Mark S. Nelson,[1] M.S.; Elizabeth A. Benzinger,[4] Ph.D.; Michael J. Budzynski,[1] B.S.; Mark T. Boodee,[1] M.S.; Anita Matthews,[1] M.S., Eric Buel,[2] Ph.D.; Margaret B. Schwartz,[2] Ph.D.; Cecilia von Beroldingen,[3] Ph.D.; Randall L. Wampler,[3] B.S.; Terry M. Coons,[3] M.S.; James Bixby,[3] B.S.; William E. Frank,[4] B.S.; and D.A. Metzger,[4] B.S.

Validation of Probe EFD52 (D17S26) for Forensic DNA Analysis

REFERENCE: Nelson, M. S., Benzinger, E. A., Budzynski, M. J., Boodee, M. T., Matthews, A., Buel, E., Schwartz, M. B., von Beroldingen, C., Wampler, R. L., Coons, T. M., Bixby, J., Frank, W. E., and Metzger, D. A., **"Validation of Probe EFD52 (D17S26) for Forensic DNA Analysis,"** *Journal of Forensic Sciences,* JFSCA, Vol. 41, No. 4, July 1996, pp. 557–568.

ABSTRACT: Validation studies that meet TWGDAM (The Working Group on DNA Analysis Methods) and CAC (California Association of Criminalists) guidelines for RFLP (restriction fragment length polymorphism) analysis were performed with the DNA probe EFD52 (D17S26). These studies demonstrate that the probe EFD52 is suitable for forensic casework. No unexpected DNA banding patterns were obtained from controlled studies examining various tissues, sample consistency over many gels, mixtures of body fluids, various substrates, various contaminants and non-human DNA sources. Of all the animal DNAs tested, only one higher primate yielded a single band to EFD52 hybridization. The sensitivity of EFD52 was shown to be comparable to that of other forensic probes. Population frequency distribution tables were prepared from over 4000 alleles and two-locus studies were conducted on nine forensically useful probes. Black, White, Hispanic and Lumbee Indian populations were found to be in Hardy-Weinberg and linkage equilibrium. Comparisons between victim blood standards and epithelial fractions of mixed stains from sexual assault cases were used to demonstrate the robustness of the EFD52 probe in forensic casework.

KEYWORDS: forensic science, restriction fragment length polymorphism, DNA, forensic, variable number tandem repeats, EFD52, D17S26

DNA profiling by restriction fragment length polymorphism (RFLP) analysis has become an increasingly important tool in the investigation and prosecution of violent crime, as well as in the exoneration of wrongfully accused individuals. A great deal of attention has been given to issues of quality assurance in laboratories performing this powerful technique. The Technical Working Group on DNA Analysis Methods (TWGDAM) and the California Association of Criminalists (CAC) Ad Hoc Committee on DNA Quality Assurance have addressed quality assurance programs in forensic DNA typing (1,2).

One aspect of quality assurance, as recommended by TWGDAM and CAC, is the thorough evaluation of probes prior to their use in forensic settings. Data are presented here demonstrating that the probe EFD52 (3; D17S26; Promega Corp., Madison, WI) meets the requirements set forth by the above named groups and is suitable for forensic analysis. Controlled studies involving tissue comparisons, consistency of measurement over numerous different analyses, mixtures of body fluids, various substrates, various contaminants, non-human DNA sources, forensic case samples, and allelic population frequencies are described. The results are compared to previous RFLP validation studies and recommendations are made for the validation of new RFLP probes and other forensic DNA profiling methods in the future.

[1]North Carolina State Bureau of Investigation, Raleigh, NC (A.M. presently, Laboratory Corporation of America Holdings, Research Triangle Park, NC).
[2]Vermont State Police, Waterbury, VT.
[3]Oregon State Police Forensic Laboratory, Portland, OR.
[4]Illinois State Police Forensic Science Command, Springfield, IL.
Received for publication 2 Sept. 1995; revised manuscript received 28 Nov. 1995; accepted for publication 4 Dec. 1995.

Materials and Methods

DNA profiling was carried out in the four authoring laboratories using restriction endonuclease *Hae* III and the basic RFLP DNA profiling procedure developed by the FBI as described by Budowle and Baechtel (4) and Budowle et al. (5). EFD52 was purchased from Promega Corporation as a 6.4 kb purified plasmid insert and labeled with ^{32}P-dCTP. Interpretation of autoradiographs was assisted by the DNA Imaging System software provided by the Federal Bureau of Investigation (6). Population frequency tables were prepared using the fixed bin approach described by Budowle et al. (7). Bin boundaries were assigned arbitrarily on the basis of the Lifecodes Corp. 30-band molecular weight marker. The following studies were performed:

Tissue Studies

Studies to identify any tissue-specific banding differences with the probe EFD52 were conducted on liquid blood, bloodstains, saliva, vaginal secretions, semen and anagen (pulled) hairs from known volunteers. DNA was extracted from each type of tissue and comparisons were made after profiling at the D17S26 locus (Table 1).

Consistency

The ability to obtain consistent measurements over time and over numerous gels is a reflection on the reliability of both the RFLP procedure and the probe. Three sets of data were examined to determine the extent of inter- and intragel variation in banding patterns at the locus D17S26. In the first experiment, DNA from the cell line K562 and from bloodstains from one individual was extracted and typed on 166 different gels spanning a three year period (Table 2). In the second experiment, DNA from blood stains

TABLE 1—*EFD52 band measurements from various tissues from the same donor. The largest difference between the dried blood stain and any other tissue is indicated.*

Donor	Donor	Band	Blood Stain	Liquid Blood	Saliva	Hair	Semen	Vaginal Epithelial	Largest % Difference
RMT-1	A	1	3948	3894	3934	...	3916	...	1.37
		2	1371	1335	1362	...	1368	...	2.63
SS	B	1	5953	5856	5934	...	...	...	1.63
		2	1394	1402	1391	...	...	...	0.57
KJS	C	1	5068	...	5015	5003	...	4940	2.53
		2	1850	...	1839	1849	...	1839	0.59
GOR	D	1	5901	...	5876	...	5885	...	0.42
		2	4276	...	4222	...	4229	...	1.26
JAR	E	1	4966	...	...	...	...	4854	2.26
		2	1389	...	...	...	...	1343	3.31
OBC	F	1	1398	...	...	...	1397	...	0.07
		2	1341	...	...	...	1339	...	0.15
BAC	G	1	7271	7320	...	...	...	7012	3.56
		2	4073	4114	...	...	...	3990	2.04
KAP	H	1	3864	...	3849	...	...	3834	0.78
		2	1455	...	1449	...	...	1437	1.24
BAP	I	1	8770	8897	8708	...	...	...	1.45
		2	5128	5138	5091	...	...	...	0.72
RLW	J	1	5518	5551	5406	5594	...	...	2.03
		2	4910	4910	4827	4932	...	...	1.69
JIH	K	1	4510	...	4496	4506	...	4404	2.35
		2	1452	...	1451	1442	...	1427	1.72
JJH	L	1	2482	...	2512	...	2519	...	1.49
		2	1390	...	...	...	...	...	
CVB-1	M-1	1	2324	2334	2311	...	...	2266	2.50
		2	1369	1374	1364	...	...	1368	0.37
CVB-2	M-2	1	2321	2290	2310	...	...	2310	1.46
		2	2369	1353	1377	...	...	1356	1.17
LVB	N	1	1383	...	1340	...	1370	...	3.11
BCT	O	1	4886	...	4884	...	...	...	0.04
		2	4096	...	4096	...	...	...	0.00
CGS	P	1	1836	...	...	...	...	1801	1.91
		2	1375	...	...	...	...	1355	1.45
DCC	Q	1	4501	...	...	...	4539	...	0.84
		2	2243	...	...	...	2245	...	0.09
DJS-1	R	1	1391	1383	1370	...	...	1370	1.51
LVB	S	1	1397	1375	1367	...	1346	...	3.65

TABLE 2—*EFD52 sizing data from 166 separate gels for K562 and donor "MJB."*

	K562		"MJB"	
	Band 1	Band 2	Band 1	Band 2
Mean	4825.3	1366.9	4838.2	1355.4
S.D.	20.7	6.4	18.0	6.1
Low	4802	1352	4767	1347
High	4900	1383	4892	1381

of three volunteers and from the cell line K562 were electrophoresed in duplicate on each of 18 different gels (Tables 3*a* and 3*b*). In the third experiment, band measurements for human cell line K562 DNA from 535 gels prepared in the four different laboratories were analyzed (Table 4).

