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Characterization of human DNA in environmental samples

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Abstract

Environmental samples from indoor surfaces can be confounded by dust, which is composed largely of human skin cells and has been documented to contain roughly tens of micrograms of total DNA per gram of dust. This study complements previous published work by providing estimates of the quantity of amplifiable human DNA found in environmental samples from a typical indoor environment, categorized by the intensity of human traffic and visible quantity of dust. Dust was collected by surface swabbing standard 576 cm^2 areas in eight locations, and evaluated for total DNA quantity, presence of human DNA (mitochondrial and nuclear loci using conventional PCR), quantity of human nuclear DNA using quantitative PCR, and STR analysis. The total DNA content of 36 dust samples ranged from 9 to 28 ng/cm^2, and contained 0.2–1.1 pg/cm^2 of human DNA. Overall, human DNA was detected in 97% of 36 dust samples and 61% of samples yielded allele distributions of varying degrees of complexity when subjected to STR analysis. The implications of this study are twofold. First, the presence of dust in evidence can be a significant contamination source in forensic investigations because the human DNA component is of sufficient quality and quantity to produce allele calls in STR analysis. This can be effectively managed by implementing stringent protocols for collection and analysis of potential biological samples. A second implication is the use of dust as a source of evidence for identification of inhabitants within a defined location. In the latter case, a number of additional studies would be necessary to identify relevant pretreatments for environmental dust samples and to develop the necessary deconvolution techniques to separate the composite genotypes obtained.

Keywords: Dust; Human DNA; Quantitation; qPCR; STR analysis

1. Introduction

The importance of dust has long been recognized in forensic investigations and the components of indoor and environmental dust have been characterized in numerous studies. Edmond Locard published the first papers on the importance of dust in forensics in 1930, inspiring the disciplines of fiber and fingerprint analysis, among others [1,2]. Locard outlined in Part I of *The Analysis of Dust Traces* [2] that dust was composed of, among numerous other things, the ''skin-peelings'' of animals and humans. Protocols are available for isolation of important trace evidence from dust at the scene of a crime or in an environment of interest and for the extraction and quantitation of DNA found in dust [2,3]; for example, dust is routinely collected from air filters and analyzed to characterize microbial DNA in air quality studies. Such samples have been used to determine the effects of African dust events on air microbial populations in Caribbean Islands [4]. Agricultural studies have determined the impact of industrial livestock farming on air microbial content of the surrounding areas [5]. Bacterial DNA and endotoxin levels have been quantified in the dust present in homes in rural, urban, and farm settings to gauge the importance of exposure to these agents early in life in the development of allergic immunity and asthma [6]. Of specific note is that dust collected averaged 18.2 μg total DNA/g dust in urban homes, and 31.1 μg/g dust in rural homes. Although statistics published on the composition of indoor dust vary, this quantitation of total DNA in dust reveals a reservoir that potentially contains sufficient quantities of human DNA for identification and surveillance. Researchers have extensively

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documented the transfer of DNA from person to person or person to object, known as the Locard Exchange Principle [7–11]. There is no study to date, however, that describes the quantity or quality of human-specific DNA in dust. It is commonly known that humans shed keratinocytes constantly and that keratinocytes make up a substantial proportion of indoor dust. If dust does in fact harbor DNA from these shed human skin cells, there are a number of possible avenues for its quantitation and analysis. Foremost, an investigator should be aware of the range of ''background'' DNA that can be expected based on the amount of dust present at a crime scene. More importantly, human DNA in dust may contaminate the results of sensitive PCR assays used in forensic DNA analyses. Finally, if present in sufficient quantities, dust-borne human DNA can possibly aid in identification and surveillance of inhabitants of a certain space.

