

A rapid procedure for extracting genomic DNA from leukocytes

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We have developed a rapid procedure, based on standard protocols, to isolate DNA from leukocytes. The procedure yields 100 μg to 150 μg from 5 ml of blood. The DNA produced is of good quality (Fig. 1) and is suitable for restriction enzyme digestion or PCR amplification.

METHOD

Conduct all steps at room temperature unless otherwise stated.

1. Collect 5 ml of blood in a vacutainer tube (Becton Dickinson) containing EDTA and mix.
2. Make volume up to 10 ml with solution 1 (10 mM Tris pH 7.6; 10 mM KCl; 10 mM $MgCl_2$).
3. Add 120 μl Nonidet P40 (BDH) to lyse the cells. Mix well by inverting several times.
4. Spin down the nuclear pellet at 2000 rpm for 10 mins.
5. Pour off the supernatant without dislodging the pellet. The pellets can be stored frozen.
6. Gently resuspend pellet well in 800 μl of solution 2 (10 mM Tris pH 7.6; 10 mM KCl; 10 mM $MgCl_2$; 0.5 M NaCl; 0.5% SDS; 2 mM EDTA). Solution 2 will lyse the nuclei so be careful not to shear the DNA. Transfer to a 1.5 ml microcentrifuge tube.
7. Add 400 μl of distilled phenol (saturated with 1 M Tris pH 8.0) and mix well.
8. Microfuge for 1 min at 12000 rpm. Transfer upper phase to a clean microfuge tube. Do not worry about transferring a little of the interface.
9. Add 200 μl of phenol and 200 μl of chloroform:isoamyl alcohol (24:1). Mix well by inverting.
10. Spin for 1 min at 12000 rpm. Transfer upper phase to a clean microfuge tube.
11. Add 700 μl of chloroform:isoamyl alcohol and extract as above.
12. Transfer upper aqueous phase to a small clean container. Avoid removing the interface. Add 2 volumes of ice cold ethanol and mix to precipitate the DNA*.
13. With the sealed tip of a pasture pipette, transfer the DNA fibres to a microcentrifuge tube containing 1 ml of 70% ethanol. Mix well to wash the DNA.
14. Spin for 5 mins at full speed. Discard the ethanol and dry pellet in a speed vac. Resuspend DNA in sterile water at 65°C. Do not over-dry genomic DNA or it will be difficult to resuspend.

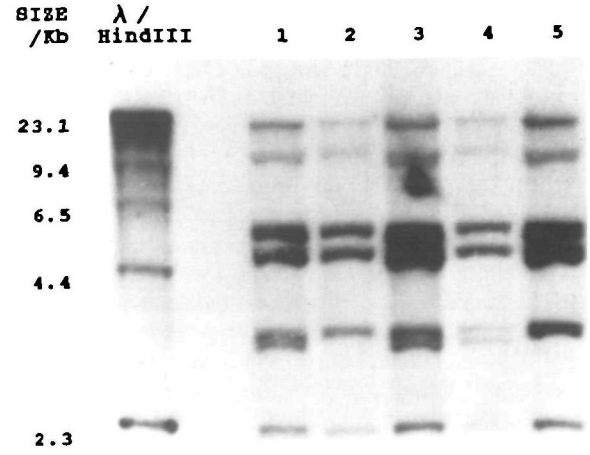

Figure 1. Southern analysis. DNA samples were digested with *Hin*dIII, electrophoresed and blotted onto a nitrocellulose membrane and hybridized with a full-length radiolabelled phenylalanine hydroxylase (PAH) cDNA probe. Lanes 1, 2 and 3 contain DNA isolated by standard procedures while lanes 4 and 5 contain DNA isolated with this protocol. The DNA in all lanes appears to be of similar quality.

*If the blood is old (1 week or so) very little DNA may precipitate out. If so, proceed as follows:

Place sample at −20°C overnight. Transfer 1.5 ml to a microcentrifuge tube and spin for 10 mins at full speed. Discard the ethanol and add the remainder of the sample. Spin as above. Discard the ethanol. Wash the pellet in 70% ethanol and continue as in step 14.

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