Mixed Specimens

A study was performed to demonstrate that mixtures of DNA from more than one donor can be resolved by the probe EFD52. Sixteen mixtures consisting of bloods, blood and semen, blood and vaginal secretions, blood and saliva, vaginal secretions and semen, and saliva and semen from known volunteers were analyzed and compared to blood standards from those volunteers (Fig. 1).

TABLE 3*a*—*Results of 144 intergel comparisons of EFD52 alleles. DNA from the cell line K562 and from bloodstains prepared from three volunteers was digested with* Hae *III and electrophoresed in duplicate on each of 18 gels. Percent differences from the mean were calculated by dividing the difference between the high and low values by the mean. The maximum intergel variation from the mean was 2.5%.*

Sample		Band 1 (kb)	Band 2 (kb)
1	Mean	5508	1490
	High	5572	1503
	Low	5467	1471
	% Diff from Mean	1.91	2.15
2	Mean	4845	1359
	High Value	4903	1374
	Low Value	4782	1343
	% Diff from Mean	2.50	2.28
3	Mean	5662	4378
	High	5739	4429
	Low	5603	4341
	% Diff from Mean	2.40	2.01
K562	Mean	4840	1356
	High	4884	1376
	Low	4786	1342
	% Diff from Mean	2.02	2.51

TABLE 3*b*—*Intragel variation for 72 pairwise sample comparisons. Each member of a pairwise comparison was bounded by separate molecular weight marker lanes.*

% Variation	# Observations
0–0.5	36
0.5–1.0	27
1.0–1.5	8
1.5–2.0	1

Environmental Insult and Matrix Effects

Liquid blood (25 μL) from known volunteers was spotted on white cotton material and spiked with 25 μL of various contaminants (Table 5*a*) or was applied to various fabrics and surfaces and allowed to dry (Table 5*b*). The DNA was extracted and analyzed to determine what effect the contaminants or the various matrices would have on DNA recovery and banding patterns observed with the probe EFD52.

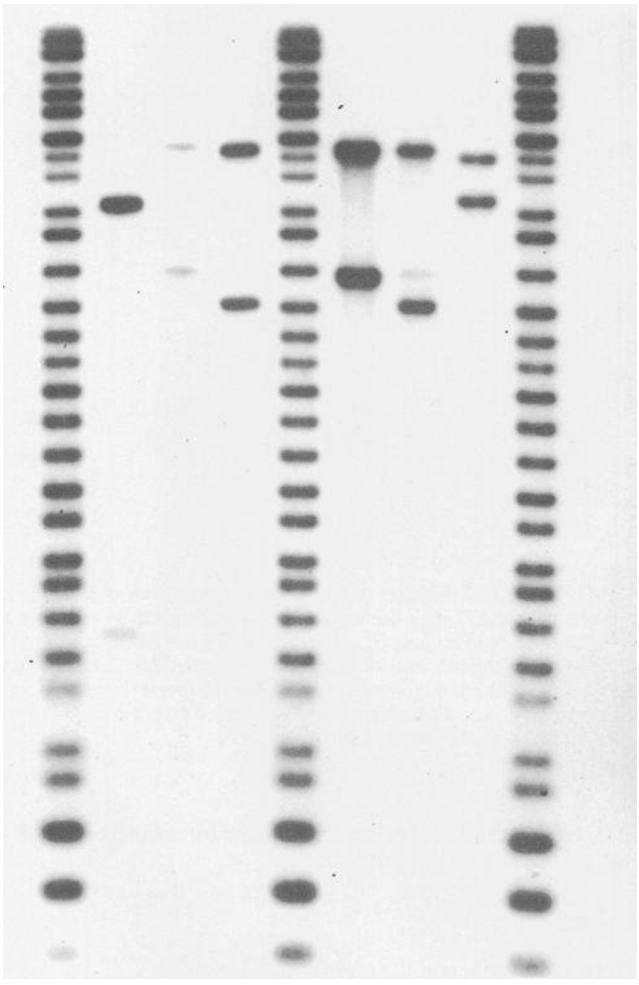

FIG. 1—*EFD52 banding patterns from donor blood samples and a mixture of semen and vaginal epithelial cells from the same sources. Lanes 1, 5 and 9, Life Technologies 30 band molecular weight ladder; lane 2, K562 control; lane 3, female donor blood; lane 4, male donor blood; lane 5, female fraction of differential extraction of postcoital vaginal swab; lane 6, male fraction from postcoital swab (with trace of female contribution); lane 7, laboratory control sample.*

TABLE 4—*Mean K562 band measurements from four different laboratories.* n = 535.

Source	Mean Band Size (kb)	*n*	SD
North Carolina	4849	210	26.0
	1366		8.0
Oregon	4857	231	28.5
	1367		6.7
Vermont	4843	21	28.6
	1368		4.9
Illinois	4839	73	21.1
	1363		4.6
Grand mean	4851		
	1366		

Forensic Samples

Forensic case samples were analyzed at the D17S26 locus. This type of sample permits the evaluation of a probe or analytical technique on actual forensic specimens in a controlled fashion. Sexual assault evidence collection kits were evaluated where comparisons were made between a victim's blood and the epithelial cell component of the vaginal or rectal swabs from the kit or the stains from the victim's underwear. The chain of custody for these evidence items documents that they originate from the same individual. Seventy-five comparisons between victim blood standards and vaginal or rectal epithelial cell fractions were made at the D17S26 locus (Table 6; Fig. 2).

Nonhuman DNA Sources

DNA extracted from the blood of 22 different vertebrate species (Table 7) was analyzed at the locus D17S26 to determine if the probe EFD52 would hybridize to DNA from these animals.

Minimum Detectable Sample Size

The sensitivity of probe EFD52 was assessed by analyzing serial dilutions of DNA from the human cell line K562 on two different sets of membranes. These membranes were hybridized with EFD52 as well as with other probes.

Population Studies

Donor samples (4378 alleles) from four geographically distinct regions were profiled at the locus D17S26 to establish population frequencies. Data was placed into 31 bins using the Lifecodes 23 kb molecular weight marker (Lifecodes Corporation, Stamford, CT) bands as bin boundaries. Populations consisted of Whites from North Carolina, Vermont, Oregon and Illinois; Blacks from North Carolina, Oregon and Illinois; Hispanics from Oregon; and Lumbee Indians from North Carolina. Binned data was used for comparisons between White and Black subpopulations (Tables 8, 9 and 10; Figs. 3 and 4). Finally, rebinning was performed so that no bin contained less than five observations.