We hypothesized that human DNA could be recovered from indoor dust samples in sufficient quantities for analysis using standard forensic typing methods. We also examined the potential effects of location, traffic, and apparent dust level on the quantity and quality of human DNA recovered. Quantitative and qualitative analyses were performed using conventional and quantitative PCR methods for both nuclear and mitochondrial loci and by multiplex STR amplification followed by both ultrathin and capillary electrophoresis. We demonstrate here that human DNA is present in genotypeable amounts in indoor surface samples composed primarily of dust.

2. Materials and methods

2.1. Sampling locations

Dust samples were collected from sites within an academic building. Choices of sites were based upon availability and prior knowledge of the number of people normally occupying the rooms (''human traffic level''), giving priority to locations with extensive flat surfaces that could reveal potential differences in the quantity of recovered DNA. ''Low traffic'' faculty offices (Offices) were used regularly by one person with frequent visitors, ''medium traffic'' research laboratories (Labs) were frequently used by 5–10 individuals, and ''high traffic'' instructional laboratories (Classrooms) were typically used by 100+ persons per day. Areas within locations (referred to as ''sublocations'') were sampled. Visible dust at each sublocation was categorized as being ''unapparent'' (no visible dust), ''low'' (light layer of dust), ''medium'' (uniform layer of dust), or ''high'' (thick dust, sometimes containing hair, lint, or dirt).

2.2. Dust collection

Sampling sites were chosen on the basis of the available open surface area. Surfaces were not cleaned or otherwise altered prior to sample collection. Areas with obvious fingerprints were excluded from sampling to avoid collecting DNA from direct skin transfer. Dust was collected from a standard-sized 18 cm × 32 cm (576 cm^2) area from three surfaces (sublocations) within each location using a sterile cotton-tipped swab moistened in 2.0 ml sample tubes filled with 1.7 ml of Cell Lysis Solution (Mo Bio UltraClean™ BloodSpin™ purification kit, Mo Bio, Inc., Carlsbad, CA). The surface was repeatedly swabbed vertically and horizontally with a single cotton-tipped applicator to ensure complete collection of dust present. Swabs were rinsed in the collection tube of cell lysis solution as necessary until the entire area was cleared of dust. Once the sample was collected, the applicator was cut and left in the 2.0 ml sample collection tube. Samples were stored at 4 °C for <2 days until DNA was extracted.

2.2.1. Experiment 1

Eight locations were selected, characterized by low, medium, or high traffic as described. Three sublocations were sampled per location, for a total of 24 samples. Traffic remained constant for each location, but dust level was variable within locations.

2.2.2. Experiment 2

To eliminate dust level as a variable, six additional locations were investigated, all visually normalized to fall into the medium dust level category. At three high traffic locations, three sublocations were sampled for a total of nine samples. Three additional low traffic locations were investigated where only one sample was taken from each location.

2.3. Purification of DNA from dust

DNA was extracted from dust samples using the Mo Bio UltraClean™ BloodSpin™ purification kit (Mo Bio, Inc., Carlsbad, CA). Prior to extraction, tubes containing lysed dust samples were centrifuged at 12,000 × *g* for 1 min and the aqueous phase was transferred to a sterile 1.5 ml tube. The manufacturer's protocol was followed with three exceptions. First, because the samples were collected in the cell lysis solution supplied with the kit, the addition of cell lysis solution to the sample as outlined in the protocol was excluded. Second, the addition of 20 μl of 10 mg/ml Proteinase K was performed after centrifugation. Finally, the purified DNA was eluted from the silica column using 50 μl of warmed elution buffer instead of 200 μl, as quantities of human DNA were expected to be low. The potential for kit-based and operator-induced contamination was assessed with each set of DNA extractions by performing extraction blanks using kit reagents only.

2.4. Screening purified samples for presence of human DNA

To assess the presence of human DNA, PCR analysis was performed using human-specific nuclear and mitochondrial primer sets. The presence of nuclear DNA was assessed in Experiment 1 in triplicate assays for the polymorphic *Alu* sequence of the Tissue Plasminogen Activator gene on chromosome 8 [12] using the following primers:

*TPA*25-F (5′-GTA AGA GTT CCG TAA CAG GAC AGC T-3′)
*TPA*25-R (5′-CCC CAC CCT AGG AGA ACT TCT CTT T-3′)

Expected products were approximately 100 and 400 bp, depending upon the presence or absence of the *Alu* insert at this locus. In Experiment 2, the presence of human nuclear DNA was assessed using quantitative real-time PCR reactions, described in detail in Section 2.5.

