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Isolation of Deoxyribonucleic Acid (DNA) from Saliva and Forensic Science Samples Containing Saliva

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ABSTRACT: Saliva and saliva-stained materials were examined as potential sources of deoxyribonucleic acid (DNA) for DNA analysis and identity testing. In this paper, the authors demonstrate that DNA was isolated and DNA banding patterns suitable for DNA typing were obtained from fresh saliva and various saliva-stained materials, such as envelopes, buccal swabs, gags, and cigarettes. Furthermore, DNA and DNA banding patterns were obtained from actual forensic evidentiary samples containing mixed saliva/semen stains. The DNA banding patterns obtained from saliva or saliva-stained material were indistinguishable from the patterns obtained from blood or hair from the same individual. Intact DNA was readily isolated and DNA banding patterns were obtained from saliva stored at −20°C and dried saliva stains stored under varying conditions. We conclude that saliva and saliva-stained material can be good sources of DNA for analysis and for DNA typing in certain forensic settings.

KEYWORDS: pathology and biology, saliva, deoxyribonucleic acid (DNA), restriction fragment length polymorphism

DNA isolated from biological materials can be analyzed by DNA profiling to help establish the source of the deoxyribonucleic acid (DNA) [*1*,*2*]. It has been demonstrated that blood, semen, hair roots, tissue, and bones are good sources of DNA for identity testing [*3*–*6*]. Most conventional uses of saliva or saliva-stained materials recovered from crime scenes rely primarily on the identification of blood group antigens, and rarely on isoenzymes and polymorphic proteins [*7*]. These approaches have several inherent limitations, especially their limited detectability because of the low concentrations of the antigens, isoenzymes, and proteins in the samples. In addition, the drying, aging, and contamination associated with saliva stains limit the number of markers that can be typed. Moreover, the potential for discrimination using these markers is less sensitive than DNA analysis. In this paper, the authors present several methods for isolating DNA from saliva and saliva stains. We demonstrate that DNA can be isolated and DNA banding patterns can be obtained from saliva and saliva-stained materials stored under various conditions.

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Materials and Methods

Extraction of DNA

Saliva was collected and centrifuged for 1 min in an Eppendorf Model 5415 centrifuge. The pellet was resuspended in 0.7 mL of lysis buffer [(10m*M* tris(hydroxymethyl)aminomethane (Tris) (pH 8.0), 10m*M* ethylenediaminetetraacetate (EDTA), 0.1*M* sodium chloride (NaCl), and 2.0% sodium dodecyl sulfate (SDS)] [*3*], and 35 μL of 20 mg/mL Proteinase K (Boehringer Mannheim Biochemicals) was added. In one experiment, the saliva cell pellets were resuspended in 305 μL of 10m*M* Tris (pH 7.6), 10m*M* NaCl, 1m*M* EDTA, 1% SDS, and 0.65 μg/mL Proteinase K in the presence or absence of 39m*M* dithiothreitol (DTT). To obtain DNA from saliva frozen at −20°C, the saliva was thawed at 20°C, 1-mL aliquots were removed to microfuge tubes, and pellets were collected and treated with lysis buffer as above.

To obtain buccal swabbings, one cotton swab was rubbed lightly several times on the inside cheek of each individual in the study and allowed to dry at room temperature overnight. For studies to determine the minimum volume of saliva required to give a DNA banding pattern, aliquots of 1, 10, 20, 30, 40, 50, 75, or 100 μL of saliva were spotted onto cotton swabs and allowed to dry at room temperature overnight. The cotton was then removed from the swab stem, transferred to a microfuge tube, and 0.7 mL of lysis buffer and 35 μL of Proteinase K (20 mg/mL) were added.

Cigarette butts were obtained from two sources. Discarded cigarettes smoked by volunteers were obtained from ashtrays within 12 h of being smoked. Cigarette butts dipped in saliva were provided by Collaborative Testing Services, Inc. (Herndon, Virginia) as part of a proficiency testing program (Physiological Fluids No. 89-13). These cigarette butts were dipped briefly in fresh saliva, allowed to air dry at 20°C overnight and stored at room temperature until they were shipped five days later. The cigarette butts were received 11 days after shipping. To obtain DNA from the cigarette butts, the filter ends of the cigarettes were removed, cut into small pieces, and incubated in lysis buffer with Proteinase K (1 mg/mL final concentration) in a microfuge tube as above.

