Low cost non-hazardous modified extraction method of DNA from human blood

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Abstract

A simple, relatively non-expensive, and non-dangerous method was used isolate DNA from human blood. Several toxic organic solvents were omitted in this procedure and compensated with salting out technique representing by utilization of NaCl instead. Since significant resolution of DNA bands were identified this procedure could be used in routine clinical laboratories and might be suitable for restriction fragment length polymorphism (RFLP) analysis.

طريقة استخلاص محورة قليلة الكلفة وغير خطرة للدنا من دم البشر

الخلاصة

تم استخدام طريقة بسيطة وغير مكلفة نسبياً وغير خطرة لعزل الدنا من دم البشر. ولم تستخدم العديد من المذيبات العضوية السامة في هذه الطريقة وتم تعويضها باستخدام تقنية التمليح الخارجي المتمثلة باستخدام كلوريد الصوديوم بدلاً عنها. وبما أن حزم مميزة من الدنا قد تم تشخيصها فأن هذه الطريقة من الممكن أن تستخدم في المختبرات السريرية الروتينية وربما تكون ملائمة لتحليل تعدد الأشكال المقطوعة بانزيمات التقييد (FRLP).

Introduction

One of the obstacles encountered during extracting DNA from a large number of samples is the cumbersome method of deproteinizing cell digests with the hazardous organic solvents such as phenol, chloroform and iso-amyl alcohol (1). Several other non-toxic extraction procedures have been published, but require either extensive dialysis (2) or the use of filters (3, 4). A safe and relatively inexpensive method was developed to simplify the deproteinization procedure. Some researchers were used salting out of the cellular proteins by dehydration and precipitation with a saturated NaCl solution, while other used ammonium acetate instead of NaCl (5). By using of NaCl, hazardous organic solvents like phenol, chloroform and isoamyl alcohol were avoided (6, 7, 8, 9). The only expensive material used in this report was proteinase K to keep the sensitivity of procedure, otherwise the method reported here is the most economical and safe for preparation of DNA from whole blood.

Materials and methods

All steps except the final one (visualizing step) were undertaken in biology department / college of science for ladies / Babylon university.

DNA isolation**:** Miller *et al.* method (10) was considered with some modifications correlated with dealing with whole blood instead of Buffy coat alone (11) and other suitable modifications: 5 ml of blood was collected from 8 donators and placed immediately in sterile EDTA tubes (vacutainer corporation, UK) and was shaked back and forth several times. Blood was placed in a sterile 10 ml centrifugation tubes and centrifuged (Gemmy industrial corp. – Taiwan) at 4000 rpm for 10 min at room temperature. Supernatant (blood

plasma) was removed. Pellet was washed with 2 ml of normal saline. Tubes were centrifuged at 2500 rpm for 10 min at room temperature. Supernatant was removed, washing and destruction of RBCs was taken place with 3 ml of distilled water. Tubes were centrifuged at 4000 rpm for 15 min at room temperature to remove supernatant. Pellet was suspended with 10 ml erythrocyte lytic buffer (0.05 M Tris-Cl, 0.5% SDS, 0.1 M NaCl, 0.001 M Na2EDTA pH 7.2) containing 100 µg / ml proteinase K. The mixture was incubated overnight in moderate temperature incubator (Memmert - Germany) at 37˚C.

After digestion was complete, 200 µl of 25% SDS and 4 ml of 5 M NaCl were added to each tube and shaken vigorously for 15 seconds, followed by centrifugation at 2500 rpm for 15 minutes. The precipitated protein pellet was left at the bottom of the tube and the supernatant containing the DNA was transferred to another 15 ml polypropylene tube. Exactly 1 volume of freeze cold (-20˚C) isoprobanol was added and the tubes were inverted several times until the DNA was precipitated. The precipitated DNA strands were removed with a Pasteur-pipette and washed with 70% ethanol, then transferred to a 1.5 ml micro-centrifuge tube containing 100 µl of TE buffer (10 mM Tris-HCl, 0.2 mM Na2EDTA, pH 7.5). The DNA was allowed to dissolve for 15 minutes at room temperature and stored in -20˚C until electrophoresis.

Agarose gel electrophoresis conditions: Electrophoresis was done in 27x17.5x10 cm tank dimensions, 15x15 cm tray dimensions, 15x1.0 mm comb dimensions (agarose electrophoresis unit was purchased from Labnet international corporation, Korea). Agarose concentration was 1%. 25 µl DNA suspension was applied to the gel after mixing with 5 µl (1X) loading buffer (40% w/v sucrose and 0.25% w/v bromophenol blue). Electrophoresis was done under 90 V, 20 mA at room temperature for 2.5 hours. Agarose gel was stained with ethidium bromide for 30 minutes, then washed and placed in UV light translluminator (Herolab – Germany) and submitted to 256 nm wavelength and photographed by 7.2 Mega pixel digital camera (Sony-China).

Results and Discussions

After blood samples were collected, it was found that dealing with Buffy coat alone was difficult particularly with specimens of small quantity. So, we have used a procedure made us capable of dealing with Buffy coat without surrendering to remove it from the whole blood. This step was avoided successfully by our mentioned procedure. That was taken place by multiple steps to destroy all escorting cells except the nucleated cells in Buffy coat in only single tube.

The DNA preparation is free of RNA and degrading enzymes. The uncut DNA is seen as a typical slow-migrating, high molecular weight and un-degraded species in an ethidium bromide-stained agarose gel. The DNA produced was of good quality and was might be suitable for restriction enzyme digestion (12).

