TECHNICAL NOTE

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Sensitive and Specific Quantification of Human Genomic Deoxyribonucleic Acid (DNA) in Forensic Science Specimens: Casework Examples

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ABSTRACT: We describe the forensic science application of a method for quantification of human genomic deoxyribonucleic acid (DNA). The two cases cited in this report involve DNA samples extracted from skin tissue and bloodstained clothing recovered from different crime scenes. High-molecular-weight DNA was recovered from both specimens, and the concentrations of these DNAs were estimated to be ~0.5 μg/μL by ethidium bromide/agarose gel electrophoresis. Using the human-specific DNA probe p17H8 (locus D17Z1) to quantify the amount of human genomic DNA in these samples, it is shown that less than 1% of the DNA isolated from the skin tissue is of human origin and that the DNA isolated from the bloodstained clothing is effectively devoid of human DNA sequences. These case examples illustrate the need to quantify not only the total amount of DNA recovered from forensic casework material, but also the proportion of the DNA that is of human origin.

KEYWORDS: pathology and biology, deoxyribonucleic acid (DNA), genetic typing, physical evidence, DNA typing, contaminating nonhuman DNA, quantification, human genomic DNA

Forensic science analysis of restriction fragment length polymorphism (RFLP) requires that high-molecular-weight genomic deoxyribonucleic acid (DNA) be isolated from a wide range of evidential materials. Prior to RFLP analysis, it is important to assess both the quantity and the quality (molecular weight) of the DNA sample. This is most easily accomplished by ethidium bromide/agarose gel electrophoresis of a small portion of the DNA sample and visualization of the DNA by ultraviolet light fluorescence [*1,2*]. This method is capable of detecting nanogram quantities of DNA as well as providing an indication of the quality or molecular weight of the DNA. What this method fails to

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provide, however, is any indication as to the species of origin. For most forensic casework specimens, the DNA is primarily of human origin, and ethidium bromide fluorescence can be used to estimate accurately the concentration of human genomic DNA. Nevertheless, there will be contaminated specimens for which the ethidium bromide fluorescence will overestimate the quantity of human genomic DNA. The cases described in this report illustrate the usefulness of a sensitive and specific method for quantifying the amount of human genomic DNA in forensic casework specimens.

Materials and Methods

Isolation of DNA

Methods for DNA isolation have been previously described [*3*]. DNA was isolated from a 1-cm^2 segment of skin tissue and a 1-cm^2 bloodstain on cloth shorts. The samples were incubated for 4 h at 56°C in a 400 μL solution of 10m*M* tris(hydroxymethyl)aminomethane (Tris) (pH 7.4), 10m*M* ethylenediaminetetraacetic acid (EDTA), 100m*M* sodium chloride (NaCl), 39m*M* dithiothreitol, and 500 μg/mL proteinase K. Insoluble material and cloth cuttings were removed by centrifugation, and the aqueous extract was treated successively with buffer-saturated phenol, phenol/chloroform/isoamyl alcohol (25:24:1), and chloroform/isoamyl alcohol (24:1). Following the solvent extractions, two volumes of 100% ethanol were added, and the DNA was precipitated by centrifugation. The DNA pellets were dissolved in TE (10m*M* Tris, at pH 7.4, and 0.1m*M* EDTA) and ethanol precipitated as above. The DNA pellets were briefly dried to remove residual ethanol and solubilized in a final volume of 30 μL of TE.

DNA Quantification by Ethidium Bromide/Agarose Gel Electrophoresis

A small portion of each DNA sample (~1 μL) was analyzed on a 0.8% agarose test gel. Following electrophoresis, the test gel was stained in a 0.5-μg/mL solution of ethidium bromide. Included in the analysis were samples containing known amounts of high-molecular-weight human genomic DNA and *Hind*III-digested lambda-phage DNA as a molecular-weight marker. Following electrophoresis, the gel was photographed under ultraviolet light (312 nm), and the relative intensity of fluorescence was used to estimate the amounts of high-molecular-weight DNA present in each sample.