Stamps and the gummed edges of envelopes were licked, sealed, allowed to dry 2 h at room temperature and then cut into small pieces and transferred to microfuge tubes or 15-mL polypropylene tubes. Lysis buffer was added to cover the samples and Proteinase K was made to a final concentration of 1 mg/mL.

To simulate a forensic science situation, two volunteers were gagged with blue cotton material. After 15 min, the gag was removed and allowed to dry at room temperature for approximately 4 h. The saliva-stained section of the material was cut into small pieces and transferred to 15-mL polypropylene tubes, and lysis buffer and Proteinase K were added as above. Saliva stains provided by Collaborative Testing Services, Inc., were prepared by spotting two to three drops of fresh saliva on clean cotton cloth. These samples were dried, stored, and shipped as described above for the cigarette butts and were processed for DNA extraction as described for the gags (above).

DNA was isolated from forensic casework evidentiary samples containing mixed saliva and semen stains as previously described [*3*] by treating the material with lysis buffer and Proteinase K at 140 μg/mL final concentration.

For all of the studies, the samples were incubated overnight at 56°C. The DNA was then extracted from each sample with an equal volume of phenol/chloroform (1:1) and with an equal volume of chloroform. One microlitre of glycogen (20 mg/mL) (Boehringer Mannheim Biochemicals) was added and the DNA was precipitated with an equal volume of isopropanol at −20°C overnight. The DNA was pelleted, washed with 80% ethanol, air dried, and resuspended in restriction buffer [60m*M* Tris (pH 7.5), 10m*M* magnesium chloride (MgCl), 100m*M* NaCl, 35m*M* 2-mercaptoethanol, and 1 mg/mL bovine serum albumin (Life Technologies, Inc.)].

DNA was isolated from whole blood, from dried blood stains (provided by Collaborative Testing Services, Inc.), and from hair as previously described [3,6].

Analysis of DNA

The DNA was digested with *Hinf* I (New England Biolabs) in the presence of 3m*M* spermidine at 37°C overnight. The digested DNA was size fractionated by electrophoresis on 0.7% agarose (Sigma Type I) gels at 75 V, 60 mA in TBE buffer (pH 8.8) (134m*M* Tris, 75m*M* boric acid, 25m*M* EDTA) until the 2.3 kb Lambda *Hin*d III fragment had migrated 20 cm from the origin. The 1-kb ladder (Life Technologies, Inc.) was also included on the gels as a molecular weight standard. After the gels had been washed for 15 min in 0.25*M* hydrochloric acid (HCl), 30 min in 0.5*M* sodium hydroxide (NaOH) and 1.5*M* NaCl, and 30 min in 0.5*M* Tris-HCl (pH 7.5) and 3.0*M* NaCl, the DNA fragments were transferred [8] to nylon membranes (Amersham Hybond N).

The membranes were prehybridized in 300 mL of 0.5*M* sodium phosphate (Na_2PO_4) (pH 7.2), 7% SDS, 1m*M* EDTA, and 0.1% bovine serum albumin (Fraction V) (Sigma) for 10 min at 65°C, and then hybridized at 65°C in 150 mL of 1 × SSC, 6% PEG 8000, and 0.5 mg sheared human placental DNA (Sigma Type XIII), with a cocktail of ^{32}P-labeled [9] single-locus DNA probes D1S7 (MS1), D7S21 (MS31), D12S11 (MS43), and D7S22 (g3) or each of the probes individually [10–12]. After an overnight hybridization, the membranes were washed at 65°C for 30 min in 500 mL of each of the following: Wash 1—1 × SSC (0.15*M* NaCl and 0.015*M* trisodium citrate) and 1% SDS; Wash 2—40m*M* Na_2PO_4 (pH 7.2) and 1% SDS; Washes 3 and 4—0.5 × SSC and 0.1% SDS; Washes 5 and 6—0.2 × SSC and 0.1% SDS. The membranes were rinsed in 1 × SSC and autoradiography was performed at −70°C for various lengths of time depending on the amount of DNA present. It has been previously reported that a minimum of 30 to 60 ng of DNA is required to give a complete DNA banding pattern with the single-locus probes used here [6,11]. Generally an exposure time of approximately 14 days is required to detect this amount of DNA. Larger amounts of DNA can be readily detected with shorter exposure times.