Statistical tests were performed by Dr. Bruce S. Weir. The similarity of geographically distinct databases within racial groups was tested by chi-square goodness-of-fit tests. Chi-square goodness-of-fit, exact and likelihood ratio tests were used to evaluate individual databases for Hardy-Weinberg expectations (Table 11). Two-locus tests were performed to verify that D17S26 allelic frequencies are

TABLE 5a—*EFD52 band sizes from blood challenged with various contaminants. The letters in parenthesis indicate the corresponding control. The % difference from the control is given.*

Treatment	Band	bp	% Difference
Control A	1	5661	...
	2	5142	...
10% EDTA (A)	1	5640	0.37
	2	5100	0.82
20% Glycerol (A)	1	5645	0.28
	2	5160	0.35
20 mM Ni (A)	1	5637	0.42
	2	5124	0.35
20 mM Cd (A)	1	5628	0.58
	2	5173	0.60
20 mM MN (A)	1	5687	0.46
	2	5132	0.19
Antifreeze (A)	1	5654	0.12
	2	5163	0.41
DMSO (A)	1	5685	0.42
	2	5132	0.19
Dioxane (A)	1	5685	0.42
	2	5162	0.39
Control B	1	7117	...
	2	5594	...
Control C	1	5473	...
	2	1488	...
Oil (B)	1	7086	0.44
	2	5585	0.16
Oil (C)	1	5497	0.44
	2	1488	0.00
Gasoline (B)	1	7301	2.59
	2	5689	1.70
Gasoline (C)	1	5594	2.21
	2	1498	0.67
Dried bleach (B)	1	7130	0.18
	2	5638	0.79
Dried bleach (C)	1	5563	1.64
	2	1488	0.00
Urine (B)	1	7215	1.38
	2	5608	0.25
Urine (C)	1	5527	0.99
	2	1502	0.94
Sweat (B)	1	7257	1.97
	2	5645	0.91
Sweat (C)	1	5571	1.79
	2	1510	1.48
Detergent (B)	1	7309	2.70
	2	5722	2.29
Detergent (C)	1	5606	2.43
	2	1508	1.34
Red clay (B)	1	7093	0.34
	2	5608	0.25
Red clay (C)	1	5496	0.42
	2	1482	0.40
Gun powder (B)	1	7247	1.83
	2	5581	0.23
Gun powder (C)	1	5520	0.86
	2	1483	0.34
Black finger print powder (B)	1	7140	0.32
	2	5574	0.36
Black finger print powder (C)	1	5501	0.51
	2	1477	0.74
Silver finger print powder (B)	1	7079	0.53
	2	5583	0.20
Silver finger print powder (C)	1	5512	0.71
	2	1473	1.01
Soil (B)	1	7234	1.64
	2	5649	0.98
Soil (C)	1	5516	0.79
	2	1488	0.00

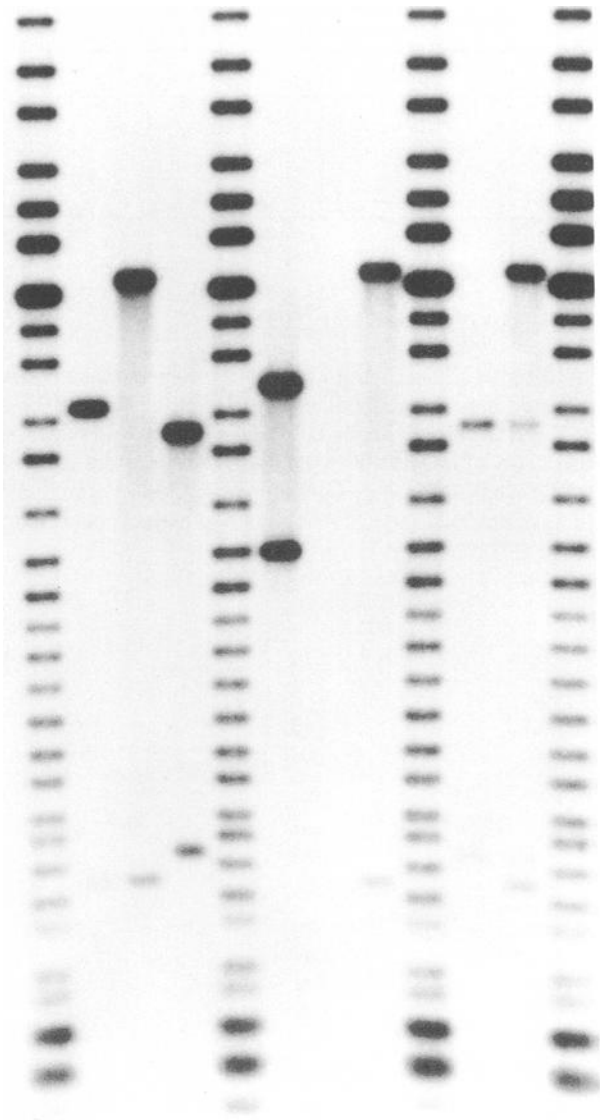

FIG. 2—*EFD52 banding patterns in samples from a forensic case. Lanes 1, 5, 9 and 12, Life Technologies 30 band molecular weight ladder; lane 2, K562 control; lane 3, sexual assault victim blood standard; lane 4, suspect 1 standard; lane 6, suspect 2 standard; lane 7, empty; lane 8, epithelial cell fraction of vaginal swab; lane 10, sperm fraction of vaginal swab; lane 11, mixed fraction of vaginal swab. Suspect 2, but not suspect 1, is excluded as the semen donor.*

independent of allelic frequencies at other forensically useful RFLP loci (Table 12).

Results

Tissue Studies

Comparisons were made between EFD52 banding patterns from blood stains, liquid blood, saliva, vaginal secretions or semen and anagen (pulled) hairs derived from the same donors. In all cases, DNA extracted from various tissues from the same individual gave the same DNA profile when probed with EFD52. EFD52 band size measurements for DNA extracted from bloodstains, liquid

TABLE 5b—*EFD52 band sizes from blood deposited on various substrates. The % difference from the control is given.*

Treatment	Band	bp	% Difference
Control	1	3911	...
	2	1366	...
Polyester/satin	1	3910	0.03
	2	1365	0.07
Polyester/course	1	3886	0.64
	2	1371	0.37
Polyester/velvet	1	3912	0.03
	2	1371	0.37
Cotton/polyester	1	3913	0.05
	2	1373	0.51
Cotton	1	3914	0.08
	2	1367	0.07
Nylon	1	3889	0.56
	2	1368	0.15
Wool	1	3904	0.18
	2	1364	0.15
Denim	1	3918	0.18
	2	1367	0.07
Rubber sole	1	3919	0.20
	2	1372	0.44
Sock	1	3913	0.05
	2	1370	0.29
Sweatshirt	1	3889	0.56
	2	1359	0.51
Sheet	1	3916	0.13
	2	1368	0.15
Pistol	1	3893	0.46
	2	1373	0.51
Knife blade	1	3905	0.15
	2	1376	0.73
Brick	1	3914	0.08
	2	1378	0.88
Glass	1	3898	0.33
	2	1371	0.37
Wood	1	3875	0.92
	2	1373	0.51
Cardboard 1	1	3921	0.26
	2	1370	0.29
Cardboard 2	1	3908	0.08
	2	1381	1.10
Fingerprint tape	1	3906	0.13
	2	1379	0.95
White paper	1	3926	0.38
	2	1376	0.73
Brown bag	1	3920	0.23
	2	1374	0.59
Floor tile	1	3896	0.38
	2	1373	0.51
Painted wall	1	3903	0.20
	2	1373	0.51
Disposable diaper	1	3909	0.05
	2	1374	0.59
Leather	1	3882	0.74
	2	weak	
Dark corduroy	1	no bands	
	2	no bands	
Shoe top	1	no bands	
	2	no bands	
Soil	1	no bands	
	2	no bands	

bloods, saliva, hair and semen or vaginal secretions from 19 different donors are displayed in Table 1. The largest difference between measurements within donors was 3.65%. These results were not unexpected. While RFLP analysis employing methylation sensitive restriction endonucleases can reveal tissue-specific banding patterns due to differences in methylation state, *Hae* III is not sensitive

TABLE 6—*Comparisons of EFD52 banding patterns in sexual assault victim blood standards and vaginal epithelial cells.*

