TECHNICAL NOTE

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Restriction Fragment Length Polymorphism DNA Analysis by the FBI Laboratory Protocol Using a Simple, Convenient Hardware System

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ABSTRACT: Restriction fragment length polymorphism analysis of human deoxyribonucleic acid (DNA) using two probes, pYNH24 and CMM101, was performed on the BIOS Timeframe™ system following the Federal Bureau of Investigation (FBI) Laboratory protocol and some variations of it. Comparable results were obtained by the different methods used.

KEYWORDS: forensic science, pathology and biology, deoxyribonucleic acid (DNA), restriction fragment length polymorphism (RFLP), DNA analysis, forensic serology, variable-number-of-tandem-repeats polymorphism

Several years ago, Jeffreys described hypervariable regions of human deoxyribonucleic acid (DNA) using multilocus probes and the applicability of these DNA typing procedures to the individualization of human blood and tissues [*1,2*]. The potential forensic science applications of DNA analysis in resolving disputed parentage cases, as well as in the individualization of blood and body fluids, were immediately recognized [*3,4*]. The extensive polymorphism detected by this type of DNA analysis consists of variable numbers of tandem repeats (VNTR) within sequences of repetitive DNA in the human genome [*5–7*]. Numerous multilocus and single-locus probes that detect VNTR polymorphisms have been found since Wyman and White first described the single-locus probe pYNH24

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[*8*]. DNA probes detecting VNTR polymorphism are widely employed in human gene mapping [*9,10*]. The use of a number of single-locus DNA probes in forensic science analysis of bloodstains and body fluid stains has recently been described [*11–13*].

The Federal Bureau of Investigation (FBI) Forensic Science Research and Training Center has developed carefully validated procedures for the restriction fragment length polymorphism (RFLP) typing of bloodstains and body fluid stains [*14–16*]. These procedures are used in the FBI Laboratory, and have been shared with forensic scientists throughout the country through extensive training programs.

In this paper, the authors report that the FBI Laboratory DNA typing procedures can be performed using a simple, convenient hardware system (the BIOS Timeframe™ system) for electrophoresis, Southern blotting [*17*], hybridization, and autoradiography.

Materials and Methods

DNA was isolated from cultured cells using standard buffered sodium dodecyl sulfate (SDS), proteinase-K incubation followed by phenol-chloroform extractions. The DNA was precipitated with cold absolute ethanol, washed with 70% ethanol, dried, and dissolved in TE buffer [10m*M* Tris and 1m*M* ethylenediaminetetraacetic acid (EDTA), at pH 7.5]. The DNA was quantitated by ultraviolet (UV) spectrophotometry. The cultured cells were from different, unrelated individuals of the "UP" series.

The DNA was digested with the restriction endonuclease *Hae*III (BIOS Corp., New Haven, CT), following the supplier's recommended conditions. A specimen of the digested DNA was electrophoresed in a 1% agarose minigel containing ethidium bromide and examined on a UV transilluminator to ensure that complete digestion had occurred.

Samples of approximately 1 μg of *Hae*III-digested DNA were mixed with a loading buffer (80% glycerol, 0.1% bromophenol blue, and 0.1% xylene cyanol) and loaded into wells of a 1% agarose gel prepared in the Timeframe gel casting assembly in TAE buffer (40m*M* Tris, 10m*M* acetic acid, 20m*M* EDTA at pH 8), containing 5 μg of ethidium bromide per 100 mL. Lane 1 contained visual and molecular weight (MW) size markers, Lane 2 contained K562 cell line DNA, and Lanes 6, 10, and 14 contained MW size markers (Lifecodes Corp., Valhalla, NY), as specified in the FBI protocol. In some experiments, Lane 2 contained 100 ng of K562 DNA "allelic control" and Lane 3 contained 1 μg of K562 DNA. In other experiments, 1 μg of K562 DNA was run in Lane 2. Electrophoresis was carried out overnight (17 h) at 30 V.

