Lorah McNally,[1] *M.S.; Robert C. Shaler,*[1] *Ph.D.;*
Michael Baird,[1] *Ph.D.; Ivan Balazs,*[1] *Ph.D.;*
Lawrence Kobilinsky,[2] *Ph.D.; and Peter De Forest,*[2] *D.Crim.*

The Effects of Environment and Substrata on Deoxyribonucleic Acid (DNA): The Use of Casework Samples from New York City

REFERENCE: McNally, L., Shaler, R. C., Baird, M., Balazs, I., Kobilinsky, L., and De Forest, P., **"The Effects of Environment and Substrata on Deoxyribonucleic Acid (DNA): The Use of Casework Samples from New York City,"** *Journal of Forensic Sciences*, JFSCA, Vol. 34, No. 5, Sept. 1989, pp. 1070-1077.

ABSTRACT: This study was designed to analyze the effects of the environment and substrata on the quality of deoxyribonucleic acid (DNA) isolated from evidentiary specimens. The quality of DNA isolated from actual casework specimens was determined by measuring its size by agarose gel electrophoresis. The information obtained could be used to predict the suitability of the DNA in the samples for restriction fragment length polymorphism (RFLP) analysis. The evidentiary specimens chosen for DNA were classified according to substrate (scrapings, plastic bags, synthetics, denim, and carpet) and according to a subjective evaluation of the condition of the stain (soiled, damp, or putrified) and to its size (small or large). The results show that DNA of sufficient quality and high molecular weight (HMW) can be reliably isolated from bloodstains deposited on evidentiary items which have an unknown environmental history and which have dried onto a variety of substrata. Subsequent RFLP analysis of a selected number of these samples verified that the DNA was suitable for this type of analysis.

KEYWORDS: criminalistics, deoxyribonucleic acid acid (DNA), restriction fragment length polymorphisms, high molecular weight, substrata

Several studies have been published recently describing the application of deoxyribonucleic acid (DNA) restriction fragment length polymorphisms (RFLPs) to forensic science [*1-5*]. Although some background work describing the reliability of this technique exists, there are no published reports which address the effect of substrata on the integrity of the DNA and on its subsequent ability to be digested with restriction endonucleases and analyzed for the presence of RFLPs. Similarly, no studies have been published that correlate the condition of casework evidentiary items to the quality of the stain as related to the molecular integrity of DNA.

A classical approach to the subject was described by Denault [*6*] in which she and her coworkers dried blood onto various laboratory-controlled surfaces. Genetic marker analysis

Received for publication 5 May 1988; revised manuscript received 18 Aug. 1988; accepted for publication 18 Nov. 1988.

[1]Forensic scientist, director of forensic science, manager of forensic and paternity laboratory, and director of laboratories, respectively, Lifecodes Corporation, Valhalla, NY.

[2]Professor of biology and immunology, and professor of criminalistics, respectively, John Jay College of Criminal Justice, City University of New York, New York, NY.

for antigens and isoenzyme phenotypes were performed after periods of exposure to laboratory simulated environmental influences, for example, humidity. A similar approach has been taken by McNally et al. for DNA RFLPs [7]. Although this approach does provide information on the stability of the markers being tested under the conditions of the experiment and reflects common environmental insults, it does not necessarily reflect the influences found in actual casework.

DNA is unique among forensically important genetic markers because the effects that the environment and substrata might have on its integrity and subsequent ability to be used in RFLP analysis can be monitored during the testing procedure [2]. In this way, an analyst can ascertain whether the condition of the sample is suitable for RFLP determination at the beginning of the analytical procedure.

This report documents the stability of DNA, as measured as high-molecular-weight (HMW) DNA, in actual casework. Casework bloodstains were examined for the presence of HMW DNA and representative samples were chosen for subsequent RFLP analysis.

Methods

Sample Selection

Bloodstains were selected from 100 different specimens submitted to the Office of the Chief Medical Examiner of the City of New York. The stains were selected from a variety of substrata.

