TECHNICAL NOTE

Carol A. Scherczinger,[1] Ph.D.; Michael T. Bourke,[1] Ph.D.; Carll Ladd,[1] Ph.D.; and Henry C. Lee,[1] Ph.D.

DNA Extraction from Liquid Blood Using QIAamp*

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ABSTRACT: The implementation of convicted felon DNA databases by increasing numbers of forensic science laboratories has engendered the need for a quick, efficient, and cost-effective method for the isolation of DNA from liquid blood samples. Because of the large numbers of samples involved, the ideal method would combine high throughput capability with maximal yield, high quality, and minimal time. We have found that the QIAGEN QIAamp Blood Kit/Tissue Kit satisfy all of these requirements. This simple, low cost spin column procedure yields purified DNA of approximately 20–30 kb that can be used directly in PCR or other enzymatic reactions without further purification.

We compared the QIAamp isolation procedure to the standard SDS-Proteinase K/organic extraction/microcon purification procedure currently used by many forensic laboratories. The QIAamp procedure consistently gave a two- to four-fold increased yield relative to the organic extraction procedure. The DNA obtained was of high molecular weight, exhibited little degradation, and was suitable for RFLP and PCR analyses. We have found QIAGEN's QIAamp DNA isolation procedure to be ideally suited for preparation of samples for DNA databasing.

KEYWORDS: forensic science, DNA extraction, liquid blood, databank samples

In order to meet the high throughput demands associated with the establishment and maintenance of a convicted felon DNA database, a quick and efficient DNA isolation method is essential. Ideally, the appropriate method should yield abundant levels of high molecular weight DNA with minimal time expenditure. In addition, the procedure must not compromise genotyping results using all RFLP and PCR systems presently available. Although effective, the standard SDS-Proteinase K/organic extraction/microcon purification procedure used by many forensic science laboratories is both time-consuming and cumbersome when processing a large number of liquid blood samples. We have investigated the utility of QIAGEN's QIAamp DNA isolation procedure for processing our convicted felon liquid blood samples, and have found the method suitable for this application.

[1]Criminalists, lead criminalist, and laboratory director and chief criminalist, respectively, Connecticut State Police Forensic Science Laboratory, Meriden, CT.

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Methods

DNA was extracted from 200 uL of liquid blood using either organic extraction or the QIAamp protocol. For organic extraction, samples were treated with Proteinase K in SDS buffer (1). Following overnight incubation at 56°C, samples were extracted twice with phenol/chloroform/isoamyl alcohol, once with butanol, and concentrated using Microcon-100 microconcentrators (Amicon, Beverly MA). For the QIAamp protocol (QIAGEN, Inc, Chatsworth CA; Fig. 1), 200 uL of liquid blood was incubated for 10 min at 70°C in QIAGEN's lysis buffer and QIAGEN Protease or Proteinase K. After addition of EtOH, the lysate was applied to a capped spin column containing a silica membrane and microcentrifuged for 1 min. Subsequently, the samples were washed twice with EtOH-containing high salt buffer followed by 1 min microcentrifugation after each wash. Finally, the DNA was eluted in 200 μL of water (preheated to 70°C) and microcentrifuged for 1 min.

DNA yields were estimated by comparison with standards after electrophoresis in 1% agarose gels and confirmed using the QuantiBlot kit according to manufacturer's instructions (Perkin Elmer/Roche Molecular Systems, Inc., Branchburg NJ). For RFLP analysis, approximately 250 ng of DNA were digested with the restriction enzyme *Hae*III (2). DNA fragments were separated in 1% agarose gels, transferred to nylon membranes and hybridized with ^{32}P-labeled probes D1S7, D2S44, and D5S110 using standard protocols (3). For DQA1 and Polymaker PCR analysis, 5–10 ng of DNA were amplified using the Amplitype PM+DQA1 kit (Perkin Elmer/Roche Molecular Systems, Inc., Branchburg NJ). Amplified DNA was detected by hybridization in reverse dot blot format using the manufacturer's protocol. For D1S80 PCR analysis, 5 ng of DNA were amplified according to standard protocols (4). Amplified DNA products were separated in non-denaturing acrylamide gels and visualized by silver staining according to standard protocols (5).