The FBI protocol recommends the use of Zetaprobe nylon membranes. One of the convenient features of the Timeframe system is the use of framed nylon membranes for Southern blotting. The standard framed membrane is Biodyne A (Pall Corp., Glen Cove, NY). The Timeframe system can be used, however, with unframed membranes as well.

In these experiments, identical blots were made on Biodyne A framed and unframed membranes and on unframed Biodyne B (Pall Corp., Glen Cove, NY) and Zetaprobe (Bio Rad Laboratories, Richmond, CA) membranes. The latter two membranes are charged, and the DNA fragments were transferred onto them in alkali [0.4*M* sodium hydroxide (NaOH)]. Biodyne A is a neutral nylon membrane, and the DNA fragments were transferred onto it in salt solution [10× standard saline citrate (SSC)] [*16*].

DNA fragments require no additional fixation to charged membranes after alkali transfer, but fragments transferred to neutral membranes in salt solutions are typically fixed to the membranes by exposure to 254-nm ultraviolet light. In the present experiments, the restriction fragments were fixed to Biodyne A by exposure to 0.2 J/cm^2 of 254-nm light.

Human DNA probes pYNH24 and CMM101 (which detect RFLP at the D2S44 and D14S13 loci, respectively) were obtained in plasmids from the American Type Culture Collection, Bethesda, MD. The inserts were isolated by digesting the plasmids with the

appropriate restriction endonucleases and recovering them from agarose gels after electrophoretic separation of the inserts from the plasmids [*18*]. The human probes were radioactively labeled by standard random primer methods [*18*] to a specific activity of $>1 \times 10^9$ counts per minute per microgram, as were λ, T7, and ϕX174 DNA for visualization of the MW size markers.

The membranes were first hybridized with pYNH24 and autoradiograms were developed. They were then stripped and reprobed with CMM101. For the Biodyne A membranes, the hybridization conditions consisted of a 2-h prehybridization; an overnight hybridization step in 6× SSC, 5× Denhardt's solution, 10% dextran sulfate, and 100 μg of salmon sperm DNA at 65°C; and subsequent washing under high-stringency conditions [*19*]. The membranes were stripped in 0.1*N* NaOH for 30 min and in 0.1× SSC, 0.5% SDS, and 0.2*M* Tris/hydrochloric acid (HCl), at pH 8.0 for an additional 30 min at room temperature before being reprobed [*19*]. For the Biodyne B and Zetaprobe membranes, the hybridization conditions and membrane stripping followed the FBI Laboratory protocol. The minimum DNA probe concentration was 1 ng/mL, and the labeled probe specific activity was routinely 1×10^9 counts per minute per microgram, determined by liquid scintillation counting.

Results and Discussion

The detailed FBI Laboratory RFLP typing techniques were followed for blots prepared in Zetaprobe and Biodyne B membranes, except that alkaline transfers were carried out for 2 h rather than for 6 h. Salt solution transfer followed by UV fixation was used for

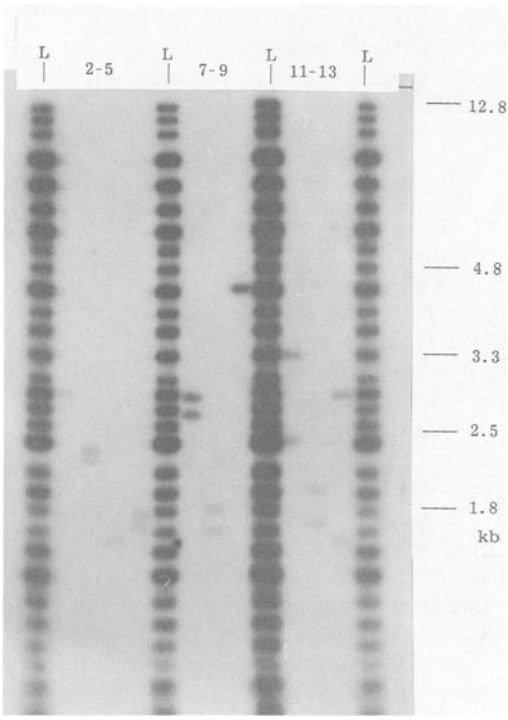

FIG. 1—*Autoradiogram of a Biodyne A membrane after hybridization with pYNH24: L, size ladders; Lane 2, K562 DNA; Lanes 2 through 5, 7 through 9, and 11 through 13. DNA from unrelated persons of the "UP" series. All the DNA was digested with* Hae*III. The approximate size of the size ladder bands is given in kilobase units on the scale at right.*