For some experiments, the amount of DNA recovered was estimated from yield minigels prior to digestion with the restriction enzyme. For some experiments, the amount of DNA recovered was determined after the DNA was digested with *Hinf*I using a Hoefer TKO 100 DNA fluorimeter.

A Bio Image Visage computerized imaging system was used to determine band sizes for a DNA banding pattern using the reciprocal method of molecular weight determination of Elder and Southern [13].

Results

DNA Banding Patterns from Saliva and Buccal Swabs

DNA was isolated from 1 mL of saliva collected from several individuals as described in the Materials and Methods section. Between 3 and 14 μg of DNA were obtained from all of the samples of saliva. The DNA extracted from the saliva of four of the individuals (Nos. 1 through 4) was digested with *Hinf* I, electrophoresed, Southern blotted, and probed with a cocktail of four single-locus probes (MS1, MS31, MS43, and g3). DNA banding patterns were obtained for each individual. Furthermore, the DNA banding patterns showed little or no evidence of degradation (Fig. 1, and data not shown).

DNA isolated from buccal swabbings from four individuals (Nos. 2 through 5) was similarly analyzed. A DNA banding pattern was obtained from the buccal swab for each individual (Fig. 1) which matched the DNA banding pattern obtained with saliva from

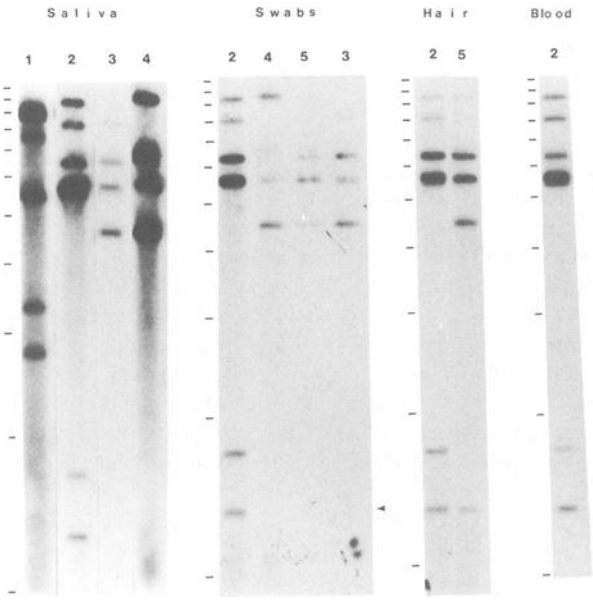

FIG. 1—*DNA banding patterns from saliva, buccal swabs, hair, and blood. DNA was isolated from 1 mL of saliva, one buccal swab, ten hairs, or blood from five individuals (Nos. 1 through 5) and analyzed as described in the Materials and Methods section. An amount of 1.5 μg of DNA extracted from blood was loaded on the gel; the other DNAs were not quantitated. Individuals 3 and 5 are the children of Individuals 2 (father) and 4 (mother). The arrow head denotes the position of a band present in the original autoradiogram for Individual 5, but not visible in this reproduction. Molecular weight standards from 2.04 to 12.22 kb (1 kb ladder; Life Technologies, Inc.) are shown on the left. Autoradiograms are shown.*

that individual (Nos. 2, 3, and 4). The DNA on the membrane was then sequentially hybridized using each of the single-locus probes individually. As expected from previous publications [*10–12*], Mendelian patterns of inheritance could be demonstrated in the mother (No. 4), Child 1 (No. 5), Child 2 (No. 3), and father (No. 2) quartet shown in Fig. 1.