DNA Isolation and Quality Determination

DNA was isolated according to standard procedures [2,8]. The quality of the isolated DNA was determined by submarine electrophoresis in 0.9% agarose gels run for 1 h at room temperature. The quality of the DNA was determined by ethidium bromide staining (0.5 mg/mL added to the gel buffer). Approximately 1% of the total sample was loaded onto the gel. The DNA was observed under ultraviolet light. The quality of the DNA for subsequent RFLP analysis was determined by estimating the degree of DNA degradation as referenced by its mobility in the gel. The quality of the isolated DNA was categorized as being HMW, HMW plus degradation, degraded, or no detectible DNA (No DNA). The DNA isolated was also categorized according to the substrata from which the stain was removed: scrapings (removed from unknown surfaces), plastic bags, synthetics (polyester, and so forth), denim, and carpet. The samples were also categorized according to a subjective determination of the quality of the stain and were classified as soiled, (dirty, greasy, and so on), damp (stains which were wet with water either before or after drying), and putrefied (odor). Since the size of the stain usually relates to the time it takes to dry, the stains were also classified as being either small (less than 1 cm in diameter) or large.

RFLP Determination

Of the stains analyzed and found to contain HMW DNA, ten were chosen for subsequent restriction endonuclease digestion and RFLP analysis.

Endonuclease digestion using Pst 1 (Bethesda Research Laboratory, BRL) was carried out at 37°C for 2 h according to the manufacturer's instructions. The restriction enzyme digested DNA was precipitated with ethanol according to standard procedures and size separated by submarine electrophoresis in 0.9% agarose gels for 66 h at room temperature [2].

Following size separation, the DNA fragments were transferred to a nylon membrane [9] and hybridized to locus specific probes as described previously [2]. The probes used, pAC255 (D2S44) [10] and pAC225 (DXYS14) [11], were chosen because of the distribution

of their alleles throughout the size range being considered (1.5 to 20 kb). Autoradiography was performed as previously described [2,8].

Results and Discussion

Quality of DNA Obtained

Specific criteria must be met before DNA RFLP analysis can be successful. First, there must be sufficient DNA present and second, the DNA isolated must be of sufficient quality, that is, HMW. Both conditions are easily monitored using agarose gel electrophoresis. Examples of HMW DNA are shown in Fig. 1, Lanes 4, 5, 7, and 11; HMW with partial degradation in Fig. 2, Lane 7; and degraded DNA in Fig. 1, Lanes 9 and 10. No detectable DNA (no DNA) is illustrated in Fig. 2, Lanes 2 to 6.

The tabulated results of the examination of the 100 case bloodstains for the presence of HMW DNA are shown in Table 1. The row labeled "All" shows the cumulative results of the data obtained. The results indicate that 41 samples had HWM DNA with no degradation. Partial degradation was seen in 11, and 12 had degraded DNA. No detectable DNA (No