Results and Discussion

DNA was extracted from the blood of two individuals of known and four individuals of unknown genotypes using the QIAGEN QIAamp kit. The quality and quantity of DNA isolated by both purification procedures were compared directly by UV visualization of EtBr-stained agarose gels (Fig. 2). In addition, the quantity

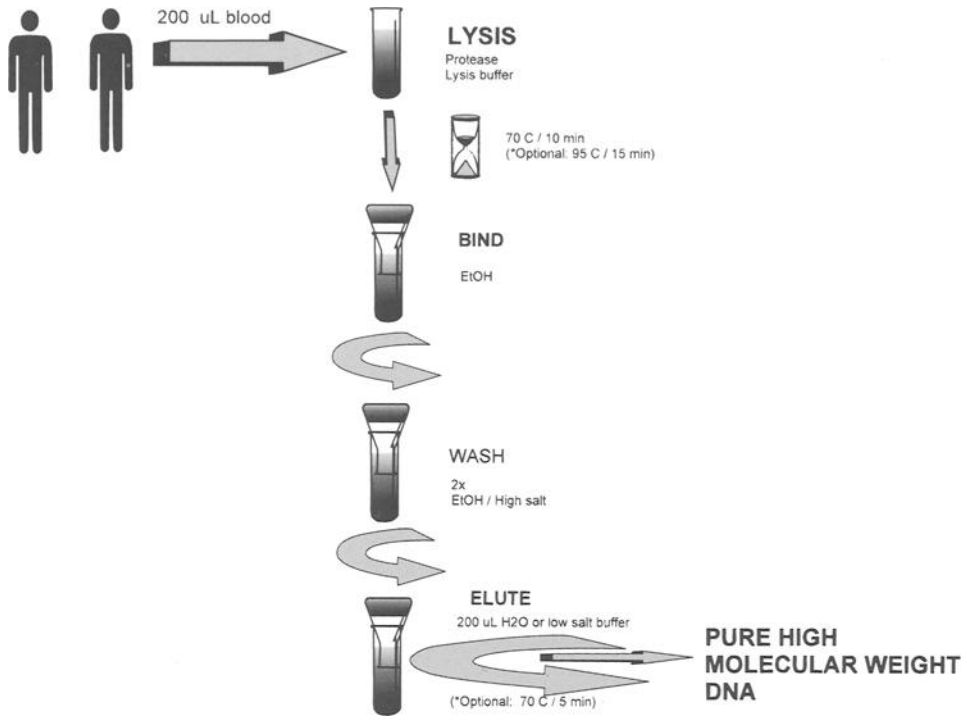

FIG. 1—*Schematic summary of the QIAamp liquid blood extraction protocol.*

of the extracted DNA was verified using the QuantiBlot kit (Table 1). DNA isolated by both methods was of high molecular weight and displayed little degradation. Moreover, the QIAamp procedure consistently gave a two- to four-fold increased yield relative to the standard procedure in considerably less time. Eighteen samples can be processed by this method in approximately 1 hr, in contrast to the several hours necessary for organic extraction and concentration using microconcentrators. We also determined DNA yields using the QIAamp procedure with and without the optional 5 min 70°C incubation at the elution stage; no apparent difference was noted when this step was included (data not shown). Because DNA yields from fresh liquid blood samples are generally high, this step is unlikely to further increase yield. However, this additional incubation might be more useful to increase recovery from evidentiary samples routinely encountered in forensic casework analysis, which often contain a limited quantity of DNA.

RFLP analysis was performed on DNA from unknown individuals purified by the two extraction procedures. The results of RFLP analysis using the D2S44 locus are presented in Fig. 3. Digestion of all DNA samples appeared complete, and the duplicate samples gave identical genotypic results regardless of the isolation method.