To verify that the DNA banding pattern obtained from saliva or buccal swabs is the same as the pattern obtained from other cells of that individual, similar analyses were performed on DNA isolated from either hair or blood. As seen in Fig. 1, the DNA banding pattern for Individual 2 is the same whether the source of DNA is from saliva, a buccal swab, hair, or blood. Likewise, the DNA isolated from both the buccal swab and hair of Individual 5 yields indistinguishable DNA banding patterns.

Based on the amount of DNA obtained from saliva (see above) and the previous reports [*6,11*] that approximately 60 ng of DNA are required to give a complete DNA banding pattern with the single-locus probes used here, one would predict that a minimum of 10 to 20 μL of saliva is needed to obtain a complete DNA banding pattern. To test this prediction and to test the recovery of DNA from saliva on swabs, aliquots (1, 10, 20, 30, 40, 50, 75, and 100 μL) of saliva were spotted onto cotton swabs, and DNA was isolated and analyzed as described in the Materials and Methods section. As expected, small amounts of DNA were detected by minigel analysis, and complete DNA banding patterns were obtained from all of the samples containing 20 μL or more of saliva (data not shown). These studies demonstrate that sufficient amounts of DNA for identity testing can be obtained from very small amounts of saliva and that the yield of DNA from saliva spotted onto cotton swabs is high.

Stability of DNA in Saliva

The foregoing results show that freshly isolated saliva and saliva-stained swabs are good sources of intact DNA for identity testing. However, for use in forensic science testing, it is important to determine whether the DNA in saliva remains intact under various storage conditions. DNA was isolated as described from aliquots of saliva stored at −20°C from Individuals 1 and 2, and analyzed as described above. The DNA banding patterns obtained after two or three weeks of storage of saliva at −20°C are virtually indistinguishable from the patterns obtained from fresh isolates of saliva (Fig. 2). Degradation of DNA would result in the loss of high molecular weight DNA and would be detected as a preferential loss of signal in the high molecular weight bands of the DNA banding pattern relative to the bands of lower molecular weight. As seen in Fig. 2, there may be some decrease in the intensity for the top two bands relative to the smaller bands in the DNA banding pattern from Individual 2. Although this may suggest that some DNA degradation has resulted from long-term storage (20 or 14 days at −20°C), this observation does not seem to hold true for the DNA from Individual 1 stored under the same conditions. This result indicates that there has been no significant degradation of the DNA with storage and that meaningful DNA banding patterns can be obtained.

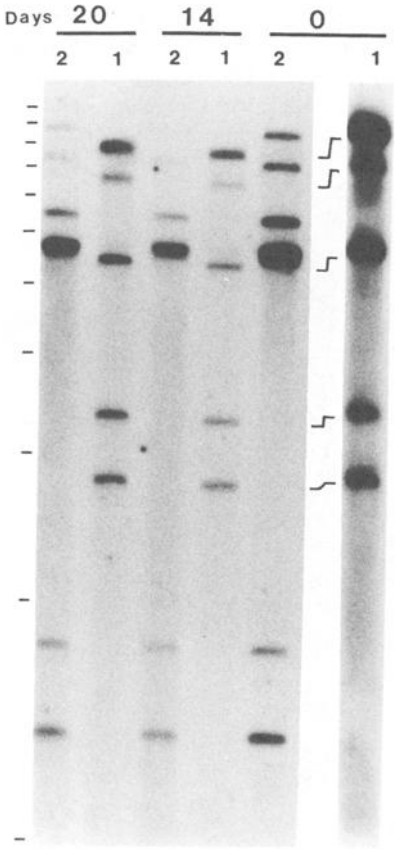

FIG. 2—*DNA banding patterns from saliva stored at −20°C. DNA was extracted from freshly isolated saliva (Day 0) and from saliva stored at −20°C for 14 or 20 days from two individuals (1 and 2) and analyzed as described in the Materials and Methods section. The pattern shown for Individual 1, Day 0 is from another gel. The molecular weight standards are as described in Fig. 1. An autoradiogram is shown.*

Preliminary results from studies on the effects of storage conditions indicate that intact DNA can be isolated and DNA banding patterns can be obtained from cotton swabs containing saliva stored for up to one week at 4 or 20°C under both dry and humid conditions. However, there is more evidence of DNA degradation at 20°C than at 4°C. Not surprisingly, cotton swabs containing saliva stored at 37°C for as little as four days yield large amounts of high molecular weight DNA but no DNA banding pattern. The DNA isolated may be predominantly of bacterial origin.