Sample	Victim Standard	Vaginal Epithelial	% Difference
1	4949	4883	1.33
	1483	1485	0.13
2	6102	6030	1.18
	5197	5182	0.29
3	7610	7512	1.29
	4181	4099	1.96
4	5666	5639	0.48
	1817	1813	0.22
5	6168	6156	0.19
	3682	3666	0.43
6	4848	4809	0.80
	1357	1362	0.37
7	2015	2001	0.69
	1330	1318	0.90
8	11221	11139	0.73
	1493	1491	0.13
9	5257	5219	0.72
	2316	2316	0.00
10	3936	3928	0.20
	1370	1365	0.36
11	5311	5263	0.90
	1569	1572	0.19
12	4835	4855	0.41
	...	...	...
13	1368	1378	0.73
	...	...	...
14	6144	6145	0.02
	1734	1739	0.29
15	6144	6145	0.02
	1734	1741	0.40
16	6144	6155	0.18
	1734	1743	0.52
17	2033	2019	0.69
	1356	1355	0.07
18	6297	6315	0.29
	5348	5318	0.56
19	6642	6695	0.80
	1371	1362	0.66
20	6932	6986	0.78
	5481	5545	1.17
21	2750	2758	0.29
	1597	1592	0.31
22	2750	2760	0.36
	1597	1594	0.19
23	3607	3605	0.06
	1563	1553	0.64
24	3943	3913	0.76
	2520	2527	0.28
25	11410	11237	1.52
	5909	5906	0.05
26	4537	4538	0.02
	2304	2288	0.69
27	2185	2177	0.37
	1331	1311	1.50
28	5788	5808	0.35
	4965	4960	0.10
29	2138	2145	0.33
	1362	1362	0.00
30	2138	2133	0.23
	1362	1359	0.22
31	2831	2833	0.07
	2336	2319	0.73
32	4090	4081	0.22
	1382	1397	1.09
33	5859	5860	0.02
	1435	1439	0.28
34	1724	1723	0.06
	1355	1357	0.15
35	5556	5532	0.43
	1950	1962	0.62

TABLE 6—*(Continued)*

Sample	Victim Standard	Vaginal Epithelial	% Difference
36	1959	1978	0.97
	1544	1561	1.10
37	1737	1736	0.06
	1344	1347	0.22
38	6691	6660	0.46
	6126	6061	1.06
39	1353	1355	0.15
	...	...	...
40	3447	3414	0.96
	2544	2528	0.63
41	5065	5033	0.63
	3406	3396	0.29
42	5041	5011	0.60
	2476	2510	1.37
43	4677	4649	0.60
	3945	3964	0.48
44	5149	5084	1.26
	3888	3882	0.15
45	5466	5421	0.82
	1436	1436	0.00
46	10838	10678	1.48
	4566	4530	0.79
47	1906	1904	0.10
	1372	1369	0.22
48	5201	5205	0.08
	1448	1445	0.21
49	4529	4578	1.08
	1349	1354	0.37
50	2921	2873	1.64
	1501	1490	0.73
51	3809	3814	0.13
	1761	1767	0.34
52	1510	1502	0.53
	1337	1350	0.97
53	2740	2736	0.15
	1369	1364	0.37
54	2331	2338	0.30
	1362	1360	0.15
55	2014	2016	0.10
	1327	1324	0.23
56	3263	3286	0.70
	1955	1963	0.41
57	2311	2291	0.87
	1613	1592	1.30
58	1366	1348	1.32
	...	...	...
59	3832	3824	0.21
	1351	1366	1.11
60	1993	1996	0.15
	1367	1366	0.07
61	5800	5802	0.03
	1372	1364	0.58
62	3296	3309	0.39
	1441	1436	0.35
63	6004	5974	0.50
	2710	2673	1.37
64	4051	4028	0.57
	1786	1776	0.56
65	3924	3890	0.87
	3502	3487	0.43
66	6050	6004	0.76
	1695	1686	0.53
67	1574	1564	0.64
	1368	1363	0.37
68	1424	1425	0.07
	1353	1341	0.89
69	2357	2362	0.21
	1736	1734	0.12
70	5861	5769	1.57
	5248	5229	0.36

TABLE 6—*(Continued)*

Sample	Victim Standard	Vaginal Epithelial	% Difference
71	3829	3836	0.18
	3310	3299	0.33
72	6431	6512	1.26
	4525	4552	0.60
73	3364	3353	0.33
	3203	3207	0.12
74	5697	5662	0.61
	1324	1319	0.38
75	2764	2779	0.54
	1965	1986	1.07

TABLE 7—*Vertebrate DNAs probed with EFD52. Weak hybridization was observed only for rhesus monkey DNA at 1078 base pairs, below the size range normally observed for humans.*

Bear	Goat
Baboon	Horse
Bushbaby	Lemur
Cat	Pig
Chicken	Rabbit
Cockatoo	Sheep
Cow	Turkey
Deer	African green monkey
Dog	Rhesus monkey
Donkey	Fascicularis monkey
Duck	

to mammalian methylation motifs (5). In human DNA, methylation occurs in the form of 5-methyl cytosine in a subpopulation of 5′-CG dinucleotides. A portion of the *Hae* III recognition sites (5′-GGCC) would then be methylated at the 3′ cytosine. However, *Hae*III is only inhibited by methylation of the inner cytosine, which is not observed in humans.

Consistency

In the first study of intergel variation, 166 intergel comparisons of bloodstains from the same individual were made over a span of three years. All D17S26 allele measurements were within ±2.5% of the mean value (Table 2). The EFD52 profile of donor "MJB" is similar to that of the cell line K562. However, "MJB" and K562 possess dissimilar RFLP profiles at other loci studied.

In a second test of intergel variation, a total of 144 comparisons of DNA extracted from the cell line K562 and from 3 individuals were made to measure intergel variation in allele sizing. The maximum amount of intergel variation detected across 144 samples and 18 gels was 2.5% (Table 3*a*). Of 72 pairwise intragel comparisons, half fell within 0.5% of each other and no two band measurements exceeded 2.0% variation (Table 3*b*).

In the third study, large and small cell line K562 RFLP bands derived from all four laboratories (535 measurements total) were found to have mean sizes of 4851 and 1366 bp, respectively. The inter-laboratory variation between means was 0.37% or less (Table 4).

Mixed Specimens

Sixteen mixtures consisting of bloods, blood and semen, blood and vaginal secretions, blood and saliva, vaginal secretions and

TABLE 8—*D17S26 binned population frequency distribution for four different U.S. White populations. VT = Vermont, IL = Illinois, NC = North Carolina, OR = Oregon.*

Bin	Range (bp)	Frequency VT	IL	NC	OR
1	0– 639	.000	.000	.000	.000
2	640– 772	.000	.000	.000	.000
3	773– 871	.000	.000	.000	.000
4	872– 963	.000	.000	.000	.000
5	964– 1077	.000	.000	.000	.000
6	1078– 1196	.000	.000	.000	.000
7	1197– 1352	.009	.041	.057	.045
8	1353– 1507	.270	.243	.231	.246
9	1508– 1637	.036	.015	.025	.016
10	1638– 1788	.014	.020	.015	.013
11	1789– 1924	.036	.019	.036	.027
12	1925– 2088	.063	.030	.036	.030
13	2089– 2351	.018	.027	.019	.027
14	2352– 2522	.005	.015	.012	.016
15	2523– 2692	.027	.018	.011	.013
16	2693– 2862	.005	.008	.016	.013
17	2863– 3033	.014	.008	.000	.011
18	3034– 3329	.005	.019	.012	.007
19	3330– 3674	.014	.012	.011	.018
20	3675– 3979	.023	.044	.036	.036
21	3980– 4323	.036	.034	.041	.050
22	4324– 4821	.041	.067	.068	.056
23	4822– 5219	.095	.094	.096	.090
24	5220– 5685	.077	.060	.074	.048
25	5686– 6368	.113	.112	.094	.129
26	6369– 7241	.059	.060	.060	.065
27	7242– 8452	.023	.037	.029	.027
28	8453–10089	.005	.010	.007	.009
29	10090–14999	.018	.008	.015	.011
30	15000–23409	.000	.000	.000	.000
31	23410–	.000	.000	.000	.000
Number of alleles		222	734	732	558

semen, and saliva and semen from known volunteers were analyzed and compared to known blood standards from those volunteers. In all cases, banding patterns matching that of the donors were observed in the mixed samples (Fig. 1).

