# A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies

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In genetic linkage studies using the restriction fragment length polymorphism (RFLP) technique, it is essential to process effectively large numbers of blood samples. One of the problems faced when extracting DNA by standard methods is the requirement of deproteinizing cell digests with hazardous organic solvents like phenol, chloroform and isoamyl alcohol (1-3). The method described in this report avoids the use of any organic solvents. This is achieved by salting out the cellular proteins by dehydration and precipitation with a saturated sodium chloride solution (4). Most of the procedures also involve prolonged incubation with proteinase K (1, 4, 5). Our procedure eliminates completely the use of proteinase K treatment. The method reported here is the most economical, safe and rapid for preparation of DNA from whole blood.

## PROCEDURE

1. Collect whole blood in a Vacutainer tube (purple-stoppered) containing 100 μl of 15% EDTA.
2. Transfer 5 ml of blood into a 15 ml centrifuge tube and add 5 ml of low salt buffer containing 10 mM Tris-HCl pH 7.6, 10 mM KCl, 10 mM $MgCl_2$ and 2 mM EDTA (TKM1).
3. Add 125 μl of Nonidet P-40 (NP-40, Sigma) to lyse the cells. Mix well by inversion several times.
4. Centrifuge at 2200 RPM for 10 min at room temperature (RT) in a Beckman table-top centrifuge (model TJ-6).
5. Slowly pour off the supernatant and save the nuclear pellet (the small pellet at the very bottom of the tube) and wash the pellet in 5 ml of TKM1 buffer and centrifuge as before.
6. Gently resuspend the pellet in 0.8 ml of high salt buffer containing 10 mM Tris-HCl pH 7.6, 10 mM KCl, 10 mM $MgCl_2$, 0.4 M NaCl and 2 mM EDTA (TKM2).
7. Add 50 μl of 10% SDS and then mix the whole suspension thoroughly by pipetting back and forth several times and incubate for 10 min at 55°C.
8. Add 0.30 ml of 6 M NaCl in the tube and mix well.
9. Centrifuge at 12000 RPM for 5 min, in microcentrifuge.
10. Save the supernatant containing DNA and discard the precipitated protein pellet at the bottom of the tube.
11. To the supernatant add 2 volumes of 100% ethanol at RT and invert the tube several times until the DNA precipitates.
12. Remove the precipitated DNA strands and put them in a microcentrifuge tube containing 1 ml of ice-cold 70% ethanol.
13. Microfuge for 5 min at 12000 RPM at 4°C.
14. Dry the pellet in a Speed-vac and resuspend DNA in 0.5 ml of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 at 65°C for 15 min.
15. Measure the concentration of DNA by taking $A_{260}$ and $A_{280}$ and checking the quality of DNA by agarose gel electrophoresis.

## RESULTS AND DISCUSSION

1. In our hands, this procedure yields more DNA than any other methods (1-5), generally in the range of 130-160 μg from 5 ml of blood. We have also found more yield and less time to extract DNA from whole blood by this 'Rapid method' than using an A.S.A.P. DNA isolation kit (5). Our method has worked successfully with 20 samples of blood from different persons.
2. The DNA preparation is free of RNA, protein and degrading enzymes. The uncut DNA is seen as a typical slow-migrating, high molecular weight and undegraded species in an ethidium bromide-stained agarose gel. The DNA produced is of good quality and is suitable for restriction enzyme digestion. We have used *Bam*HI, *Eco*RI, *Hind*III, *Kpn*I, *Pst*I, *Pvu*II, *Sac*I and *Taq*I restriction enzymes to digest the DNA successfully. We have found that the 'Rapid method' can yield high molecular weight DNA that can be analyzed in standard Southern blot experiments.
3. Sometimes the plasma is used for other purposes and only cells are available for DNA extraction. We have obtained by this 'Rapid method' an adequate amount of DNA from packed cells.
4. This procedure works equally well with fresh blood samples, with those that are stored at 4°C, and with those stored at -70°C.

## SIGNIFICANCE

1. This procedure eliminates completely the use of any toxic reagents such as phenol, chloroform and isoamyl alcohol.
2. The 'Rapid method' is complete in less than an hour.
3. It eliminates the step of prolonged digestion of samples with proteinase K, thus saving the cost and time of operation.
4. This method yields 150 μg of DNA from 5 ml of blood.

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