These results suggest that an adequate long-term storage condition for saliva is −20°C, but that short-term storage of saliva on cotton swabs at 4 or 20°C can yield DNA suitable for analysis (also see below).

DNA Banding Patterns from Mock Forensic Samples Containing Saliva

The possibility of obtaining DNA banding patterns from samples containing saliva which may be encountered in forensic science casework was examined. Saliva stains from envelopes, stamps, discarded smoked cigarette butts, and gags were used as potential sources of DNA. Although sufficient amounts of high molecular weight DNA for detection on a minigel were obtained from the six legal-size (9½ × 4 in.) and the six small (4 × 7½-in.) envelopes, six stamps, three of six cigarette butts, and both of the gags, complete DNA banding patterns were obtained from only three of the legal-size envelopes and the single gag examined (Fig. 3). Only faint and incomplete DNA banding patterns were seen from the other three legal-sized envelopes and from the six small envelopes (data not shown). No DNA banding patterns were obtained from any of the stamps or cigarette butts analyzed.

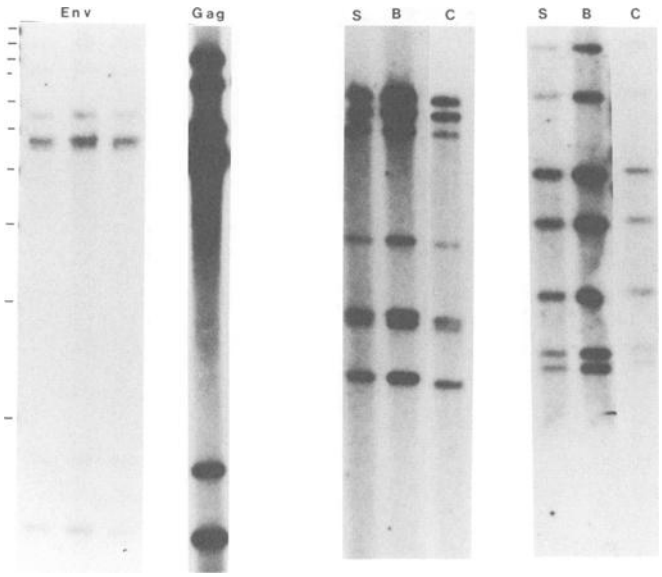

FIG. 3—*DNA banding patterns from mock forensic science samples. DNA extracted from mock forensic science samples containing saliva (large envelopes, a gag, saliva-stained material, and cigarette butts) and from blood-stained material was analyzed as described in the Materials and Methods section. The envelopes (Env) were licked and the gag was worn by Individual 2 (in Figs. 1 and 2). Each of the sets of cigarette butts* (C), *the saliva-stained materials* (S), *and the blood-stained materials* (B) *were prepared using saliva or blood from the same individual (samples provided by Collaborative Testing Services). The molecular weight standards are as described in Fig. 1 except that the 2.04 kb band is not shown. Autoradiograms are shown.*

While it was not possible in these studies to obtain DNA banding patterns from the discarded butts of smoked cigarettes, probably because of the limited amount of saliva and DNA, it is possible to obtain complete DNA banding patterns from cigarette butts which had been dipped in saliva. Cigarette butts dipped in the saliva were provided by Collaborative Testing Services, Inc. (Herndon, Virginia), along with saliva-stained and blood-stained material from two individuals. DNA was isolated from each of the samples and analyzed. As seen in Fig. 3, complete DNA banding patterns were obtained from the cigarette butts (C) and these patterns matched the patterns obtained from the blood (B) and saliva (S) stains. Therefore, the absence of a DNA banding pattern from the smoked cigarette butts is not due to the presence of the cigarette materials during the extraction.