Environmental Insult and Matrix Effects

None of the contaminants nor matrices tested (Tables 5*a* and 5*b*) materially changed the results of EFD52 banding patterns. The banding patterns observed were similar to those obtained from pristine samples. The maximum variation observed was 2.7%. However, poor DNA recovery and weak banding patterns were noted from leather. DNA recovered from heavily dyed corduroy and red clay was resistant to digestion with *Hae*III. Additional bands due to partial digestion were noted on samples from the denim, sweatshirt material, and cardboard. In previously reported validation studies, certain environmental insults or substrates have been observed to limit the recovery of high molecular weight human DNA from forensic samples or to inhibit restriction digestion (8–11). However, as was the case for other probes and other published studies, we have found that no banding pattern alteration occurs due to substrate or environmental insults.

Forensic Samples

Seventy-five comparisons were made between victim blood standards and epithelial fractions of vaginal or rectal swabs or stains from the victim's underwear in sexual assault cases. In all of the 75 forensic cases that were analyzed at the locus D17S26, the EFD52 banding pattern detected in the DNA extracted from the forensic specimen was consistent with that of the victim's blood standard (Table 6; Fig. 2). The largest difference measured between a victim blood standard and forensic specimen was 1.96%. The mean difference was 0.53% and the SD of the % difference was 0.42.

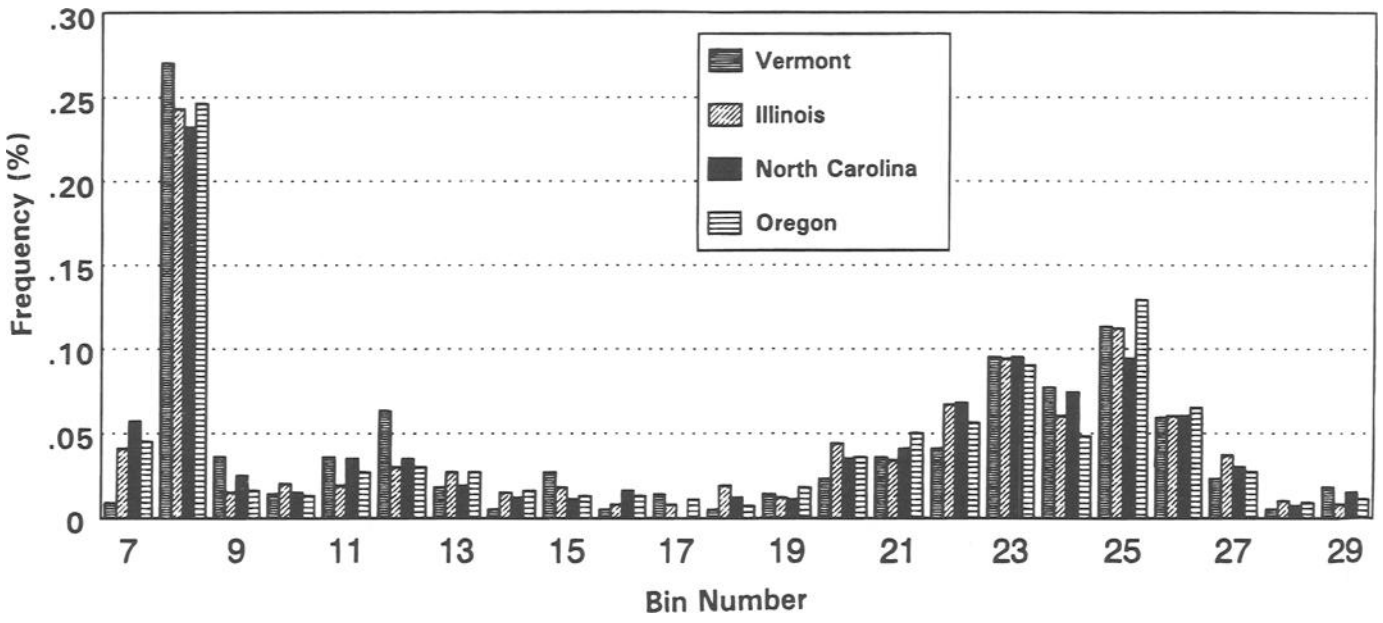

FIG. 3—*Comparisons of D17S26 allele frequency distributions in four White populations.*

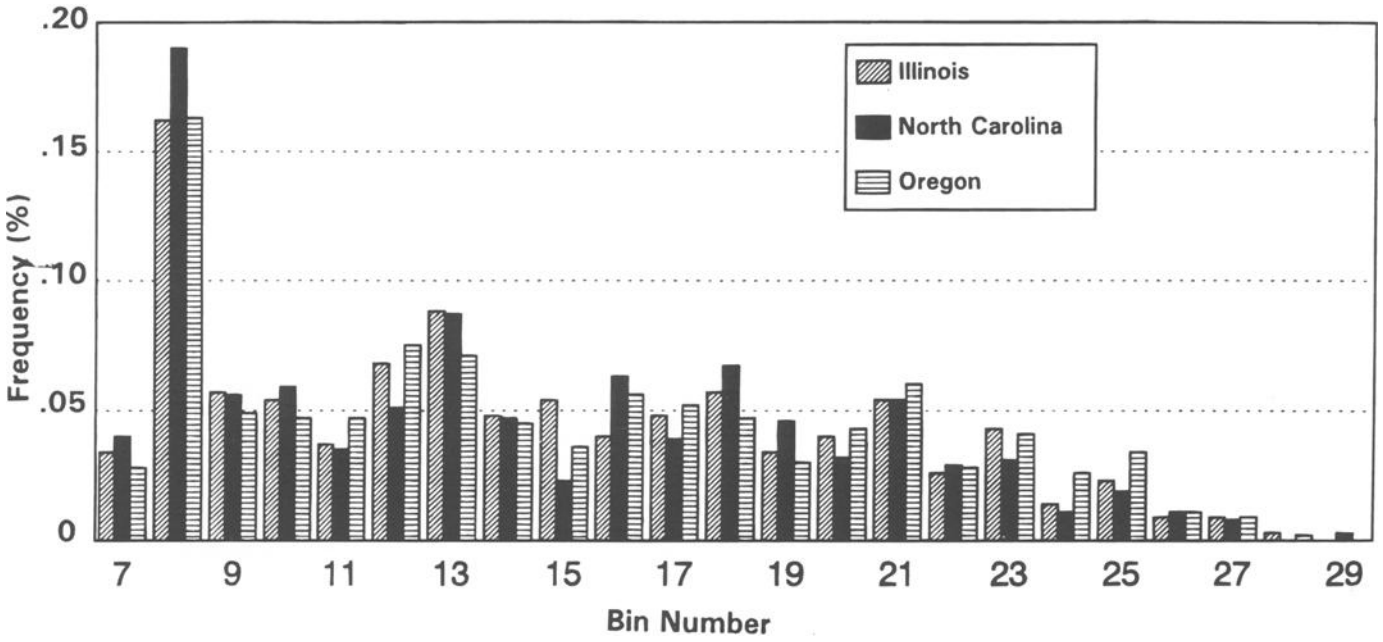

FIG. 4—*Comparisons of D17S26 allele frequency distributions in three Black populations.*