Specific Quantification of Human Genomic DNA

The procedure used to quantify human genomic DNA has been described in detail elsewhere [*4*]. Briefly, a small portion of each sample and standards containing known amounts of high-molecular-weight human genomic DNA were applied directly to a nylon membrane (Hybond-N, Amersham) using a slot-blot vacuum chamber (Schleicher and Schuell). The membranes were prehybridized at 65°C for ~15 min in a solution of 6× standard saline citrate (SSC) (1× SSC is 150m*M* NaCl and 15m*M* sodium citrate), 0.5% sodium dodecyl sulfate (SDS), 5× Denhardt's (50× Denhardt's is 1% ficoll, 1% polyvinylpyrolidone, and 1% bovine serum albumin), and 100 μg/mL denatured salmon sperm DNA and hybridized at 65°C for 1 to 2 h in the same solution containing 10% dextran sulfate and 10^6 counts per minute (cpm)/mL of phosphorus-32 (^{32}P)-labeled p17H8 (Oncor, Gaithersberg, Maryland). Alternatively, this quantification protocol can be used in conjunction with charged membranes and a hybridization buffer containing 7% SDS, 10% polyethylene glycol, 250 mg/mL denatured salmon sperm DNA, and 1.5 × saline sodium phosphate EDTA (SSPE) [1 × SSPE is 150m*M* NaCl, 10m*M* sodium dihydrogen orthophosphate (monobasic) ($NaH_2PO_4 \cdot H_2O$), and 1m*M* EDTA].

The probe, which corresponds to a highly repetitive satellite DNA specific for human chromosome 17 [5], was labeled using the random primer method of Feinberg and Vogelstein [6]. Following hybridization, the membranes were washed at 65°C for 15 min in a solution of 2× SSC and 0.5% SDS and exposed at −70°C to XAR5 X-ray film (Kodak) sandwiched between two intensifying screens (Cronex Lightning Plus, Dupont). The entire procedure (including hybridization, washing, and autoradiography) can be completed in less than 4 h. Using these methods, autoradiographic exposure times as short as 15 to 30 min are capable of detecting subnanogram amounts of human genomic DNA.

Southern Hybridization

Human genomic DNA was digested with the restriction endonuclease *Hae*III, subjected to electrophoresis through 1.0% agarose gels in 1× TBE (89m*M* Tris/borate, 89m*M* boric acid, and 1m*M* EDTA), transferred to nylon membranes (Hybond-N, Amersham) [1,7], and hybridized successively with ^{32}P-labeled probes corresponding to loci D7Z2 [8] (Oncor, Gaithersberg, Maryland) and D17S79 [9] (Lifecodes, Valhalla, New York). The membranes were prehybridized for 1 to 2 h at 52°C in a solution of 3× SSC/50% formamide/10× Denhardt's, and hybridized overnight at 52°C in the same solution containing 10% dextran sulfate and 10^6 cpm/mL ^{32}P-labeled probe. Following hybridization, the membranes were washed at 65°C to a final stringency of 0.1× SSC and 0.1% SDS. Autoradiography was conducted as described above. Radioactive probes were stripped from the membrane by incubation for 20 min at >90°C in 1m*M* EDTA and 0.1% SDS.

Results and Discussion

Two DNA typing cases have been chosen to illustrate the importance of sensitive and specific quantification of human genomic DNA. *Case No. 1* dealt with the murder of a young woman whose partially clothed body was found in a garbage dumpster. The cause of death was manual strangulation, and it appeared that areas of the body had been scalded, causing considerable amounts of skin to slough off. A sample of skin tissue was recovered from the bathtub drain of a nearby residence. The maximum temperature of water from the bathtub faucet was measured at 71.5°C. It was thought that the woman had been beaten and strangled and that, subsequently, her body had been scalded during a prolonged attempt to "clean" the body using the bathtub shower. The skin sample and blood taken from the deceased at autopsy were submitted for DNA analysis to determine whether the skin sample could have originated from the deceased.