DNA Banding Patterns from Forensic Casework

Three pieces of evidence containing mixed saliva and semen stains were received for analysis. DNA was extracted from the evidence (E) and analyzed as described in the Materials and Methods section. DNA was also extracted from blood (B) from the three known female victims and similarly analyzed (Fig. 4). For each of the cases, one of the DNA banding patterns obtained from the evidence matches the DNA banding pattern obtained from the blood of the corresponding victim.

In subsequent extractions of the evidence in the presence of DTT, which lyses sperm and releases the sperm DNA, a second DNA banding pattern was obtained that matched the DNA banding pattern obtained from blood of a suspect (data not shown). Similar results were obtained in three other cases having mixed saliva/semen samples (data not shown). It is apparent from these studies that DNA and DNA banding patterns can be readily obtained from both the saliva and semen components of a mixed saliva/semen stain and that the DNA from the epithelial cells in the saliva and the DNA from the sperm in the semen can be partitioned using a differential lysis procedure. Furthermore, it is of interest that all three of these evidentiary samples had been stored at −20°C for 2 to 2½ years before DNA isolation.

Discussion

The studies presented here demonstrate that saliva and saliva-stained material are good sources of DNA. Furthermore, the DNA isolated from these sources can be readily digested with a restriction enzyme and hybridized with single-locus probes to generate DNA banding patterns that are suitable for identity testing. The amount of DNA obtained from 1 mL of saliva is clearly sufficient for analysis (Fig. 1), but as little as 100 μL or less of saliva may be adequate in some situations. The DNA banding patterns obtained from saliva collected from an individual at different times are indistinguishable from each other and from the banding patterns obtained from blood, hair, or buccal swabs from the same individual (Figs. 1 and 2). Moreover, Mendelian patterns of inheritance could be demonstrated from saliva-extracted DNA in the same way as for other cell sources. This finding suggests that saliva sources in a forensic setting may be useful for identifying relatives even in the absence of a body.

The DNA banding pattern obtained from fresh saliva is also indistinguishable from that obtained from dried saliva found on mock forensic science samples such as cotton swabs, envelopes, or gags (Fig. 3). In addition, the DNA banding patterns generated from dried saliva/semen stains from casework material are the same as the patterns obtained from known blood samples (Figs. 3 and 4). Although smoked cigarette butts, small envelopes, and stamps were also processed, insufficient amounts of DNA were obtained to yield complete DNA banding patterns.

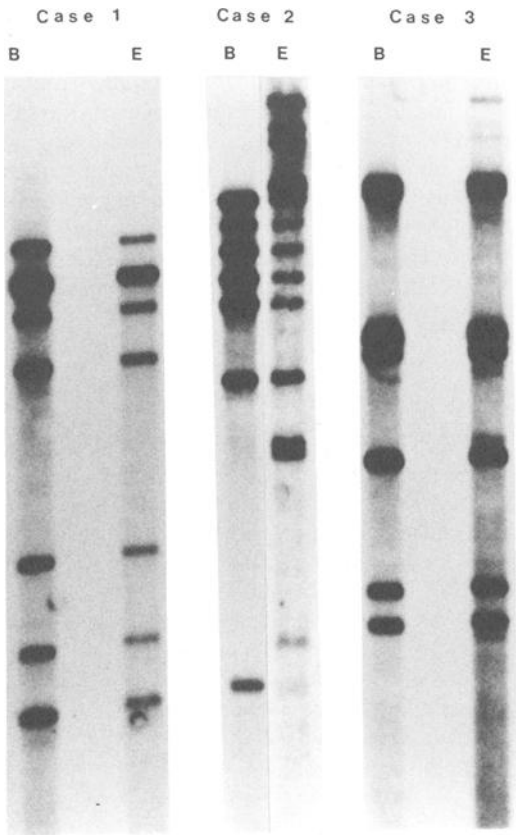

FIG. 4—*DNA banding patterns from forensic science casework. Three pieces of evidence* (E) *containing mixed saliva/semen stains were submitted for analysis. DNA was extracted from the mixed stains on sweatshirts (Cases 1 and 2) and a sweater (Case 3) and from known blood standards from each of the victims* (B) *and analyzed as described in the Materials and Methods section. In Case 2 the additional bands present in the evidence not matching the victim's bands match the DNA banding pattern for the suspect. Autoradiograms are shown.*

