

2004

DNA Preparation from Blood

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Ammonium chloride (NH₄Cl)

Chloroform

Mallinckrodt, Cat. 4440

Ethanol, absolute

HCl

Isoamyl alcohol

Sigma, Cat. I-3643

Isopropanol (2-Propanol)

Phenol

Potassium carbonate (KHCO₃)

Proteinase K

EM Science, Gibbstown, WV, Cat. 24568-2 (100 mg)

Sodium acetate

Sodium chloride

Sodium EDTA (Na₂EDTA)

Sodium dodecyl sulfate (SDS 10%)

Digene Diagnostics, Inc, Cat. 3400-1016

Tris EDTA buffer, pH 8.0

Preparation

Lysis Buffer

NH₄Cl 8.29 g f.c. [155 mM]

KHCO₃ 1 g f.c. [10 mM]

Na₂EDTA 0.034 g or 200 μ l 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]
Na₂EDTA 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 CH₃COOH

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 5 ml SE-buffer and 10 ml phenol, shake by hand for 10 min, and centrifuge at 3000 rpm for 5 min at 10°C.
6. Transfer the supernatant into a new tube, add 10 ml phenol/chloroform/isoamyl alcohol (25:24:1), shake by hand for 10 min, and centrifuge at 3000 rpm for 5 min at 10°C.

7. Again transfer the supernatant into a new tube, add 10 ml chloroform/isoamylalcohol (24:1), shake by hand for 10 min, and centrifuge at 3000 rpm for 5 min at 10°C.
8. Transfer the supernatant into a new tube, add 300 μ l 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 0.5-1 ml TE-buffer 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 in a spectrophotometer (Pharmacia, GeneQuant) and run 200 ng on a 1% agarose gel.