Rapid Isolation of PCR-Ready DNA from Blood, Bone Marrow and Cultured Cells, Based on Paramagnetic Beads

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ABSTRACT

PCR-based methods for the analysis of genomic DNA are becoming increasingly common both in research and for routine purposes. A rapid, small-scale DNA isolation method is needed to take full advantage of the speed and automation potential of the PCR technology. We demonstrate the use of Dynabeads® DNA DIRECT™, a kit for the isolation of PCR-ready genomic DNA from whole blood, bone marrow or cultured cells in less than 10 min. The method is based on adsorption to magnetic beads prior to magnetic separation and involves no centrifugation steps or organic solvents. The yield and quality of the DNA is comparable to traditional large-scale methods. One isolation is enough for at least ten PCRs.

INTRODUCTION

Traditionally, DNA extraction from whole blood has been a time-consuming process. For diagnostic and forensic purposes, polymerase chain reaction (PCR) has become a method of choice for analysis of genomic DNA. Since PCR is a fast and often automated technique, genomic DNA isolation is a rate-limiting step.

Several short protocols for the isolation of PCR-ready genomic DNA from blood have been published during the last few years. The most widely used is probably the cetyltrimethylammonium (CTAB) method of Gustincich and coworkers (1), which is not recommended for blood containing heparin. This is a weakness that is common among short protocols.

The commercial products available may be divided into column-based products and products that are based on precipitation steps. Differences within these groups are considerable, but two general points can be made: (i) precipitation steps are often rate limiting and always difficult to automate, as they include centrifugation and (ii) columns, though automated with relative ease, are often difficult to scale down to small sample volumes.

In this article we evaluate Dynabeads® DNA DIRECT™, a product with which a unique combination of surface chemistry and lysis solution allows binding of soluble genomic DNA to the surface of superparamagnetic, monodisperse particles. Purification of the DNA is performed by two washing steps and a resuspension. Optionally, DNA can be eluted from the solid phase. All steps are simplified by the paramagnetic properties of the beads.

MATERIALS AND METHODS

Biological Material

Blood from healthy donors, except from fresh capillary blood, was kindly provided by Professor Peter Kierulf at Ullevål Hospital in Vacutainer® tubes with anticoagulants (Vacutainer Systems; Becton Dickinson, Rutherford, NJ, USA). Anticoagulants used were heparin, EDTA, citrate and anhydrous citrate dextrose (ACD). The anticoagulant citrate phosphate dextrose (CPD) was obtained from Baxter Healthcare (Thetford, England, UK) and was mixed with fresh capillary blood in the ratio 1:9. Bone marrow samples from healthy donors were kindly provided by Professor Steinar Funderud, the Norwegian Radium Hospital. Cultured cells used in this study were of the Burkitt’s lymphoma cell line Daudi (CCL 213; ATCC, Rockville, MD, USA), grown to a density of $5 \times 10^5$ cells/mL in RPMI 1640 supplemented with 9% fetal bovine serum (FBS) and 1 mM sodium pyruvate.

Phenol Extraction/Ethanol Precipitation-Based Isolation of DNA

Control DNA for comparison with DNA DIRECT-isolated DNA was isolated according to the method of John and coworkers (2).
Isolation of DNA with the Dynabeads DNA DIRECT Kit

The Dynabeads DNA DIRECT kit is commercially available from Dynal AS (Oslo, Norway). Lysis of DNA-containing cells was obtained by mixing 200 µL (one sample test) of Dynabeads DNA DIRECT with 5 µL heparinized blood, 10 µL of other blood samples or 1–5 µL of heparinized bone marrow in a 1.5-mL microcentrifuge tube. Lysates were then left at room temperature for 5 min to allow adsorption of genomic DNA to the Dynabeads.

The DNA/Dynabeads complex was attracted to a magnet (the Dynal Magnetic Particle Concentrator [MPC®], which conveniently combines a magnet with a tube rack), and the lysate was aspirated and discarded.

The complex was then washed twice in washing buffer (supplied in the kit) by attracting it to the tube wall in the Dynal MPC and discarding the supernatant. Finally, the complex was resuspended in 20–40 µL of TE, pH 8.0 (provided in the kit). We used 40 µL as standard volume, but 20 µL if the starting material was heparinized blood. Unless otherwise stated, both PCR and agarose gel electrophoresis were performed directly on the DNA suspension in TE with the Dynabeads present. The initial heating step of a normal PCR is sufficient to release the DNA from the beads, as is the electric field of agarose gel electrophoresis.

When DNA was isolated from blood diluted by a factor of a hundred or more, only 100 µL (0.5 sample test) of DNA DIRECT were used, and the washing and resuspension volumes were adjusted accordingly. In such experiments, the DNA/Dynabeads complex was resuspended in water, and the DNA was eluted from the beads by heating to 65°C for 5 min prior to PCR.

DNA isolation from 4 × 10^6 culture cells (cell line Daudi) was performed as described for undiluted samples, except that 1 mL (5 sample tests) of Dynabeads DNA DIRECT was used. Accordingly, the washing steps were performed in 1 mL washing buffer. The DNA/Dynabeads complex was resuspended in 120 µL TE, and no elution step was performed after the resuspension.