These studies also demonstrate that saliva and saliva-stained material provide a stable source of DNA if adequately stored. Frozen (−20°C) aliquots of saliva gave banding patterns that were indistinguishable from those of fresh saliva (Fig. 2). Materials containing dried saliva stored under varying conditions were studied, and DNA banding patterns were often obtained that showed little or no sign of degradation. For example, saliva from Individual 2 which was dried overnight on cotton swabs, on envelopes, or on material (gags) for several hours at 20°C gave complete DNA banding patterns (Figs. 1 and 3). Saliva dried on cigarette butts and analyzed over two weeks later gave DNA banding patterns showing no evidence of degradation. In addition, in casework samples, epithelial cell DNA and sperm DNA were successfully separated using a differential lysis procedure from dried mixed saliva and semen stains that had been stored at −20°C for up to 2½ years; these DNAs yielded DNA banding patterns that showed little sign of degradation.

Generally, fresh whole blood or blood-stained material is the primary source of an individual's DNA used as a standard for comparison to evidentiary material in DNA typing. The findings presented here allow the use of saliva as an alternative source of

DNA for known standards. Fairly large volumes of saliva can be collected in a noninvasive manner without any pain. In fact, saliva and buccal swabbings were easily obtained from a 6-month-old child for these studies (Fig. 1, Individual 3). Although the extraction and analysis of DNA from materials that may be present in a forensic setting, such as cigarettes, envelopes, stamps, gags, or other fabric, were explored here, there are many other potential sources of saliva-stained materials.

References

[1] *Banbury Report 32: DNA Technology and Forensic Science*, J. Ballantyne, G. Sensabaugh, and J. Witkowski, Eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.

[2] *Genetic Witness: Forensic Uses of DNA Tests*, Congress of the United States, Office of Technology Assessment, U.S. Government Printing Office, Washington, DC, 1990.

[3] Gill, P., Jeffreys, A. J., and Werret, D. J., "Forensic Applications of DNA 'Fingerprints,' " *Nature*, Vol. 318, Dec. 1985, pp. 577–579.

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

[5] 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.

[6] Hopkins, B., Morten, J. E. N., Smith, J. C., and Markham, A. F., "The Development of Methods for the Analysis of DNA Extracted from Forensic Samples," *Technique*, Vol. 1, No. 2, Oct. 1989, pp. 96–102.

[7] Gaensslen, R. E., *Sourcebook in Forensic Serology, Immunology and Biochemistry*, National Institute of Justice, Washington, DC, 1983.

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

[9] Feinberg, A. P. and Vogelstein, B., "A Technique for Radiolabelling DNA Restriction Endonuclease Fragments to High Specific Activity," *Analytical Biochemistry*, Vol. 137, 1984, pp. 266–267.

[10] Wong, Z., Wilson, V., Jeffreys, A. J., and Thein, S. L., "Cloning a Selected Fragment from a Human DNA 'Fingerprint': Isolation of an Extremely Polymorphic Minisatellite," *Nucleic Acids Research*, Vol. 14, No. 11, 1986, pp. 4605–4616.

[11] Wong, Z., Wilson, V., Patel, I., Povey, S., and Jeffreys, A. J., "Characterization of a Panel of Highly Variable Minisatellites Cloned from Human DNA," *Annals of Human Genetics*, Vol. 51, 1987, pp. 269–288.

[12] Smith, J. C., Anwar, R., Riley, J., Jenner, D., Markham, A. F., and Jeffreys, A. J., "Highly Polymorphic Minisatellite Sequences: Allele Frequencies and Mutation Rates for Five Locus-Specific Probes in a Caucasian Population," *Journal of Forensic Sciences*, Vol. 30, 1990, pp. 19–32.

[13] Elder, J. K. and Southern, E. M., "Measurement of DNA Length by Gel Electrophoresis: II. Comparison of Methods for Relating Mobility to Fragment Length," *Analytical Biochemistry*, Vol. 128, 1983, pp. 227–231.

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