Mitochondrial DNA (mtDNA) from the Hyper-Variable One (HV1) region of the human mitochondrial genome was detected using previously published primer sequences [13]:

Forward 16140 (5′-TACTTGACCACCTGTAGTAC-3′)
Forward 16190 (5′-CCCCATGCTTACAAGCAAGT-3′)
Reverse 16420 (5′-TGATTTCACGGAGGATGGTG-3′)

Two separate mtDNA targets were investigated using combinations of these three primers. The F16140/R16420 primer set, referred to as mtDNA-A, was expected to amplify a 281 bp fragment, and the F16190/R16420 set, mtDNA-B, was expected to amplify a 231 bp fragment. In Experiment 1, three replicate reactions for each mtDNA target were prepared for each dust sample. In Experiment 2, the mtDNA reactions were duplexed and only one set of duplexed reactions was carried out for each specimen.

All PCR reactions were assembled under a laminar flow hood and each reaction consisted of three parts JumpStart™ REDTaq™ ReadyMix™ PCR Reaction Mix (Sigma, St. Louis, MO), 1 part forward and reverse primer mix at 5 μM concentration each primer (Integrated DNA Technologies, Coralville, IA) and two parts template DNA in a total reaction volume of 10 μl. Reactions were performed in a calibrated PTC-100 Thermal Cycler (MJ Research, Inc., Watertown, MA) with an initial polymerase activation step of 94 °C for 3 min 30 s, followed by 40 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 20 s. Following amplification, reactions were stored at 4 °C (short term) or at −20 °C (long term). Absence of PCR master mix contamination was verified by

including two or more negative control reactions with molecular biology grade DNase-free sterile water with every set of reactions. All *TPA25* PCR reactions were visualized on 6% polyacrylamide (PAGE) gels in TAE; mtDNA reactions were visualized on 6% PAGE gels or 2% e-Gels (Invitrogen Corporation, Carlsbad, CA). Polyacrylamide gels were stained with 1× Sybr® Green (Invitrogen); 2% e-Gels were supplied with ethidium bromide incorporated in the gel. All gels were illuminated and photographed with UV light.

2.5. Quantitation of total and human DNA purified from dust samples

DNA isolated from dust was diluted 1:20 with DNase-free sterile water and loaded into an optically clear 384-well plate. Absorbances of samples, DNA standards (dilutions ranging from 5 μg/μl to 50 pg/μl), and blanks were determined using a μQuant MQX200 plate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). Purity and concentration of total DNA was estimated using KC4 software, and further quantitation of the human DNA component was performed using the Quantifiler™ Human DNA Quantification Kit (Applied Biosystems, Inc., Foster City, California). Quantitative PCR (qPCR) reactions were assembled under a laminar flow hood and cycling was accomplished using a BioRad iCycler iQ™ Real-Time PCR Detection System (BioRad, Hercules, California). The thermal cycling protocol was programmed as detailed for the ABI apparatus (Quantifiler™ User's Manual) except that the minimum number of thermal cycles was empirically determined to be 45 as opposed to the manufacturer's recommendation of 40, to maximize the detection and quantitation of the small amounts of human DNA found in the dust samples.