PCR Amplification

All PCRs were performed in a 50-µL reaction volume, 10× PCR buffer (Perkin-Elmer, Norwalk, CT, USA) was added to a final concentration of 1×, dNTPs (Pharmacia Biotech, Piscataway, NJ, USA) were added to a final concentration of 0.2 mM and 1 U of AmpliTaq® (Perkin-Elmer) was used per reaction. Five picomoles each of primers GAPDH-forward (5′-ACAGTCCATGCCATTGCC-3′) and GAPDH-reverse (5′-GCCTGCTTCACCACCTTCTTG-3′) were added per reaction for amplification of an amplicon in the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. PCR was performed on either a Perkin-Elmer GeneAmp® PCR System 9600 or System 2400. PCR conditions were 4 min at 94°C; 34 cycles of 30 s at 94°C, 30 s at 61°C and 1 min at 72°C; and 10 min at 72°C, except for the PCRs started by isolations from blood diluted by a factor of a hundred or more. When the template was isolated from diluted blood, PCRs were allowed to proceed for 40 cycles.

Agarose Gel Electrophoresis and Documentation

Both genomic DNA and PCR products were visualized on ethidium bromide-stained 1.5% agarose gels. Electrophoresis was performed in 1× TAE buffer, and the results were documented with a DS34 Polaroid® camera and Polaroid 667 film (Polaroid Ltd, St. Albans, England, UK).

RESULTS AND DISCUSSION

Comparison Between DNA DIRECT and Phenol-Based DNA Isolation

Using a traditional method based on phenol extraction and ethanol precipitation, genomic DNA was isolated from 5 mL of blood that had been treated with the anticoagulant EDTA. Four isolations from 10 µL of the same blood sample were performed using Dynabeads DNA DIRECT. The DNA from two of the isolations was eluted for 5 min at 65°C, while the DNA from the other two isolations was left in the presence of the Dynabeads. All of the DNA from the four Dynabeads DNA DIRECT isolations was loaded onto an agarose gel, as was 0.2% of the DNA isolated by the traditional method. The fraction of the traditionally isolated DNA loaded corresponded to the yield from 10 µL of blood (0.2% of 5 mL). The result of this experiment is shown in Figure 1A. The yield per microliter of blood was similar with the two methods (lanes 1–4 vs. lanes 5 and 6), and very little DNA was lost during the elution step (lanes 1 and 2 vs. lanes 3 and 4). The molecular weight of the DNA from both methods was more than 20 kb, as it ran slower than the 23.13-kb band of the λ HindIII molecular weight marker.

Effective Range of DNA DIRECT

Fresh capillary blood from a healthy donor with a white blood cell count of 4.9 × 10^6 mL⁻¹ was diluted with sterile phosphate-buffered saline (PBS) to give suspensions of 500, 50 and 10 white cells per µL. From each dilution, and from PBS, two 10-µL samples were used as starting material for DNA isolation with DNA DIRECT. All of the DNA from each isolation was used as starting material for PCR amplification of the GAPDH amplicon (40 cycles). Figure 1B shows 10 µL of the 50-µL PCRs visualized on agarose gel. Isolations from 500 cells yielded enough DNA to drive the PCRs to saturation (lanes 1 and 2), and even 10 cells were sufficient to give detectable amounts of PCR products (lanes 5 and 6). The absence of contaminating DNA was demonstrated by the lack of detectable PCR products in the reactions started with

![Figure 1. DNA yield with Dynabeads DNA DIRECT. Panel A shows the amount of genomic DNA isolated from 10 µL of whole blood with Dynabeads DNA DIRECT including the optional elution step (lanes 1 and 2), with Dynabeads DNA DIRECT with the elution step omitted (lanes 3 and 4) and with traditional DNA isolation (lanes 5 and 6). The molecular weight marker in lane 7 is λ HindIII. Panel B shows 10 µL of PCRs started with DNA preparations from whole blood diluted with PBS to 500 white cells per isolation (lanes 1 and 2), 50 white cells per isolation (lanes 3 and 4) and 10 white cells per isolation (lanes 5 and 6). Ten microliters of PCRs, started with dummy DNA preparations from PBS, are run in lanes 7 and 8.](image-url)
control isolations from PBS (lanes 7 and 8).

Experiments similar to those described above, but with high volumes of starting material, should theoretically be sufficient to establish the DNA binding capacity of the DNA DIRECT beads. However, viscosity of the lysate, not the binding capacity, is the factor determining the upper limit of the sample size. Separation of lysate and DNA/Dynabeads complex becomes technically challenging if more than $10^5$ DNA-containing cells are used per sample test of DNA DIRECT.