The DNA from known and unknown individuals was analyzed using the PCR systems DQA1, Polymaker, and D1S80. DNA isolated by the QIAamp protocol amplified with all of these systems. DNA from the known blood samples gave the expected genotypes, and DNA from the unknown blood samples yielded identical genotypes for both extraction methods (data not shown).

The QIAamp protocol recommends sample incubation at 95°C for 15 min following the lysis step in order to inactivate potentially infectious agents. When this additional step was included in the extraction procedure, the DNA obtained appeared denatured in comparison with that obtained by lysis at 70°C only (Fig. 4).

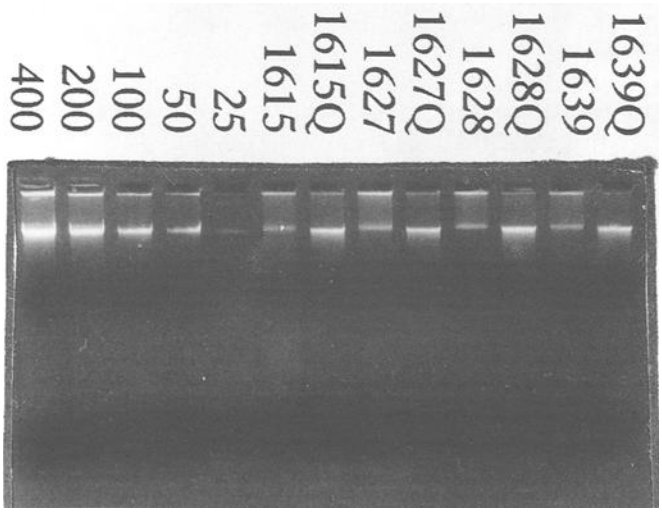

FIG. 2—*Comparison of the QIAamp protocol with the organic extraction procedure. Samples designated "Q" were isolated by the QIAamp protocol; all others were isolated by standard SDS-Proteinase K/organic extraction. All samples were resuspended in 200 μL of water, and equal volumes of each sample were loaded. Lanes 1–5 are yield standards (ng).*

TABLE 1—*Comparison of yields with both extraction procedures.*

Sample	Yield, μg
1615	2.5
1615Q	5.0
1627	2.5
1627Q	5.0
1628	1.25
1628Q	5.0
1639	2.5
1639Q	5.0

NOTE—Samples are the same as Fig. 1.