2.6. STR analysis

Human loci were amplified in multiplex PCR reactions using AmpFℓSTR® Profiler Plus™ PCR Amplification Kit (Applied BioSystems, Inc., Foster City, California). The manufacturer's protocols were followed precisely as outlined in the Profiler Plus™ User Manual. Where sufficient human DNA was present, the appropriate amount of template was added to the Profiler Plus reaction (1–2.5 ng). However, where insufficient template concentrations were available, only the maximum allowable volume (20 μl per 50 μl reaction) was added regardless of human DNA quantities present; i.e., no attempt was made to further concentrate extracts with low quantities of human DNA (Tables 1 and 2). Amplification was performed in a calibrated PTC-100 Thermal Cycler (MJ Research, Inc., Watertown, MA) using the manufacturer's recommended thermal cycling profile. Amplicons were resolved using automated detection and genotyping on an MJ BaseStation 51™ DNA fragment analyzer (MJ Bioworks, Inc., Sauk City, WI) and 9 of the 36 Profiler Plus© reactions were also analyzed using an ABI Prism® 3100-Avant to evaluate concordance of STR analysis results across two platforms. Two allelic ladders were employed with each set of reactions to calibrate allele sizes and each reaction (standard and unknown) contained 0.5 μl GeneScan™-500 ROX internal size standard (Applied Biosystems, Foster City, CA). Genotypes collected on the MJ BaseStation 51™ were scored using Cartographer® v1.2.6sg with a baseline threshold of 200 relative fluorescence units (RFU). Samples analyzed on the ABI Prism® 3100-Avant were injected using the standard (default) STR injection parameters and data were collected using ABI Prism® 3100-Avant Genetic Analyzer Data Collection Software v2.0. Analysis of STR data was performed with ABI GeneMapper™ *ID* Software v3.2 using a threshold of 75 RFU, following a protocol validated for casework samples. Individual allele calls for both data sets were checked by two operators for accuracy and consistency, and results where the same reactions were resolved on both machines were compared to check for concordance of results across digital and mechanical platforms.

2.7. Contamination controls

A number of control measures were implemented to ensure that recovered human DNA originated from experimental surface swabbing only and not from prior handling of the sampling apparatus or reagents. All pre-amplification protocols were carried out under a laminar flow hood. Protective garments and gloves were worn at all times. All surfaces and instruments were cleaned repeatedly with 10% bleach and 70% ethanol. Aerosol-barrier pipette tips and other sterile consumables were opened, used, and closed by one technician only. Sample-free DNA extraction blanks and template-free negative PCR control reactions confirmed the absence of human DNA contamination in reagents and sampling materials.

2.8. Data analyses

To determine if recovery of human DNA varied among locations, the proportion of positive dust samples was compared using a one-way ANOVA (SPSS v14.0). Dust samples that tested positive for human DNA in at least one of the three replicate reactions were considered ''positive'' and any negative replicate reactions of the same sample were counted as ''false negatives.'' Dust samples were considered ''negative'' if all three replicate reactions tested negative for human DNA. False negative rates were calculated by estimating the proportion of negative assays for positive samples with at least one human PCR result. The effect of location on the number of genotypeable STR loci per sample was examined using one-way ANOVA.

3. Results

3.1. Presence of human DNA in dust

Over a range of traffic and dust levels (Experiment 1), 100% of the 24 dust samples tested positive in at least one of three reactions for the human nuclear *TPA25* locus. The location of sample collection did not affect the detection of human nuclear DNA ($P = 0.42$). Similarly, 22 of the 24 samples (91.7%) evaluated for human mtDNA showed positive amplification, and there was no significant difference in the mean percent of positive samples among locations ($P > 0.74$). The rate of false negatives for mtDNA was lower than for the nuclear locus: mitochondrial targets mtDNA-A (F16140/R16420) and mtDNA-B (F16190/R16420) had false negative rates of 9.3% (±20.5%) and 8.0% (±19.9%), respectively, whereas the *TPA25* locus had a false negative rate of 29.3% (±22.2%). When dust level was normalized and two traffic levels were compared (Experiment 2), 92% of 12 dust samples tested positive in at least one test for either human mitochondrial or nuclear DNA; 7 of 9 dust samples (78%) collected from high traffic locations were positive for the human mtDNA, 2 of 3 dust samples (67%) from low traffic locations were mtDNA-positive, and all but 1 of the 12 samples (92%) contained human DNA that was detectable by qPCR. The false negative rate for the second experiment was 0. All template-free PCR control reactions (nuclear and mtDNA) were negative.