**Reproducibility**

Five DNA isolations were performed from each of two citrate-treated blood samples of relatively low white blood cell counts (donor I: $3.6 \times 10^6$ cells/mL; donor II: $2.6 \times 10^6$ cells/mL). Each isolation was resuspended in 40 µL, and 4 µL were used as starting material for PCR amplification of the GAPDH amplicon. Half of the DNA obtained from 10 µL of blood is shown in the upper part of Figure 2; 5 µL of the product from PCRs started with 4 µL of the isolated DNA are shown in the lower part of Figure 2. No significant variation between the different isolations was observed. Similar results were obtained with other anticoagulants, donors with higher white blood cell counts and with as little as 2.5% of the isolations as starting material (data not shown).

**The Effect of Anticoagulants**

Dynabeads DNA DIRECT was used to isolate DNA from untreated whole blood as well as blood anticoagulated with EDTA, citrate or heparin. Sample volumes were 10 µL, except for heparinized blood, where sample volume was 5 µL. From each type of starting material, two separate isolations were performed using blood from different donors. Each isolation was resuspended in 40 µL (20 µL for the heparin samples), and 4 µL were used as starting material for PCR amplification of the GAPDH amplicon. One-quarter of the DNA obtained from 10 µL of blood (one half for the heparin samples) is shown in the upper part of Figure 3; 5 µL of the product from PCRs are shown in the lower part of the Figure 3. It is apparent that the type of anticoagulant used does not significantly affect the result.

In the experiment just described, lithium heparin was used,
with results similar to those obtained with sodium heparin. The former, however, has been shown to have inhibitory effects in other systems (3). Dynabeads DNA DIRECT also performed well on bone anticoagulated with ACD (Figure 1B) or CPD (data not shown).

**The Effect of Sample Storage Conditions**

Dynabeads DNA DIRECT was used to isolate DNA from EDTA-treated blood from two different donors. The remainder of the blood samples was then divided into two, one part that was stored at +4°C and one that was stored at -20°C. After 4 days, the frozen sample was thawed, and DNA was isolated from both the thawed frozen sample and the sample that had been kept at +4°C. Each of the 6 isolations (fresh, refrigerated and frozen) were resuspended in 40 µL, and 4 µL were used as starting material for PCR amplification of the GAPDH amplicon. Half of the DNA obtained from 10 µL of blood is shown in the upper part of Figure 4A; 5 µL of the product from the PCRs are shown in the lower part of Figure 4A. No adverse effect of 4-days storage at +4 or -20°C was observed in this system. We routinely use blood that has been frozen for several months, but blood stored at +4°C for more than one week gives more variable results with Dynabeads DNA DIRECT (data not shown).

DNA was isolated from two citrate-treated blood samples, and from the same two samples, 10 µL were spotted on a plastic surface and allowed to air-dry at room temperature. The dried blood spots were transferred to 1.5-mL tubes, 40 µL of PBS were added and the tubes were left at room temperature with gentle agitation for 90 min before DNA was isolated. Each of the 4 isolations (fresh and dried) were resuspended in 40 µL, and 4 µL were used as starting material for PCR amplification of the GAPDH amplicon. Half of the DNA obtained from 10 µL of blood is shown in the upper part of Figure 4B; 5 µL of the product from each PCR are shown in the lower part of Figure 4B. The yield from dried blood is good, and the isolated DNA is suitable for PCR.

**DNA Isolations from Bone Marrow**

From each of two healthy donors, 1, 2 and 5 µL of heparinized bone marrow were used as starting material for DNA isolation with Dynabeads DNA DIRECT. Each of the 6 isolations were resuspended in 40 µL, and 4 µL were used as starting material for PCR amplification of the GAPDH amplicon. Half of the DNA obtained in each isolation is shown in the upper part of Figure 5A; 5 µL of the product from the PCRs are shown in the lower part of Figure 5A. Yield per volume starting material is higher from bone marrow (Figure 5A) than from blood (Figures 1–4). This is to be expected, since the concentration of DNA-containing cells is much higher in bone marrow than in blood. Bone marrow samples of 5 µL are close to the upper limit of what can be handled by one sample test of Dynabeads DNA DIRECT. A good correlation between sample size and DNA yield is observed in the 1–5-µL sample size interval, but even the yield from 1 µL is sufficient for at least 10 PCRs.

**DNA Isolation from Cultured Cells**

Two samples of 4 × 10⁵ Daudi cells were used as starting material for DNA isolation with Dynabeads DNA DIRECT. From each of the isolations, less than 1% was used as starting material for PCR amplification of the GAPDH amplicon. One-tenth of the DNA obtained from each isolation is shown in the upper part of Figure 5B, and 5 µL of the product from the PCRs are shown in the lower part of Figure 5B, demonstrating that more than one hundred PCRs may be run from an isolation of this scale.

**CONCLUSION**

Dynabeads DNA DIRECT consistently isolates enough PCR-ready genomic DNA for at least 10 PCRs from 10 µL of whole blood or an equivalent amount of other blood products. The yield per microliter of blood is as good as that obtained by traditional large-scale methods, and the method performs well with all anticoagulants tested. Freezing, drying or limited storage at 4°C does not interfere with the performance of Dynabeads DNA DIRECT.

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**REFERENCES**


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