TABLE 9—*D17S26 binned population frequency distribution for three different U.S. Black populations. IL = Illinois, NC = North Carolina, OR = Oregon.*

Bin	Range (bp)	Frequency IL	NC	OR
1	0– 639	.000	.000	.000
2	640– 772	.000	.000	.000
3	773– 871	.000	.000	.000
4	872– 963	.000	.000	.000
5	964– 1077	.000	.000	.000
6	1078– 1196	.000	.000	.000
7	1197– 1352	.034	.040	.028
8	1353– 1507	.162	.190	.163
9	1508– 1637	.057	.056	.049
10	1638– 1788	.054	.059	.047
11	1789– 1924	.037	.035	.047
12	1925– 2088	.068	.051	.075
13	2089– 2351	.088	.087	.071
14	2352– 2522	.048	.047	.045
15	2523– 2692	.054	.023	.036
16	2693– 2862	.040	.063	.056
17	2863– 3033	.048	.039	.052
18	3034– 3329	.057	.067	.047
19	3330– 3674	.034	.046	.030
20	3675– 3979	.040	.032	.043
21	3980– 4323	.054	.054	.060
22	4324– 4821	.026	.029	.028
23	4822– 5219	.043	.031	.041
24	5220– 5685	.014	.011	.026
25	5686– 6368	.023	.019	.034
26	6369– 7241	.009	.011	.011
27	7242– 8452	.009	.008	.009
28	8453–10089	.003	.000	.002
29	10090–14999	.000	.003	.000
30	15000–23409	.000	.000	.000
31	23410–	.000	.000	.000
Number of alleles		352	746	466

TABLE 10—*D17S26 binned population frequency distribution for Oregon Hispanic and North Carolina Lumbee Indian populations. NC = North Carolina, OR = Oregon.*

Bin	Range (bp)	Frequency NC Lumbee Indian	Oregon Hispanic
1	0– 639	.000	.000
2	640– 772	.000	.000
3	773– 871	.000	.000
4	872– 963	.000	.000
5	964– 1077	.000	.000
6	1078– 1196	.000	.000
7	1197– 1352	.061	.073
8	1353– 1507	.192	.113
9	1508– 1637	.014	.014
10	1638– 1788	.028	.006
11	1789– 1924	.023	.023
12	1925– 2088	.065	.014
13	2089– 2351	.023	.017
14	2352– 2522	.009	.006
15	2523– 2692	.009	.006
16	2693– 2862	.042	.000
17	2863– 3033	.000	.008
18	3034– 3329	.103	.014
19	3330– 3674	.056	.017
20	3675– 3979	.070	.034
21	3980– 4323	.014	.034
22	4324– 4821	.070	.076
23	4822– 5219	.056	.076
24	5220– 5685	.028	.121
25	5686– 6368	.070	.121
26	6369– 7241	.047	.096
27	7242– 8452	.005	.073
28	8453–10089	.009	.042
29	0090–14999	.005	.014
30	5000–23409	.000	.000
31	3140–	.000	.000
Number of alelles		214	354

TABLE 11—*Hardy-Weinberg test P-values for binned populations from Vermont, North Carolina, Illinois and Oregon. Values in parentheses are for rebinned data.*

	Chi-square			Likelihood
	All	Homozygotes	Exact	Ratio
Database	276 d.f.	1 d.f.	Test	Hets
NC White	215.47	1.11	0.30 (.030)	0.54 (0.54)
VT White	504.62	1.01	0.02 (0.05)	0.11 (0.17)
IL White	295.81	3.99	0.01 (0.01)	0.09 (0.09)
OR White	249.25	3.41	0.71 (0.78)	0.77 (0.85)
NC Black	264.27	3.53	0.14 (0.15)	0.19 (0.24)
IL Black	231.89	0.08	0.07 (0.09)	0.03 (0.03)
OR Black	249.15	1.30	0.13 (0.15)	0.12 (0.13)
NC Indian	327.53	5.25	0.00 (0.00)	0.05 (0.09)
Or Hisp	249.44	0.73	0.27 (0.33)	0.53 (0.19)

TABLE 12—*Two-locus test P-values.*

D17S26	Database	*P*-value
D1S7	IL White	0.83
	NC White	0.82
	OR White	0.79
	IL Black	0.34
	NC Black	0.67
	OR Black	0.50
	NC Indian	0.40
	OR Hisp	0.49
D2S44	IL White	0.19
	NC White	0.45
	OR White	0.36
	IL Black	0.19
	NC Black	0.20
	OR Black	0.15
	NC Indian	0.31
	OR Hisp	0.32
D4S139	IL White	0.98
	NC White	1.00
	OR White	1.00
	IL Black	0.27
	NC Black	1.00
	OR Black	0.69
	NC Indian	0.96
	OR Hisp	0.69
D5S110	NC White	0.40
	OR White	0.61
	NC Black	0.96
	OR Black	0.15
	NC Indian	0.15
	OR Hisp	0.31
D7S467	NC White	0.86
	NC Black	0.71
	NC Indian	0.58
D10S28	IL White	0.62
	NC White	0.57
	OR White	0.63
	IL Black	0.21
	NC Black	0.49
	OR Black	0.17
	NC Indian	0.19
	OR Hisp	0.24
D14S13	OR White	0.61
	OR Black	0.10
	OR Hisp	0.78
D17S79	NC White	0.87
	NC Black	0.46
	NC Indian	0.42
DQa	OR White	0.23
	OR Black	0.10
	OR Hisp	0.59

Nonhuman DNA Sources

Of the 21 vertebrate species listed in Table 7, hybridization to the probe EFD52 was only observed in rhesus monkey DNA. One very weak band was measured at 1078 bp, below the size range generally observed for humans. All human samples from the databases generated by the four authoring laboratories were sized at 1197 bp or higher.

Minimum Detectable Sample Size

The probe EFD52 could detect 100 ng of genomic K562 DNA, although sensitivity ranges may vary from laboratory to laboratory, depending on the exact testing conditions. In comparison with other probes tested in these experiments, EFD52 was found to be more sensitive than the probe MS1 [D1S7; 12, 13], but not as sensitive as the probes YNH24 [D2S44; 3], pH30 [D4S139; 14], TBQ7 [D10S28; 15], LH1 [D5S110; 16], D7PAC [D7S467; 17] or V1 [D17S79; 18].

Population Studies

D17S26 fragment measurements from the nine databases were binned by the Lifecodes 23 kb marker into the 31 bins used by the FBI (7). Tables 8, 9 and 10 show the binned population frequency data for four White, three Black, one Hispanic, and one Lumbee Indian population. Figures 3 and 4 show a comparison of the different binned White and Black databases in a bar graph format. The White population possesses a bimodal distribution with peaks at Bins 8 and 25, whereas the Black population possesses a single large peak at Bin 8. After rebinning so that no bin contained fewer than five observations, at most, 23 bins had observation in a database. There is overall similarity of the frequencies for each bin, especially when comparisons are made within racial groups. This similarity is confirmed by chi-square goodness-of-fit tests. For the four White databases, the test statistic was 67.52 with 66 d.f., and for the three Black databases, the test statistic was 41.51 with 44 d.f. There is no evidence for heterogeneity among the White or Black samples, giving credence to the use of convenience samples as opposed to random samples. Even though the samples were from different states, and were collected by different means, the frequencies are the same within these two racial groups. The fears of Geisser and Johnson (19) concerning convenience samples, although legitimate, are not well-founded in practice. For all nine databases, the test statistic was 1187.92 with 176 d.f., so there is significant heterogeneity between racial groups. The striking similarity between subpopulations of the same racial groups located in different geographic regions of the United States is not unexpected. Budowle et al. (20,21) found considerable uniformity within subpopulations of the major racial groups.