3.2. Quantitation of total and human DNA in dust

Total DNA content of dust samples ranged from 14 to 19 ng/cm^2 (Table 1) where locations varied with respect to both traffic and dust (Experiment 1). When sampling was restricted to medium dust levels in high or low traffic locations (Experiment 2), total DNA ranged between 9 and 28 ng/cm^2 (Table 2). Quantitative PCR reactions revealed that samples positive for human DNA contained between 5 and 464 pg of input human DNA equating to 0.2–1.1 pg/cm^2 of human DNA in indoor environmental dust samples. The amount of human DNA present varied significantly among locations in Experi-

Table 1
Human DNA in 24 dust samples from indoor locations with variable levels of human traffic and dust (Experiment 1)

Sample name	Replicate	Traffic level	Dust level	*TPA25* detected (%)	mtDNA detected (%)	Total DNA (ng/μl)	Total DNA (ng/cm^2)	Human DNA (ng/μl)	Human DNA (ng/cm^2)	% Human DNA in sample
Classroom 1	1	High	Unapp	33.33	100.00	166 ± 8.4853	14.4097	0.0257	0.0022	0.0155
Classroom 1	2	High	High	66.67	33.33	177 ± 4.2426	15.3646	0.0522	0.0045	0.0295
Classroom 1	3	High	Medium	66.67	100.00	167 ± 7.0711	14.4965	0.0132	0.0011	0.0079
Office 1	1	Medium	High	66.67	100.00	177 ± 1.4142	15.3646	0.0223	0.0019	0.0126
Office 1	2	Medium	Low	66.67	100.00	174 ± 0.0000	15.1042	0.0080	0.0007	0.0046
Office 1	3	Medium	High	66.67	66.67	174 ± 2.8284	15.1042	0.0460	0.0040	0.0264
Office 2	1	Medium	High	66.67	100.00	176 ± 2.8284	15.2778	0.1190	0.0103	0.0676
Office 2	2	Medium	High	100.00	100.00	184 ± 2.8284	15.9722	0.2790	0.0242	0.1516
Office 2	3	Medium	Low	33.33	0.00	167 ± 7.0711	14.4965	0.1450	0.0126	0.0868
Office 3	1	Medium	Unapp	100.00	100.00	169 ± 4.2426	14.6701	0.0060	0.0005	0.0036
Office 3	2	Medium	Unapp	66.67	100.00	163 ± 1.4142	14.1493	0.0087	0.0008	0.0053
Office 3	3	Medium	Low	66.67	100.00	160 ± 0.0000	13.8889	0.0734	0.0064	0.0459
Lab 1	1	Low	Medium	66.67	100.00	170 ± 2.8284	14.7569	0.0329	0.0029	0.0194
Lab 1	2	Low	High	33.33	100.00	170 ± 0.0000	14.7569	0.0243	0.0021	0.0143
Lab 1	3	Low	High	66.67	100.00	173 ± 1.4142	15.0174	0.0194	0.0017	0.0112
Lab 2	1	Medium	Low	100.00	100.00	174 ± 2.8284	15.1042	0.0129	0.0011	0.0074
Lab 2	2	Medium	Low	66.67	100.00	202 ± 33.9411	17.5347	0.0129	0.0011	0.0064
Lab 2	3	Medium	Low	100.00	100.00	175 ± 4.2426	15.1910	0.0171	0.0015	0.0098
Lab 3	1	Low	Low	66.67	100.00	222 ± 2.8284	19.2708	N/A	N/A	N/A
Lab 3	2	Low	Low	100.00	100.00	177 ± 1.4142	15.3646	0.0132	0.0011	0.0075
Lab 3[a]	3	Low	Medium	66.67	100.00	165 ± 1.4142	14.3229	0.0101	0.0009	0.0061
Lab 4	1	Medium	Low	100.00	100.00	164 ± 2.8284	14.2361	0.0272	0.0024	0.0166
Lab 4	2	Medium	Unapp	66.67	100.00	164 ± 8.4853	14.2361	N/A	N/A	N/A
Lab 4	3	Medium	Medium	66.67	0.00	173 ± 1.4142	15.0174	0.0206	0.0018	0.0119

