Semnan BioBank

MANUAL DNA EXTRACTION FROM BLOOD through SALTING OUT PROCEDURE

One of the obstacles encountered when extracting DNA from a large number of samples is the cumbersome method of DNA extraction with the organic solvents phenol and chloroform.
This method is rapid, safe and does not require expensive and environmentally hazardous reagents and equipment.

Equipment and Materials

- Polypropylene tubes 15ml
- Lysis buffer (10mM Tris-HCL,400mM NaCl, 2mM Na_2EDTA, pH 8.2)
- SDS 10%
- Proteinase K solution (1 mg proteinase K in 1% SDS and 2 mM Na_2 EDTA).
- Centrifuge
- Absolute ethanol
- TE buffer (10mM Tris-HCL, 0.2mM Na_2 EDTA, pH 7.5)
- Disposable gloves
- Gilson pipette

Procedure

1. Resuspend the buffy coats of nucleated cells obtained from blood with anticoagulents (ACD or EDTA) with 3ml of nuclear lysis buffer.
2. Digest the cell lysates, with 0.2 ml of 10% SDS and 0.5 ml of proteinase K solution, overnight at 37 °C.
3. Add 1ml of saturated NaCl (6M) to each tube and shake vigorously for 15 seconds.
4. Centrifuge for 15 minutes at 2500 rpm.
5. Transfer the supernatant containing the DNA to another 15ml polypropylene tube, the precipitated protein pellet is left behind at the bottom of the tube.
6. Add 2 volumes of absolute ethanol and invert the tubes several times until the DNA precipitates.
7. Remove the precipitated DNA with a plastic spatula or pipette and transfer to a 1.5ml microcentrifuge tube containing 100-200 microliter TE buffer
8. Dissolve the DNA for 2 hours at 37°C
9. Store the tube at +4 or –20°C.
10. Check quantity/quality of DNA (see QUALITY CONTROL OF DNA protocol)

Reference

Miller S.A, Dykes D.D, Polesky H.F : *A simple salting out procedure for extracting DNA from human nucleated cells*. Nucleic Acids Research 1988; V16 Number 3 : 1215