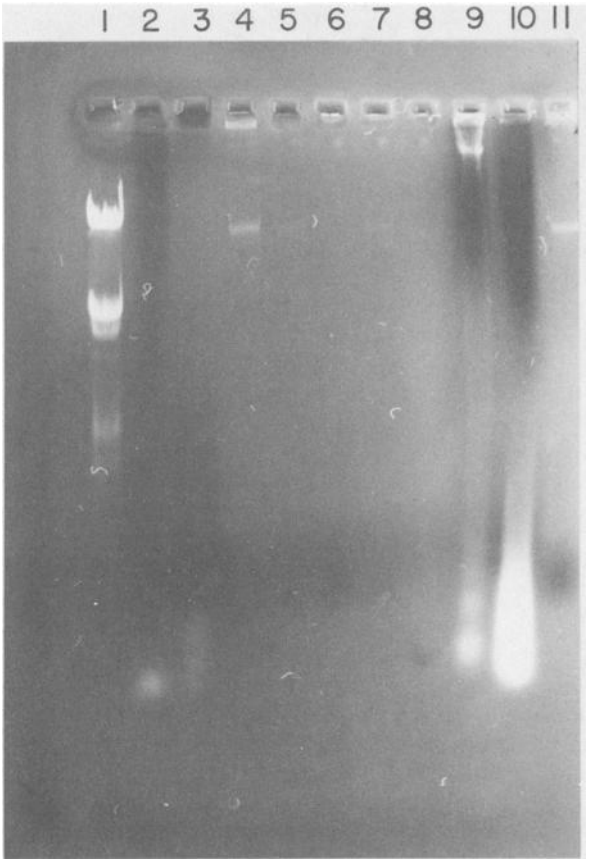

FIG. 1—*Yield gel of DNA isolated from casework bloodstains. DNA was isolated from bloodstains as described in Methods. Lane 1, Lambda DNA standard; Lanes 2, 3, and 6, No DNA observed; Lanes 4, 5, 7, and 11, HMW DNA; Lanes 9 and 10, Degraded DNA.*

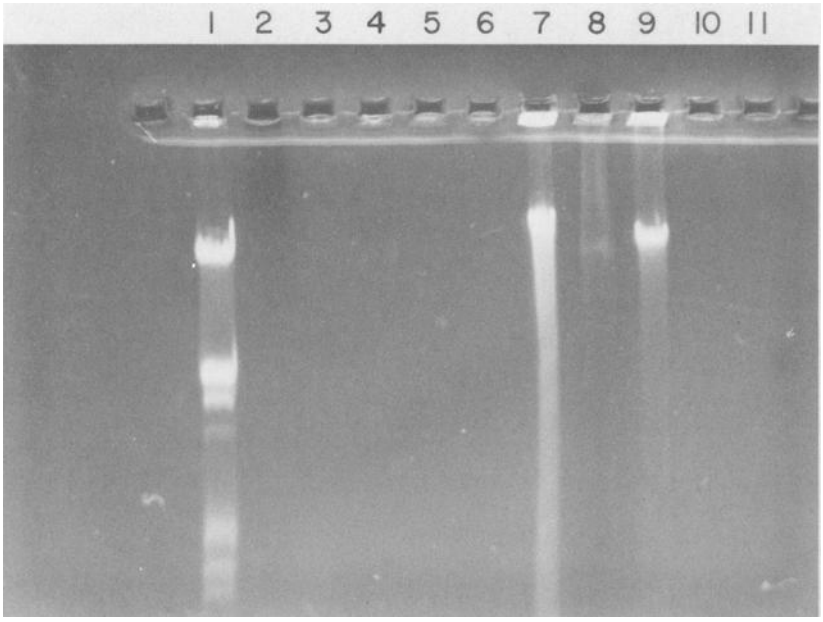

FIG. 2—*Yield gel of DNA isolated from casework bloodstains. DNA was isolated from bloodstains as described in Methods. Lane 1, Lambda DNA standard; Lanes 2-6, No DNA observed; Lanes 7 and 8, Partially Degraded DNA; Lane 8, HMW DNA.*

TABLE 1—*Substrata effects.*

	Number of Total Analyzed				
Category	No. of Samples	HMW	HMW plus Degradation	Degraded	No DNA
Scrapings	13	7	1	1	4
Plastic bag	2	1	...	...	1
Synthetics	59	26	6	7	20
Denim	7	3	1	...	3
Carpet	4	...	...	...	4
all	100	41	11	12	36

DNA) was seen in 36 of the samples. In this latter category 78% were either from small-volume stains or from samples which were visibly soiled. Because the DNA on the agarose gel contains only 1 to 2% of the total sample, the inability to visualize these samples using ethidium bromide does not imply that RFLP analysis will be unsuccessful.