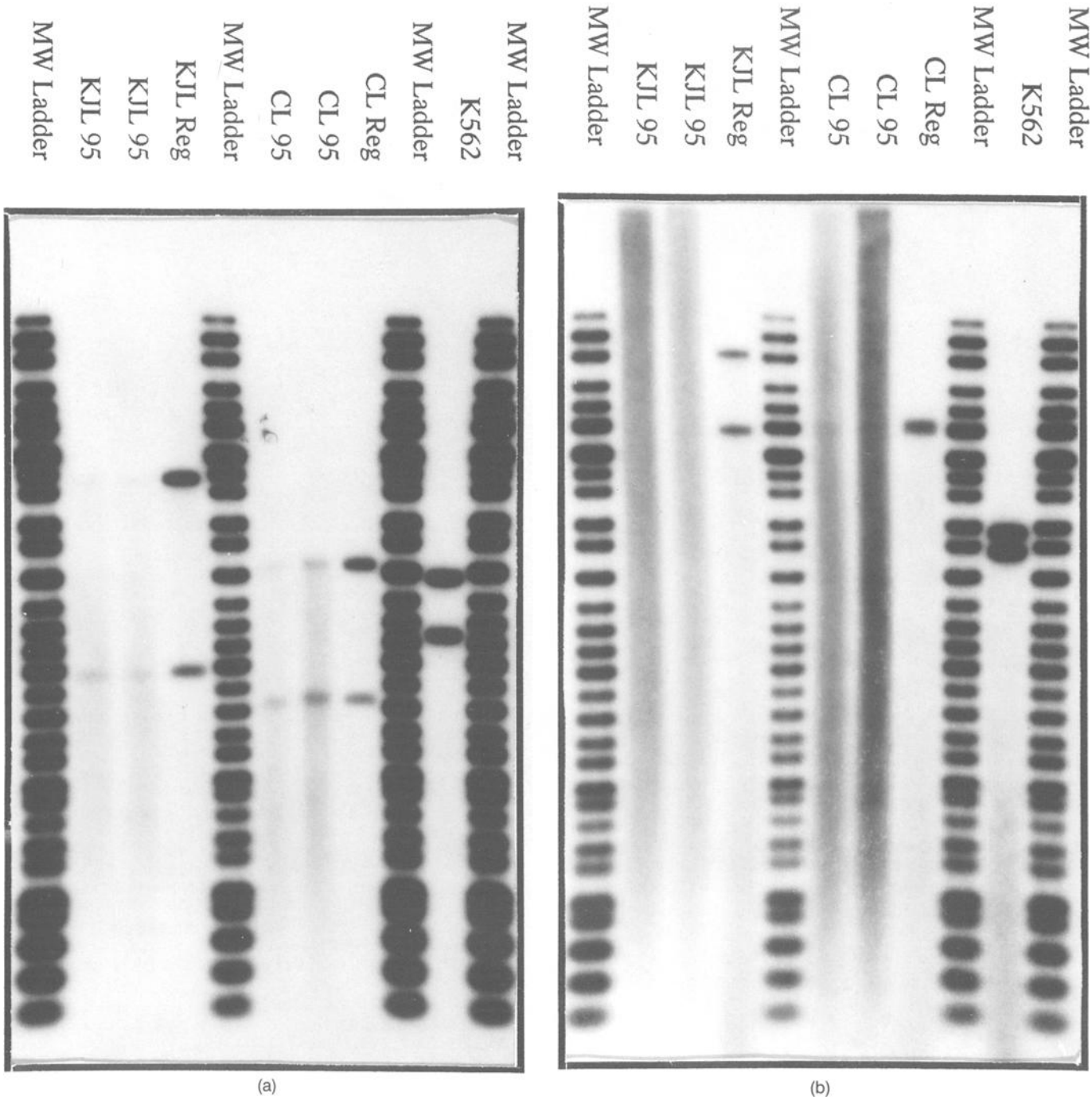

FIG. 5—*RFLP analysis of samples isolated with/without the optional 95°C incubation. Sample designations are as in Fig. 4. K562 is a cell line control. The probes were D5S110 (A) and D1S7 (B).*

Predictably, there was no effect when these samples were analyzed utilizing the three PCR systems (data not shown). However, the effect on RFLP analysis was significant (Fig. 5). The genotypes at the D5S110 locus (Fig. 5A) are barely discernible, and no bands could be distinguished at the D1S7 locus (Fig. 5B). Therefore, the consequence of sample denaturation by incubation at 95°C is more apparent for high molecular weight DNA fragments.

We have found QIAGEN's QIAamp DNA isolation procedure to be ideally suited for preparation of blood samples for DNA databasing. DNA isolated by this method is of high molecular weight, and the yield is two- to four-fold greater than for organic extraction. The DNA is of suitable quality for digestion with restriction enzymes and amplification with several different PCR systems. However, we do not recommend the optional incubation at 95°C during lysis for samples to be analyzed by RFLP, because increased sample degradation as a consequence of apparent denaturation makes genotype determination difficult or impossible.

