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High-Quality Genomic DNA from Human Whole Blood and Mononuclear Cells

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The development of efficient and cost-effective technologies for DNA extraction will become of paramount importance considering the thousands of patients and control samples that eventually will be analyzed to identify disease-

causing genes and for new drug targets. Moreover, genetic testing for early detection of viral genomes and bacterial contamination of donors' blood is also being universally implemented to assure the greatest safety of blood products and to decrease transfusion risks.

Human genomic DNA isolation by classical protocols has always been a tedious, laborious, and time-consuming procedure involving protease digestion, organic solvent extraction, alcohol precipitation, and centrifugation (12). This multi-step method is largely unsuitable for high-throughput screening. Recent-

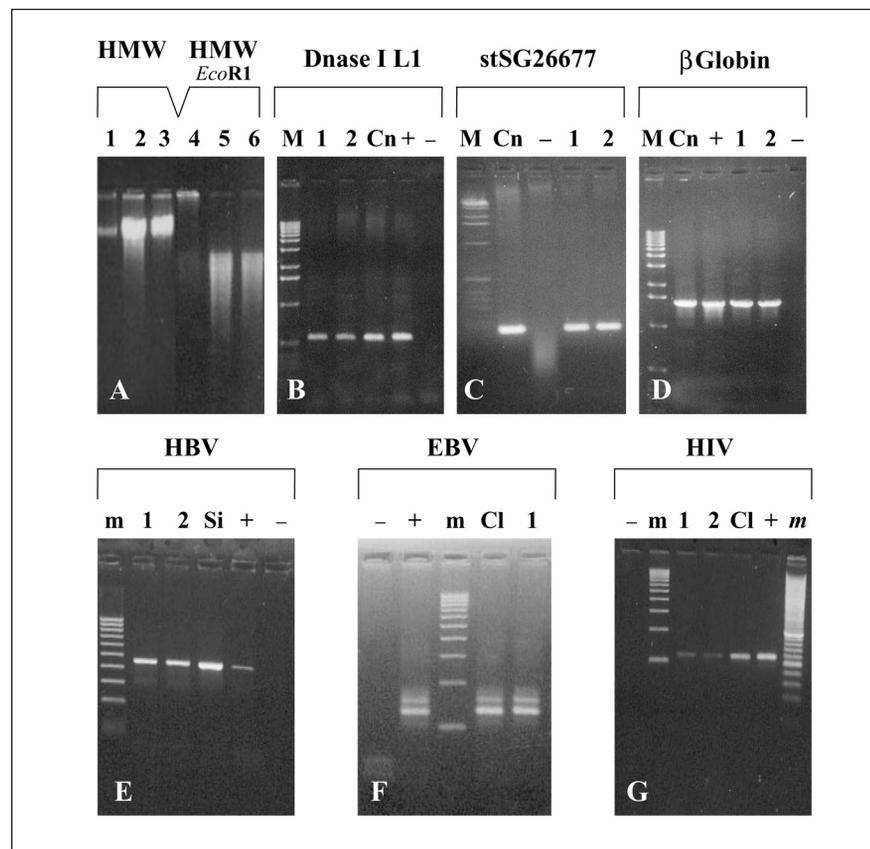


Figure 1. Purification, enzymatic treatment, and PCR amplification of human and viral DNA. (A) High molecular weight (HMW) DNA extracted from human mononuclear cells with a commercially available kit (lane 1) and with the silica-based method (lanes 2 and 3); the same DNA digested with *EcoRI* (lanes 4, 5, and 6). Equal amount of DNA was loaded on a 0.9% agarose gel. (B-G) DNA extracted from mononuclear cells by the silica-based method and amplified with DNase I L1-specific primers (panel B, lane 2; expected fragment size, 566 bp); stSG26677-specific primers (panel C, lanes 1 and 2; expected fragment size, 189 bp); β -globin gene-specific primers (panel D, lanes 1 and 2; expected fragment size, 1520 bp). Genomic DNA purified with phenol:chloroform (panel B, C, and D, lanes Cn) or with commercially available kits (panel B, lane 1) and DNA plasmids bearing target amplification sequences (panels B and D, lane +) were used as positive controls. For viral DNA detection, the target amplification sequences were: HIV proviral DNA (HIV; panel G, lanes 1 and 2; expected fragment size, 115 bp); EBV DNA (EBV; panel F, lane 1; expected fragment size, 129 bp; upper band is a nonspecific PCR product); HBV DNA (HBV; panel E, lanes 1 and 2; expected fragment size, 452 bp). Commercially available kits for viral DNA extraction and detection (Sigma and Clonit, respectively, lanes Si and Cl) and DNA plasmids bearing target amplification sequences (lanes +) were used as positive controls. M, 1-kb ladder; m, 100-bp ladder; m, 20-100 bp ladder; -, negative control.

Table 1. Genomic DNA Extraction Protocol

1. 200 μ L whole blood mixed with 100 μ L lysis buffer^a or 25 μ L mononuclear cells were added to 100 μ L binding solution^b. Mix and incubate for 3 min at room temperature. Centrifuge for 15 s at 65 \times *g* and discard supernatant.
2. Add 100 μ L lysis buffer and mix. Centrifuge for 15 s at 65 \times *g* and discard supernatant. Repeat once.
3. Add 100 μ L washing solution^c and mix. Centrifuge for 15 s at 65 \times *g* and discard supernatant. Repeat once.
4. Add 100 μ L absolute ethanol and mix. Centrifuge for 15 s at 65 \times *g*. Discard supernatant and vacuum-dry the pellet.
5. Add 50 μ L elution buffer^d, resuspend the pellet, and incubate for 3 min at 65°C. Centrifuge for 1 min at 65 \times *g*, and transfer supernatant in a clean tube.