Inspection of the left side of Table 1 shows the categorization of the stains according to class of substrata. The quality of DNA obtained was classified as described above. Samples which have sufficient HMW human DNA would be expected to give DNA RFLP patterns. "HMW plus Degradation" refers to the presence of HMW DNA as well as some degradation; samples of this type are typically useful for RFLP analysis.

The results showed that from the cumulative total of samples analyzed approximately one half gave HMW DNA and would be expected to give RFLP results. Degraded DNA was

found in 12% of the samples where DNA RFLP patterns would not be expected. It is unknown whether the remaining 36% of the samples had HMW DNA. However, because most of these samples were from small stains, and most likely dried faster, the DNA present would not be expected to be biased toward degradation.

The number of samples containing either HMW DNA or HMW and partially degraded DNA isolated was approximately half from each of the substrata categories: scrapings, plastic bags, synthetics, and denim. This is in agreement with the cumulative total of the samples analyzed and indicates that none of the substrata were biased in favor of providing either predominately HMW DNA or degraded DNA.

HMW DNA was not obtained from the four carpet samples analyzed. This may simply reflect a small sample size or the condition of these carpet samples. The carpet samples used in this study were badly soiled with dirt. Other work has shown that soil may adversely affect the integrity of DNA [*7*].

The samples analyzed from scrapings showed a tendency toward HMW DNA. The relative number of scrapings with either degraded DNA or partially degraded DNA were less than that from the cumulative total of the 100 samples.

From denim, the number of samples in the No DNA category appears large related to the number found in the cumulative total of the 100 samples. This may be due to a statistically small sample size or to the difficulty encountered in obtaining white blood cells, and thus DNA, from cotton-based fibers [*12*].

The same 100 samples were also categorized according to a subjective evaluation of the condition of the stain. These results are given in Table 2.

From soiled samples, the relative number yielding partially degraded DNA is higher than for the total of the samples. This suggests that soiled subtrata may adversely affect the quality of DNA. The relative number of samples analyzed with completely degraded DNA was approximately the same as in the cumulative total.

Of the four samples which originated from substrata which were wet either before or after stain deposition, two yielded HMW DNA. Whether or not this DNA is suitable for obtaining human specific RFLP patterns is not known: they were not chosen for RFLP analysis. Since microrganisms will grow in a moist environment containing blood, it is not known whether the HMW DNA being isolated was human or bacterial in origin.

Putrefied samples, that is, those which had a characteristic odor of decayed biological material, gave no DNA. The DNA in these samples was most likely completely degraded.

The small and large stains categories were included to give a measure of drying time on the integrity of DNA. Samples which take longer to dry might be expected either to undergo autolysis or to have bacteria or other contaminants present to a greater extent than stains which dry faster, or both. Smaller stains will normally dry faster and would be expected to have a higher level of non-degraded DNA present. The results of this study seem to substan-

TABLE 2—*Stain condition.*

	Number of Total Analyzed				
Category	No. of Samples	HMW	HMW plus Degradation	Degraded	No DNA
Soiled	19	6	4	2	7
Damp	4	2	...	...	2
Putrefied	3	...	...	...	3
Small	54	22	5	3	24
Large	46	19	6	9	12
all	100	41	11	12	36

tiate this reasoning. Large bloodstains, for which the drying time would be expected to be at least several hours, yielded DNA which was not as high a quality overall. A relatively high number of the samples had degraded DNA present. Thus in the context of this analysis there does appear to be a difference between large and small stains in the quality of obtainable DNA.

