	1 g	f.c. [10 mM]
Na_2EDTA	0.034 g or 200 µl EDTA 0.5 M	f.c. [0.1mM]

Fill to 1000 ml with distilled water
Adjust to pH 7.4 with 1 M HCl or NaOH for each use

Chloroform/Isoamyl alcohol 24:1

Chloroform	24 ml
Isoamylalcohol	1 ml

SE-Buffer
NaCl 4.39 g f.c. [75 mM]
Na_2EDTA 8.41 g or 50 ml EDTA 0.5 M f.c. [25 mM]
Fill to 1000 ml with distilled water
Adjust to pH 8.0 with 1 M NaOH for each use

Sodium acetate
3 M Sodium acetate 246 g/L
Adjust to pH 5.2 with CH_3COOH

Proteinase K (10mg/ml)
Dissolve 100 mg Proteinase K in 10 ml TE for 30 min at room temperature
Aliquot and store at –20˚C

Procedure

1. To 10 ml whole blood (EDTA, heparin, citrate) add 30 ml lysis buffer, shake gently, incubate for 30 min on ice, and centrifuge at 1200 rpm for 10 min at 4° C.

2. Remove supernatant (blood waste), add 10 ml lysis buffer, resuspend the pellet, and centrifuge for 10 min at 4°C (1200 rpm).

3. Remove supernatant (blood waste), add 5 ml SE-buffer, resuspend the pellet, and centrifuge for 10 min at 4°C (1200 rpm).

4. Remove supernatant (blood waste).
   (It is possible to store the pellet at -80°C. To do so, add 1 ml SE-buffer and resuspend the pellet. Use a cryo-tube and centrifuge at 1200 rpm for 10 min at 4°C. Remove the supernatant and freeze the pellet.)
   Add 5 ml SE-buffer and resuspend the pellet, add 40 μl proteinase K (10 mg/ml) and 250 μl 20% SDS, shake gently, and incubate overnight at 37°C in a water bath.

5. Add 3 ml phenol, shake by hand for 10 min, and centrifuge at 3,000 rpm for 5 min at 10°C.

6. Transfer the supernatant into a new tube, add 1.5 ml phenol (check the volume) and 1.5 ml chloroform/isoamyl alcohol (25:24:1), shake by hand for 10 min, and centrifuge at 3,000 rpm for 5 min at 10°C.

7. Again transfer the supernatant into a new tube, add 3 ml (check the volume) chloroform/isoamylalcohol (24:1), shake by hand for 10 min, and centrifuge at 3,000 rpm for 5 min at 10°C.

8. Transfer the supernatant into a new tube, add 300 µl 3 M sodium acetate (pH 5.2) and 10 ml isopropanol, shake gently until the DNA precipitated, use a glass pipette, make a hook over a bunsen burner, and capture the DNA.

9. Wash the DNA in 70% ethanol and dissolve the DNA in ddH_2O overnight at 4°C on a rotating shaker. (If the DNA is not dissolved leave it longer at 4˚C on the rotating shaker).

10. Measure the DNA concentration and run 1-5 µl (approximately 200 ng) for Gel electrophoresis on agarose gel (1%) in 1xTAE buffer. Also, measure the DNA with NanoDrop and print out for future reference.