In a second unrelated case, *Case No. 2*, the body of a male child was discovered in a garbage dumpster, and the autopsy examination revealed that the victim has been sexually assaulted and suffered death by strangulation. The child's underwear and shorts were bloodstained as a result of rectal trauma. Conventional serological testing (hemochromogen and anti-human globulin tests) confirmed the presence of human blood on the clothing, but seminal fluid was not detected. The bloodstained clothing and autopsy blood from the victim were submitted for DNA analysis, together with known blood samples taken from several accused individuals.

For both of the above cases, DNA was extracted from the evidence samples (skin and bloodstained clothing) as well as from blood taken from the deceased individuals at autopsy. Figure 1 (*upper panel*) shows the results of DNA quantification by ethidium bromide/agarose gel electrophoresis and ultraviolet light fluorescence. Substantial amounts of high-molecular-weight DNA were recovered from each of the samples, and the DNA concentrations were estimated to be approximately 0.5 μg/μL.

Figure 1 (*lower panel*) shows the results of slot-blot quantification of human genomic DNA. The concentrations of human genomic DNA in the autopsy blood samples were

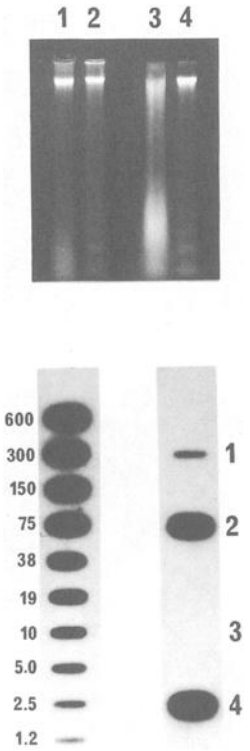

FIG. 1—*Quantification of DNA isolated from forensic casework samples.* (Upper panel) *Ethidium bromide/agarose gel electrophoresis quantification. Approximately 1/30 of each sample was analyzed. Samples 1 and 2 were isolated from skin tissue and the corresponding autopsy blood sample, respectively, of Case No. 1. Samples 3 and 4 were isolated from bloodstained clothing and the corresponding autopsy blood sample, respectively, of Case No. 2.* (Lower panel) *Slot-blot quantification of human genomic DNA. Approximately 1/30 of each sample was analyzed. The amounts of control human genomic DNA are indicated in nanograms. Samples 1 through 4 are as indicated above.*

estimated to be ~0.5 μg/μL (Fig. 1, *lower panel*), which is in agreement with the estimate derived using ethidium bromide/agarose gel electrophoresis. In contrast, the amount of human genomic DNA isolated from the skin sample was several orders of magnitude lower than that predicted by ethidium bromide/agarose gel electrophoresis, and the total amount of human genomic DNA was estimated to be 75 ng (~2.5 ng/μL, out of a total volume of 30 μL). Thus, it is apparent that a large proportion (>99%) of the DNA extracted from the skin sample was of nonhuman origin. The extent of contamination was even greater for the DNA extracted from the bloodstained clothing of the young child, since slot-blot analysis failed to detect sequences of human origin (Fig. 1). For these evidence samples, ethidium bromide/agarose gel electrophoresis substantially overestimated the amount of human genomic DNA.

DNA typing of polymorphic loci was used to ascertain the possible origins of the skin sample in Case No. 1, despite the observation that <1% of the DNA isolated from this sample was of human origin. Figure 2 shows the results of Southern hybridization analysis of varying amounts of each DNA sample using probes against repetitive and single-copy loci. Locus D7Z2 is characterized by a repetitive 2731 base pair (bp) *Hae*III fragment