Both the Oregon Hispanic and the North Carolina Indian samples had significantly different frequencies from the Black and White samples. There is no formal Federal recognition of the Lumbee Indian group. This population has been reported to consist of an admixture and the group has been shown by Chakraborty (22) to be more closely related to White groups than to Amerindians from Arizona, Minnesota or Oregon. The Oregon Hispanic population is presumably of Mexican decent. However, frequency differences for individual alleles in different racial groups do not imply differences in profile frequencies large enough to be misleading in the sense that a profile would appear to be rare in one group and common in another (23).

There is now a substantial literature showing the overall consistency of forensic VNTR databases with Hardy-Weinberg genotypic

proportions. Several tests were applied to the present databases, resulting in the *P*-values shown in Table 11. The first column shows the chi-square goodness-of-fit test statistic for observed to Hardy-Weinberg expected counts over all 276 genotypes defined on 23 bins. The second column shows the single d.f. test statistic for comparing the total counts of homozygotes with Hardy-Weinberg expectations. The test of homozygotes is performed for historical interest (24) but it is not a test for Hardy-Weinberg equilibrium.

The third test is an exact test (25) over all genotypes, and appears to be the best multiple-allele test for Hardy-Weinberg (26). The three significant results were investigated further after rebinning, consistent with the use of rebinned data in forensic applications. Rebinning was by the ad-hoc criterion of requiring at least five observations per bin. The Illinois White and North Carolina Indian results remained significant after rebinning.

The final test is the one most appropriate for VNTR loci when the Southern transfer procedure can produce single-band patterns for heterozygous individuals. Due to the phenomena of coalescence of similarly sized bands and the loss of small bands off of the gel, heterozygous individuals may be scored as *apparent* homozygotes. Single-band proportions ranged from 1.8% in the Oregon Black database to 13.5% in the Vermont White database. The problem has been discussed previously (23) and a likelihood ratio test performed only on heterozygotes was performed. This test indicates no significant heterozygote departures from Hardy-Weinberg at the 1% level, although the Illinois Black value was significant at the 5% level. It should be noted that the Illinois State Police use a different binning ladder (Life Technologies 22kb Molecular Weight Marker) in casework, and use of that ladder gives a test statistic with the much higher *P*-value of 0.29 [B.S. Weir, unpublished].

If D17S26 is to be used as a component of forensic DNA profiles, it needs to have frequencies independent of those at other loci in the profile. Whenever the locus is scored in the same individuals as other loci, two-locus tests can be conducted. As the issue is to decide whether two-locus genotypic frequencies can be constructed as the products of the corresponding allelic frequencies, the test proceeds by comparing observed and expected two-locus counts. It is not correct to refer to the test as being one of linkage disequilibrium.

In Table 12, the *P*-values are shown for the tests that could be conducted within these data sets. A likelihood ratio test on double heterozygotes only (23) was employed. The tests demonstrate independence of alleles within two-locus genotypes.

Discussion

A number of published studies have assessed the results of DNA profiling by RFLP on non-pristine samples using probes other than EFD52. These studies can be divided into two categories: those using simulated samples and those using actual forensic specimens.

The use of simulated samples permits repetition and the isolation of specific variables so that the exact effects of treatments or contaminants on subsequent RFLP testing can be determined. The use of actual forensic samples provides a wider range and combination of variables than would be possible in controlled studies since it is *never* possible to know the complete history of a forensic specimen.

Numerous controlled studies have examined the effects of specific insults on specific tissues. Kanter et al. (27) demonstrated that aging of dried blood stains did not alter the RFLP banding patterns of the extracted DNA. Giusti et al. (28) compared RFLP banding patterns derived from postcoital semen samples to those of pristine blood samples. The RFLP banding patterns observed in the DNA extracted from the postcoital semen samples were consistent with those from the pristine blood samples. Schwartz et al. (29), studied RFLP profiling of extracted teeth that had been subject to various treatments including aging, submersion, and burial in soil for various periods of time. DNA was subsequently extracted from the dental pulp and subjected to RFLP profiling. Those samples that had been subjected to longer or more severe conditions and which contained more degraded DNA were less likely to produce RFLP patterns. No material differences were found between blood exemplars and those dental pulp samples giving RFLP patterns. However, some anodal band shifting of the dental pulp DNA was reported in the more degraded samples.

Webb et al. (30) reported on the effects of mixing human DNA with DNA from various bacterial and fungal species commonly associated with the human body (and therefore with body fluid and tissue samples). Contamination of human DNA with DNA from *Streptococcus, Bacillus, Escherichia, Staphylococcus, Candida, Aspergillus* and *Mucor* species did not lead to any false-positive or false-negative RFLP results in this study. Adams et al. (8) examined the effects on RFLP profiling results of mixing various body fluids together and exposing body fluid samples to sunlight, various temperatures, microorganisms and non-biological chemical contaminants. In all cases, the challenged samples either did not produce RFLP results or produced RFLP patterns that were consistent with untreated controls. McNalley et al. (9) examined the effects of ultraviolet light, humidity, heat and soil contamination on RFLP profiling of DNA contained in dried blood stains. Once again, the more severe treatments resulted in DNA degradation and a fading or loss of RFLP banding patterns, but it did not alter the banding patterns in any way that would lead to a false match or false exclusion. Most recently, Laber et al. (31) demonstrated that contaminants, environmental insults and electrophoresis of varying amounts of DNA do not materially affect the results of RFLP analysis.

McNalley et al. (10) reported that reliable RFLP typing was possible in forensic casework samples which had been recovered from various substrata and exposed to various environmental conditions.

This study demonstrates that the probe EFD52 can be used reliably for forensic casework. No unexplained altered banding patterns were obtained from any of the studies conducted. The same banding pattern was found in DNA extracted from different tissues from the same donor. Repetitive measurements of EFD52 banding patterns from the same DNA sources were consistent between many different gels and between different laboratories. Some contaminants did degrade the DNA in a sample to the point where no bands or only the smaller molecular weight allele was present. Other contaminants inhibited the restriction endonuclease (*Hae* III) and resulted in partial digestion of the DNA. Leather, denim, heavily dyed fabric and soil were detrimental to the recovery and/or digestibility of the DNA. Problems caused by these materials are expected and have been observed with other probes used in forensic DNA profiling. While they may on occasion prevent DNA profiling results from being obtained, they do not materially change the results so as to result in an erroneous conclusion.

Seventy-five forensic specimens were examined where matching EFD52 banding patterns were observed between the victim blood

standard and the corresponding epithelial cells from panties or vaginal or rectal swabs from the victim. The probe EFD52 is highly specific for human DNA. After hybridization to DNA from 21 different vertebrates, only one weak band was observed in the DNA of the rhesus monkey.

The White population from Vermont, the White and Black populations from Illinois, the White, Black, and Lumbee Indian populations from North Carolina and the White, Black and Hispanic population from Oregon were examined and found to be in Hardy-Weinberg Equilibrium. Furthermore, no evidence was found of two-locus associations between D17S26 and the forensically useful loci D1S7, D2S44, D4S139, D5S110, D7S467, D10S28, D14S13, and D17S79. The finding of independence of allele frequencies between D17S26 and D17S79 is especially noteworthy, since they are located 6.5 cM apart on the long arm of chromosome 17 (32).

This study as well as other studies referenced here, have demonstrated that the RFLP procedure is robust and can be expected to perform with the same rigor regardless of the probe. Future studies with genomic probes new to forensics should not be concerned with the laborious analysis of all of the possible environmental insults and contaminants that have been studied here and elsewhere. The effects of these insults on DNA from body fluid and tissue samples are known and understood. DNA degradation will cause a weakening of RFLP patterns which is manifested first in the larger DNA fragments, but it will not cause a material change in the RFLP banding pattern. Rather, the focus should be on population studies, including Hardy-Weinberg equilibrium and two-locus analysis, probe sensitivity and specificity and, particularly, actual forensic samples. Numerous studies have by now established that no known treatment or insult can change RFLP banding patterns such that false matches or exclusions will be made by trained forensic scientists. The most valuable use of resources is to test new probes on forensic samples to assess the robustness of the probe when applied to actual casework situations.