RFLP Analysis

Samples were chosen for RFLP analysis to determine whether the assessment that DNA categorized as either HMW DNA and HMW and partially degraded DNA would give readable RFLP patterns. The results of part of this analysis are shown in Fig. 3. The results of the DNA probe recognizing the D2S44 locus are seen at the top (anodic) portion of the gel where one or two bands are seen per lane. The results of the DNA probe recognizing the DXYS14 locus are seen in the lower part of the gel (cathodic) and, in these instances, are seen as one to

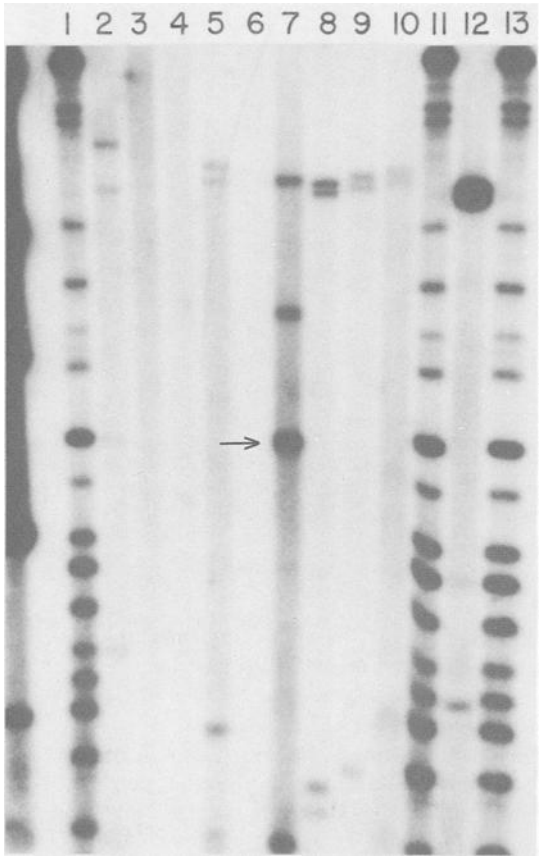

FIG. 3—*Autoradiograph of selected samples hybridized to single-locus DNA probes, D2S44 and DXYS14. Isolated DNA was restriction enzyme digested with Pst 1, size separated by electrophoresis, transferred to nylon, and probed with a cocktail single-locus probe recognizing the D2S44 and DXYS14 loci. Lanes 1, 11 and 13, molecular weight markers: Lane 2, edge of large stain from army jacket (HMW + part.); Lanes 3–7, stains prepared from postmortem blood; Lane 8, sample from denim (2.5-cm stain) (HMW); Lane 9, shorts (1-cm stain, 65% polyester, 35% cotton) (HMW + part.); Lane 10, edge of bra (51% Antron, 49% nylon) (HMW).*

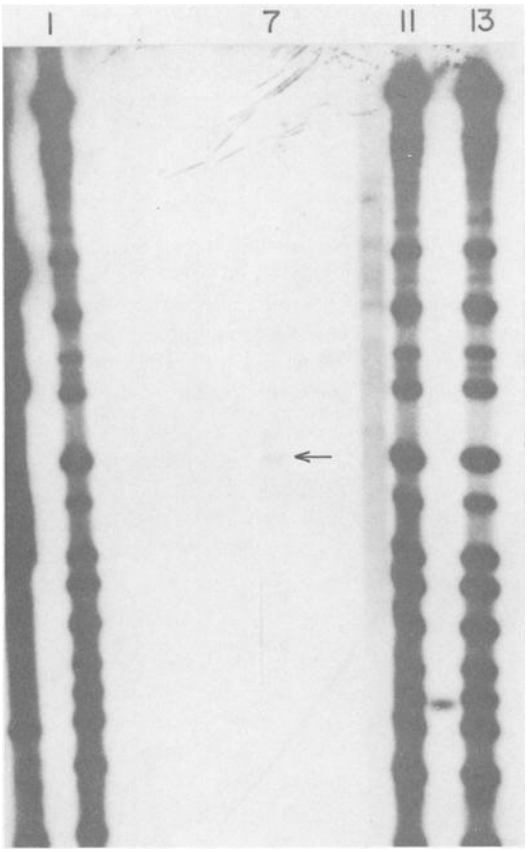

FIG. 4—*Autoradiograph of selected samples hybridized to probes recognizing bacterial ribosomal genes and plasmid sequences. Isolated DNA was restriction enzyme digested with Pst 1, size separated by electrophoresis, transferred to nylon, and probed with a cocktail of probes recognizing bacterial DNA genes and cloning vectors. Lanes 1, 11, and 13, molecular weight markers; Lane 7, arrow points out DNA fragment reacting with probe.*

three bands per lane. The results of this study complement the work of McNally et al. [7] and demonstrate that HMW DNA can be isolated from evidentiary samples exposed to environmental conditions found in actual casework. Further, the HMW and HMW with partial degradation give clearly identifiable RFLP patterns.