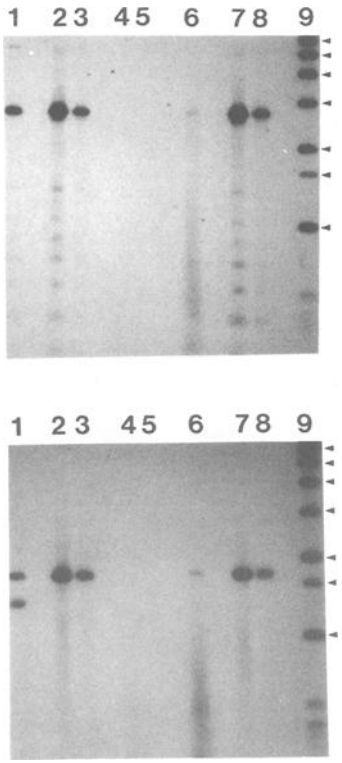

FIG. 2—*Southern hybridization analysis of DNA isolated from skin tissue and the corresponding autopsy blood sample of Case No. 1.* (Upper panel) *Analysis of monomorphic locus D7Z2: Lane 1, 250 ng of control human genomic DNA; Lane 2, 1.0 μg of autopsy blood DNA; Lane 3, 250 ng of autopsy blood DNA; Lane 4, 1.0 μg of skin DNA; Lane 5, 250 μg of skin DNA; Lane 6, 8.0 μg of skin DNA; Lane 7, 1.0 μg of autopsy blood DNA; Lane 8, 250 ng of autopsy blood DNA; Lane 9, size markers (in descending order: 6108, 5090, 4072, 3054, 2036, 1636, and 1018 base pairs).* (Lower panel) *Subsequent analysis of the same membrane for VNTR locus D17S79: The lanes and markers are as indicated above.*

that is specific for human genomic DNA [*8*]. For this locus, intense hybridization signals were detected from 1.0-μg and 250-ng amounts of autopsy blood DNA, whereas no signal could be detected from the corresponding amounts of DNA from the skin sample. However, a faint hybridization signal could be detected from a much larger amount of the skin DNA sample (~8.0 μg), containing the equivalent of ~40 ng of human genomic DNA (as estimated by slot-blot analysis). This confirms that the amount of human genomic DNA present in the skin DNA sample was substantially lower than that estimated by ethidium bromide/agarose gel electrophoresis. Subsequent hybridization to locus D17S79 [*9*], a variable number of tandem repeats (VNTR) locus [*10*], detected a hybridization pattern from 8.0 μg of the DNA isolated from the skin sample. More importantly, the RFLP pattern of the skin sample DNA matched that of the autopsy blood, thereby establishing a link between the victim, the scalded condition of her body, and the accused's residence.

The casework examples presented in this report illustrate the importance of quantifying the amount of human genomic DNA in forensic casework DNA samples. Samples may be heavily contaminated with nonhuman DNA, yet be virtually indistinguishable from pure human genomic DNA based on ethidium bromide/agarose gel electrophoresis (Fig.

1). For those samples that are contaminated with nonhuman DNA, it is essential that the degree of contamination be determined prior to DNA typing. For the cases described in this report, DNA typing results could not be obtained by routine Southern hybridization analysis of typical working quantities of DNA (250 ng and 1.0 μg). However, VNTR typing results could be obtained by increasing the absolute quantity of DNA so that the human genomic DNA component is represented in quantities sufficient for Southern hybridization analysis (~40 ng). This illustrates the principal advantage of knowing the precise amount of human genomic DNA available for analysis.

Forensic evidence samples are often contaminated with nonhuman material, the nature and degree of which may not be predictable. Therefore, we recommend that the quantity and quality of human genomic DNA be estimated using both ethidium bromide/agarose gel electrophoresis and the slot-blot hybridization method described in this report. The routine application of these techniques could serve to maximize the overall efficiency of DNA typing by (1) avoiding analysis of samples that contain insufficient quantities of human genomic DNA (for example, the DNA isolated from the bloodstained clothing) and (2) increasing the likelihood of obtaining RFLP data for samples that are heavily contaminated with nonhuman DNA (for example, the DNA isolated from the skin tissue).

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