Acknowledgments

We thank Dr. Bruce S. Weir for statistical analysis of the Illinois, North Carolina, Oregon and Vermont population data. A portion of this data was presented at the Fourth International Symposium on Human Identification in October 1993, in Scottsdale, AZ.

References

(1) Technical Working Group on DNA Analysis Methods (TWGDAM) and California Association of Criminalists Ad Hoc Committee on DNA Quality Assurance (CAC). Guidelines for a quality assurance program for DNA analysis. Crime Lab Digest 1991;18:44–75.

(2) Technical Working Group on DNA Analysis Methods (TWGDAM). Guidelines for a quality assurance program for DNA analysis. Crime Lab Digest 1995;22:21–43.

(3) Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, Culvier M, Martin C, Fujimoto E, Hoff M, Kumlin E, White R. Variable number of tandem repeat (VNTR) markers for human gene mapping. Science 1987;235:1616–22.

(4) Budowle B, Baechtel FS. Modifications to improve the effectiveness of restriction fragment length polymorphism typing. J Appl Theoret Electrophoresis 1990;1:181–87.

(5) Budowle B, Waye JS, Shuttler GG, Baechtel FS. *Hae* III—A suitable restriction endonuclease for restriction fragment length polymorphism analysis of biological evidence samples. J Forensic Sci 1990;35:530–36.

(6) Monson KL, Budowle B. A system for semi-automated analysis of DNA autoradiograms. Proc Int Symp Forensic Aspects of DNA Analysis. Government Printing Office, Washington, DC, 1989; 127–32.

(7) Budowle B, Giusti AM, Waye JS, Baechtel FS, Fourney RM, Adams DE, Presley LA, Deadman HA, Monson KL. Fixed-bin analysis for statistical evaluation of continuous distributions of allelic data from VNTR loci, for use in forensic comparisons. Am J Hum Gen 1991;48:841–55.

(8) Adams DE, Presley LA, Baumstark AL, Hensley KW, Hill AL, Anoe KS, Campbell PA, McLaughlin CM, Budowle B, Giusti AM, Smerick J B, Baechtel FS. Deoxyribonucleic acid (DNA) analysis by restriction fragment length polymorphisms of blood and other body fluid stains subjected to contamination and environmental insults. J Forensic Sci 1991;36:1284–98.

(9) McNally L, Shaler RC, Baird M, Balazs I, Koblinsky LK, DeForest P. The effects of environment and substrata on deoxyribonucleic acid (DNA): the use of casework samples from New York City. J Forensic Sci 1989;34:1070–77.

(10) McNally L, Shaler RC, Baird M, Balazs I, DeForest P, Koblinsky LK. Evaluation of deoxyribonucleic acid (DNA) isolated from human bloodstains exposed to ultraviolet light, heat, humidity and soil contamination. J Forensic Sci 1989;34:1055–69.

(11) Prinz M, Berghaus G. The effect of various stain carriers on the quality and quantity of DNA extracted from dried bloodstains. Z Rechtsmed 1990;103:191–97.

(12) Wong Z, Wilson V, Jeffreys AJ, Thein SL. Cloning selected fragment from a human DNA fingerprint: isolation of an extremely polymorphic minisatellite. Nucl Acids Res 1986;14:4605–16.

(13) Wong Z, Wilson V, Patel I, Povey S, Jeffreys AJ. Characterization of a panel of highly variable minisatellites cloned from human DNA. Ann Hum Gen 1987;51:269–88.

(14) Milner ECB, Lotshaw CL, Willems van Dijk K, Charmley P, Concannon P, Schroeder HW Jr. Isolation and mapping of a polymorphic DNA sequence pH30 on chromosome 4 [HGM provisional no. D4S139]. Nucl Acids Res 1989;17:4002.

(15) Bragg T, Nakamura Y, Jones C, White R. Isolation and mapping of a polymorphic DNA sequence (cTBQ7) on chromosome 10 [D10S28]. Nucl Acids Res 1988;16:11395.

(16) Eisenberg AJ, Clement M, Gaskill ME, Carlson DP, Klevan L. Characterization of a new single-locus VNTR probe for RFLP analysis: LH1 (D5S110). Proceedings of the Second International Symposium on the Forensic Aspects of DNA. March 29; Quantico, (VA), 1993.

(17) Leuthier V, Vergnaud G. CEB13 detects a VNTR locus (Het: 93%) on chromosome 7q. Hum Molec Gen 1992;1:64.

(18) Balazs I, Baird M, Clyne M, Meade E. Human population genetics studies of five hypervariable DNA loci. Am J Hum Gen 1989;44:182–90.

(19) Geisser S, Johnson W. Testing independence when the form of the bivariate distribution is unspecified. Statistics in Medicine 1995;14: 1621–39.

(20) Budowle B, Monson KL, Giusti AM, Brown BL. The assessment of frequency estimates of *Hae* III-generated VNTR profiles in various reference databases. J Forensic Sci 1994;39:319–52.

(21) Federal Bureau of Investigation, Washington, D.C., VNTR Population Data: A Worldwide Study. Vol. I–IV, 1993.

(22) Chakraborty R. Panel discussion: population genetics—basic issues relating to forensic applications for estimating frequencies of DNA profiles. Proc Second Int Sym Forensic Aspects of DNA; March 29; Quantico, (VA), 1993.

(23) Weir BS. Independence of VNTR alleles defined as fixed binds. Genetics 1992;130:873–87.

(24) Lander ES. DNA fingerprinting on trial. Nature 1989;39:501–05.

(25) Zaykin D, Zhivotovsky L, Weir BS. Exact tests for association between alleles at arbitrary numbers of loci. Genetica Vol 96:169–78.

(26) Maiste PJ, Weir BS. A comparison of tests for independence in the FBI RFLP databases. Genetica, Vol. 96:125–38.

(27) Kanter E, Baird M, Shaler R, Balazs I. Analysis of restriction fragment length polymorphisms in deoxyribonucleic acid (DNA) recovered from sperm. J Forensic Sci 1986;31:403–08.

(28) Giusti A, Pasquale S, Balazs I, Glassberg J. Application of deoxyribonucleic acid (DNA) polymorphisms to the analysis of DNA recovered from sperm. J Forensic Sci 1986;31:409–17.

(29) Schwartz T, Schwartz EA, Mieszerski L, McNally L, Kobilinsky L. Characterization of deoxyribonucleic acid (DNA) obtained from

teeth subjected to various environmental conditions. J Forensic Sci 1991;36:979–90.
(30) Webb MBT, Williams NJ, Sutton MD. Microbial challenge studies of variable number tandem repeat (VNTR) probes used for DNA profiling analysis. J Forensic Sci 1993;38:1172–75.
(31) Laber TL, Giese SA, Iveson JT, Liberty JA. Validation studies on the forensic analysis of restriction fragment length polymorphism (RFLP) on LE agarose gels without ethidium bromide: effects of contaminants, sunlight, and the electrophoresis of varying quantities of deoxyribonucleic acid (DNA). J Forensic Sci 1994;39:707–30.
(32) NIH/CEPH Collaborative Mapping Group. A comprehensive genetic linkage map of the human genome. Science 1992;258:67–86.

Address requests for reprints or additional information to
Elizabeth A. Benzinger, Ph.D.
Illinois State Police Forensic Sciences Command
2060 Hill Meadows
Springfield, IL 62702