Probing with the bacterial control probe Fig. 4, Lane 7, revealed a spurious band in only one of the duplicate specimens. The use of the bacterial control probe precludes the possibility that an anomolous band be misidentified as being human origin (unpublished results).

References

[1] Giusti, A., Baird, M., Pasquale, S., Balazs, I., and Glassberg, J., "Application of Deoxyribonucleic Acid (DNA) Polymorphisms to the Analysis of DNA Recovered from Sperm," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 409-417.

[2] Kanter, E., Baird, M., Shaler, R. C., and Balazs, I., "Analysis of Restriction Length Polymorphisms in Deoxyribonucleic Acid (DNA) Recovered from Dried Bloodstains," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 403-408.

[3] Baird, M., Giusti, A., Meade, E., Clyne, M., Shaler, R., et al., "The Application of DNA-Print for Identification from Forensic Biological Materials," in *Advances in Forensic Haemogenetics 2*, W. R. Mayr, Ed., 1988, pp. 396-402.

[4] Gill, P., Jeffreys, A., and Werrett, D., "Forensic Application of DNA 'Fingerprints'," *Nature*, Vol. 318, 12 Dec. 1985, pp. 577-579.

[5] Gill, P., Lygo, J., Fowler, S., and Werrett, D., "An Evaluation of DNA Fingerprinting for Forensic Purposes," *Electrophoresis*, Vol. 8, 1987, pp. 38-44.

[6] Denault, G. C., Takimoto, H. H., Kwan, Q. Y., and Pallos, A., "Detectability of Selected Genetic Markers in Dried Blood on Aging," *Journal of Forensic Sciences*, Vol. 25, No. 3, July 1980, pp. 479-498.

[7] McNally, L., Shaler, R. C., Giusti, A., Baird, M., and Balazs, I., "Evaluation of DNA From Human Bloodstains Exposed to Ultraviolet Light, Heat, Humidity, and Soil Contamination," *Journal of Forensic Sciences*, Vol. 34, No. 6, Nov. 1989, pp. 0000-0000.

[8] Baird, M., Balazs, I., Giusti, A., Miyazaki, L., Nicholas, L., et al., "Allele Frequency Distribution of Two Highly Polymorphic DNA Sequences in Three Ethnic Groups and Its Application to the Determination of Paternity," *American Journal of Human Genetics*, Vol. 39, 1986, pp. 489-501.

[9] Southern, E. M., "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis," *Journal of Molecular Biology*, Vol. 98, 1975, pp. 503-517.

[10] Nakamura, Y., Leppert, M., O'Connell, P., Woldd, R., Holm, T., et al., "Variable Number of Tandem Repeat (VNTR) Markers for Human Gene Mapping," *Science*, Vol. 235, 27 March 1987, pp. 1616-1622.

[11] Cooke, H., Brown, W., and Rappold, G., "Hypervariable Telomeric Sequences from the Human Sex Chromosomes are Pseudoautosomal," *Nature*, Vol. 317, 1985, pp. 687-692.

[12] Hillman, K. and Shaler, R. C., "The Rehydration and Isolation of Leukocytes from Dried Bloodstains," *Journal of Forensic Sciences*, Vol. 26, No. 3, July 1981, p. 543.

Address requests for reprints or additional information to
Lorah McNally
Lifecodes Corporation
Saw Mill River Rd.
Valhalla, NY 10595