^a3 M guanidine thiocyanate, 20 mM EDTA, 10 mM Tris-HCl, pH 6.8, 40 mg/mL Triton® X-100, 10 mg/mL DTT

^b40 mg/mL silica (Sigma) directly suspended in the lysis buffer

^c25% absolute ethanol, 25% isopropanol, 100 mM NaCl, 10 mM Tris-HCl, pH 8.0

^d10 mM Tris-HCl, pH 8.0, 1 mM EDTA

All solutions were prepared in double-distilled water and sterilized by filtration and remain stable for more than six months.

ly, several efforts have been made to develop simpler procedures both for genomic (7,8,14) and viral DNA (3,4,6,13) extraction from biological specimens (5). Several methods have been developed to meet these needs, but in most cases the high cost and limited versatility make them unsuitable to be employed in large-scale screening.

The ability of silica-resin to bind DNA in the presence of high concentrations of the guanidine thiocyanate chaotropic agent (Fluka Chemie, Bucks, Switzerland) and a nuclease inhibitor usually contained within the lysis buffer has already been described in the classical papers of Boom et al. (1,2). Here we report some modifications made to the original procedure to make it quicker, easier, less expensive, and more suitable to robotized and automated workstations.

Table 1 outlines the procedure used to extract genomic DNA. To increase the DNA yield a second elution step (without heating) may be performed, it has been found to release appreciable quantities of unshredded DNA.

The modifications made to the original Boom procedures consist of the simplification of the silica preparation/equilibration step, which is a major drawback in the Boom method. We found that resuspending silica particles

directly in a binding solution containing guanidine thiocyanate eliminates the tedious preparation of the silica particles, while the suspension retains a neutral pH and remains stable longer. The effect from batch to batch of silica particles impurities and size variation is also minimized, allowing enhanced stability of the binding mixture without the additional cost of α -casein (2), a scavenger for inhibitory substances. The concentration of guanidine thiocyanate was lowered with respect to the original paper, making the protocol considerably less expensive while maintaining the good characteristics of the method. The procedure avoids the use of reagents such as phenol, chloroform, or acetone facilitating waste solution disposal. Application of this protocol has shown that high molecular weight DNA can easily be purified from human whole blood and other biological sources in less than 30 min.

Figure 1A shows the DNA obtained; the yields depend on the number of cells present in the sample. From 25 μ L human mononuclear cells, the amount of DNA extracted ranged from 3 to 4 μ g. It is difficult to calculate accurately the percentage of DNA yield since the mononuclear cells were provided by a blood transfusion center. From 200 μ L whole blood, the yield is at least 5–7 μ g

Benchmarks

DNA, between 50% and 70% of the total DNA present in whole blood cells. In any case, starting with identical sample volumes, our silica-based procedure extracted significantly greater quantities of DNA when compared with the available commercial kit. The described protocol has been used several times in the laboratory using different biological samples. The procedure has always been compared to at least four commercial kits.

No residual RNA contamination was detected as assessed by agarose gel electrophoresis, allowing for a better spectrophotometric estimation of the actual amount of the DNA extracted.

The absence of nucleases was proved by incubating the eluted DNA at 37°C for several hours. DNA can be stored at 4°C for at least one year, without any degradation or significant variation in DNA concentration. No appreciable shearing of the DNA was observed; the size of the DNA was not determined by PDGF but was estimated being close to 50 kb by comparison with the size of phage lambda DNA. Restriction enzyme digestions (i.e., *EcoRI* and *BamHI*) were successfully performed under standard conditions (12); each treatment showed a typical pattern of genomic DNA digestion. In Figure A, the *EcoRI* digestion pattern is shown. By contrast, the DNA prepared by the commercial kit appeared degraded after restriction enzyme digestion. The silica-purified DNA was also used as a PCR template and amplified with three sets of primers targeted to different chromosome regions (Figure 1, B–D) (9–11).

In addition, this procedure allows for efficient detection of Epstein-Barr virus (EBV) particles and proviral human immunodeficiency virus (HIV) in infected blood samples as well as hepatitis B virus (HBV) DNA in human serum. For viral DNA extraction from serum (200 µL), the procedure follows that applied to the mononuclear cells. Viral DNA in human specimens was successfully detected by PCR (Figure 1, E–G). PCR was carried out with a commercial kit (AMSO-Clonit srl, Milan, Italy), and commercially available human genomic and viral DNA extraction systems (GenElute Mammalian Genomic DNA kit; Sigma; EX 02,

Clonit srl) were used as positive controls. Experiments are in progress to assess the sensitivity boundaries of this extraction protocol.

This procedure was not used to extract DNA from buccal cells or from spermatozoa, but is being used for paraffin-embedded tissues cells and some plant and cereal tissues (data not shown). It is routinely used to extract DNA from 1 µL polynucleated chicken whole blood. Finally, this method has been shown to extract plasmid, BAC, and PAC DNA efficiently from bacterial cultures. However, this application requires RNase treatment (data not shown). The amplified genomic DNA was also suitable to be used as a template in sequencing reactions using MegaBACE™ 1000 automatic sequencer (Amersham Biosciences).

Here we report the highly versatile nature of the described DNA extraction procedure. This method was automated by the use of the Multiprobe II EX Robotic Liquid Handling System (Perkin Elmer), which was modified to meet customer needs.

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