Miller et al. (10) described a similar procedure to the procedure mentioned here, they realized that DNA could be obtained in a low cost procedures, and could be yielded quantities comparable to those obtained from phenol-chloroform extractions. The 260/280 ratios were consistently 1.8-2.0, demonstrating good de-proteinization. Restrictions were performed using a number of different enzymes requiring high, medium or low salt concentrations, all resulting in complete restriction. This procedure could be used in routine clinical laboratories to diagnose several thousand blood samples for parentage, population and forensic studies. Dykes et al. (13) showed that non phenol-chloroform extraction methods was competent enough to be used in RFLP analysis.

It found that the most significant benefit of this procedure was the complete elimination of any toxic and cost effective organic solvent such as phenol, chloroform, and iso-amyl alcohol, this method could be used in clinical chemistry laboratories as well. The only prolonged step was the incubation with proteinase K overnight, otherwise the whole procedure could be finished in less then 5 hours to as many as 14 blood specimens which could be handed in one cycle. We were found that DNA profile in this method was similar to DNA profiles of the standard

methods (14). This procedure wasn't wanted large volume of blood (10 – 15 ml) like the most popular methods require (15, 16). It was mentioned that DNA obtained in some non-organic procedures was readily digestible with all restriction enzymes tested (17). But it is not known yet weather this modified procedure suitable for restriction endonuclease digestion like the standard methods. So the human blood DNA obtained in this procedure (figure 1) should be tested by the restriction endonuclease digestion.

References

1. Sambrook, J. and Russell, D. W. (2001). Molecular cloning- a laboratory manual. Cold Spring Harbor, NY: *Cold Spring Harbor Laboratory Publications* (third edition), vol. 1
2. Longmire, J., Albright, K., Lewis, A., Meincke, L. and Hildebrand, C. (1987). A rapid and simple isolation of high molecular weight cellular and chromosome-specific DNA in solution without the use of genetic solvents. *Nucleic Acids Research.* 15, 859.
3. Leadon, S. and Cerutti, P. (1982). A rapid and mild procedure for the isolation of DNA from mammalian cells. *Analytical Biochemistry;* 120 (2), 282-288.
4. Adeli, K. and Ogbonna, G (1990). Rapid Purification of Human DNA from Whole Blood for Potential Application in Clinical Chemistry Laboratories. *Clinical Chemistry*; 36/2: 261-64.
5. Blin N. and Stafford D. W. (1976). A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Research. 3: 2303-2308.
6. Gross-Bellard, M., Ouder, P. and Chambon P. (1973). Isolation of high molecular weight DNA from mammalian cells. European Journal of Biochemistry. 36: 32-38.
7. John. S.W.M. G., Weitzner, R., Rozen, and Scriver, C.R. (1991). A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acids Research. 19: 408.
8. Madisen.L., Hoar, L. D., Holroyd, C.D., Crisp, M., Hodes, M. E. (1987). DNA banking: the effect of storage of blood and isolated DNA on integrity of DNA. *American Journal of Medicinal Genetics*. 27: 379-390.
9. Siafakas N, Burnett L, Bennetts B, Proos A (1995). Nonenzymatic extraction of DNA from blood collected into serum separator tubes [Technical Brief]. *Clinical Chemistry;* 41:1045–6.
10. Miller S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells (1988). *Nucleic Acids research;* 16:1215.
11. Al Faisal, A. (1999). A. Laboratory techniques in genetic engineering. A laboratory manual, first edition, *Al-Daar Al-Ahlia for publication and distribution, Amman, Jordon* (In Arabic).
12. Lahiri D, Nurnberger J. (1991). A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Research*; 19:5444.
13. Dykes, D., Fondell, J., Watkins, P. and Polesky, H. (1986) The use of biotinylated DNA probes for detecting single copy human restriction fragment length polymorphisms separated by electrohoresis. *Electrophoresis*; 7, 278-282.
14. Tas S. (1990). Purification of DNA from clotted blood [Technical Brief]. *Clinical Chemistry;* 36:1851.
15. Kunkel, L. M., Smith, K. D., Boyer, S. H. (1977). Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. *Proceeding National Academic of Science USA*; 74: 1245-9.
16. Goossems, G. M., Kan, Y. Y. (1981). DNA analysis in the diagnosis of hemoglobin disorders. *Methods in Enzymology*; 76: 805-17.

17. Grimberg J., Nawoschik S., Belluscio, L., McKee, R., Truck, A. and Eisenberg, A (1989). A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucleic acid research*; 8: 8390.

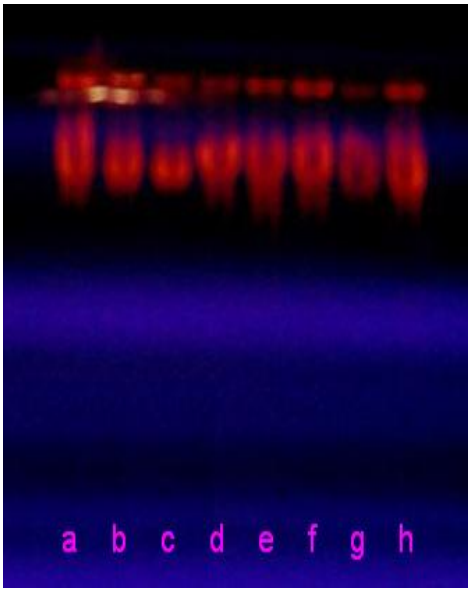

Figure (1): Electrophoresis of 1% Agarose gel electrophoresis displaying genomic DNA prepared from whole blood using the low cost non-hazardous DNA extraction method. All lanes (from a to h) were run at 90 V/ 20 mA for 2.5 hrs. DNA specimens were made visible after staining with ethidium bromide and visualization under 256 nm. The direction of electrophoresis was from top to bottom.