One advantage of the QIAamp procedure is the elimination of organic extractions and precipitation/concentration steps. In addition, there are fewer tube changes; the lysate is transferred

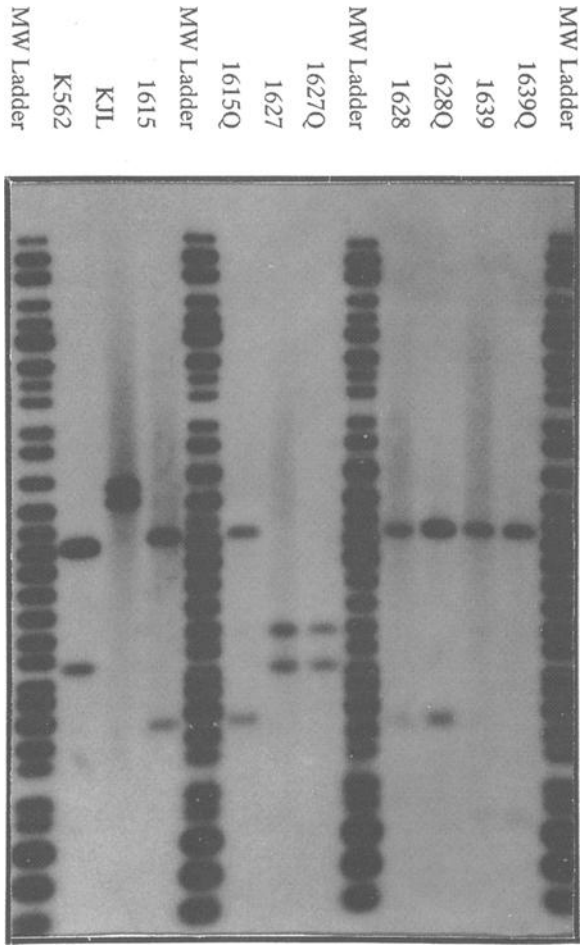

FIG. 3—*RFLP analysis of extracted DNA. Sample designations are as in Fig. 1. K562 is a cell line control and KJL is an internal laboratory standard. The probe was D2S44.*

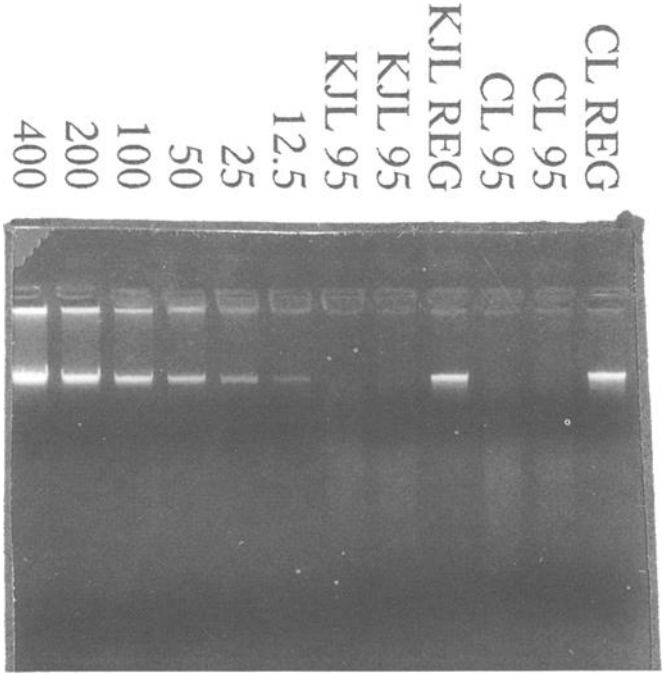

FIG. 4—*QIAamp extraction protocol with/without the optional 95°C incubation during lysis. Samples designated "95" were heated to 95°C for 15 min after incubation at 70°C during the initial lysis step (see Fig. 1). Samples designated "REG" were incubated only at 70°C. Lanes 1–6 are yield standards (ng).*

from a microfuge tube directly to a sealed spin column, and all subsequent steps are performed in the spin column. Consequently, the risks of sample handling errors, aerosol cross-contamination and exposure to infectious agents are minimized. Finally, the short time required and the low cost per sample make this method a very viable option for DNA preparation from large numbers of liquid blood samples.

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Additional information and reprint requests:
Carol A. Scherczinger, Ph.D.
Connecticut State Police Forensic Laboratory
278 Colony St.
Meriden, CT 06451