blots prepared on Biodyne A. Comparable results were obtained when the same DNA samples were run using the Biodyne A, Biodyne B, and Zetaprobe membranes. Figure 1 shows a representative autoradiogram obtained on Biodyne A after hybridization of DNA restriction fragments with pYNH24. Figure 2 shows a representative autoradiogram obtained after stripping a Biodyne B membrane (after probing with pYNH24 and development) and reprobing with CMM101.

In several experiments using the same set of DNA samples, somewhat clearer autoradiograms were obtained with Biodyne A membranes than with either Biodyne B or Zetaprobe ones, which gave comparable results. Somewhat higher backgrounds were seen in some experiments on autoradiograms from Biodyne B and Zetaprobe membranes, more so with pYNH24 than with CMM101. Minor variations in the membranes actually used might account for these differences, and they might well be minimized by minor adjustments of the washing conditions.

The 100-ng K562 DNA sample run in several experiments was not detected in 3-day exposures, but could be detected in 7-day exposures with all the membranes. The 50-ng *Hae*III-digested K562 DNA fragments were detected on Lifecodes Corp. hybridization test strips after 13-day exposures.

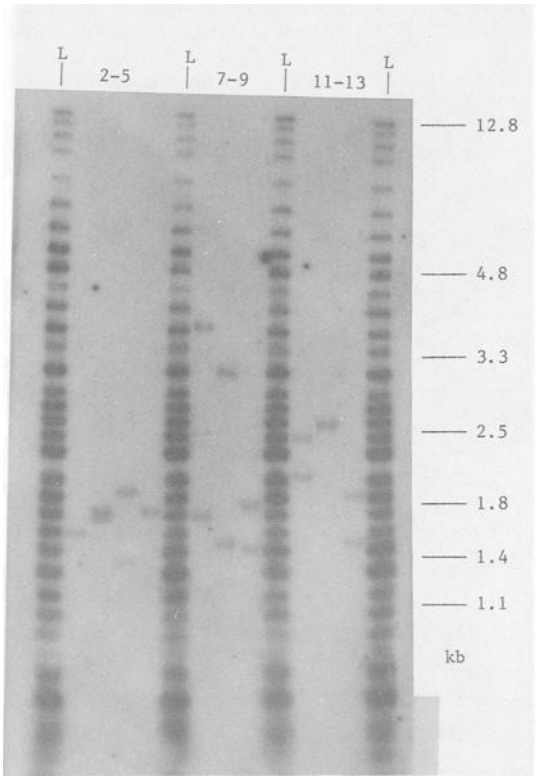

FIG. 2—*Autoradiogram of a Biodyne B membrane after hybridization with pYNH24, stripping, and rehybridization with CMM101: L, size ladders; Lane 2, K562 DNA; Lanes 2 through 5, 7 through 9, and 11 through 13, DNA from unrelated persons of the "UP" series. All the DNA was digested with* Hae*III. The approximate size of the size ladder bands is given in kilobase units on the scale at right.*

Conclusions

The data indicate that the Timeframe system provides an efficient and reproducible way to carry out RFLP typing following the FBI protocol, and that the standard, framed Biodyne A nylon transfer membranes yielded results comparable to those obtained with Biodyne B and Zetaprobe membranes. Other experiments on the RFLP typing of DNA from postmortem blood, bloodstains, and human bone tissue, the results of which will be reported elsewhere, are in progress using the techniques described here.

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