Data are shown for percent positive replicate nuclear (*TPA25*) and mitochondrial DNA (mtDNA) PCR reactions, quantity of total DNA by spectrophotometry (A_{260}; converted to ng/μl and ng/cm^2), quantity of human DNA determined by Quantifiler™ assay (ng/μl and converted to ng/cm^2), and percentage of human DNA in total environmental DNA. Levels of traffic and dust are low, medium, and high and for dust, "Unapp" indicates that dust was not apparent on the surface.
[a] STR amplification results shown in Fig. 4.

ment 1 ($P < 0.001$), due largely to one location that had at least a fivefold greater amount of human DNA than the other locations (Fig. 1). In Experiment 2, dust samples originating from high traffic locations had similar quantities of human DNA (41–290 pg/μl, $P = 0.725$), whereas the amount of human DNA detected in dust samples from low traffic locations varied significantly (5–396 pg/μl, $P = 0.020$; Fig. 2). Overall, no clear trends were found among location, traffic level, and total DNA ($P = 0.503$), quantity of human DNA ($P = 0.280$), or percent human DNA to total DNA ($P = 0.180$) in either Experiment 1 or Experiment 2 (Figs. 1 and 2, respectively).

Table 2
Human DNA in 12 dust samples collected from locations with high or low human traffic levels and medium dust level (Experiment 2)

Sample name	Replicate	Traffic level	mtDNA detected (%)	Total DNA (ng/μl)	Total DNA (ng/cm^2)	Human DNA starting qty (ng/μl)	Human DNA (ng/cm^2)	% Human DNA in sample
Classroom 2	1	High	0	318 ± 31.1127	27.6042	N/A	N/A	N/A
Classroom 2	2	High	100	112 ± 4.2426	9.7222	0.1223 ± 0.0691	0.0106	0.1092
Classroom 2	3	High	100	104 ± 1.4142	9.0278	0.2896 ± 0.1742	0.0251	0.2785
Classroom 3[a]	1	High	100	106.5 ± 0.7071	9.2448	0.2424 ± 0.0509	0.0210	0.2276
Classroom 3	2	High	0	125 ± 4.2426	10.8507	0.0408 ± 0.0348	0.0035	0.0326
Classroom 3	3	High	100	124 ± 2.8284	10.7639	0.1128 ± 0.0849	0.0098	0.0910
Classroom 4	1	High	100	181.5 ± 102.5305	15.7552	0.0570 ± 0.0088	0.0049	0.0314
Classroom 4	2	High	100	104 ± 2.8284	9.0278	0.1138 ± 0.0215	0.0099	0.1094
Classroom 4	3	High	100	112 ± 1.4142	9.7222	0.1022 ± 0.0021	0.0089	0.0912
Office 4	–	Low	100	116.5 ± 2.1213	10.1128	0.2472 ± 0.0690	0.0215	0.2122
Office 5	–	Low	0	107 ± 2.8284	9.2882	0.0474 ± 0.0248	0.0041	0.0443
Office 6	–	Low	100	102 ± 1.4142	8.8542	0.0031 ± 0.0002	0.0003	0.0031

Data are shown for percent positive replicate PCR reactions for mitochondrial DNA (mtDNA), quantity of total DNA determined by spectrophotometry (A_{260}; converted to ng/μl and ng/cm^2), quantity of human DNA determined by Quantifiler™ (ng/μl and converted to ng/cm^2), and percentage of human DNA detected in the total environmental DNA. "N/A" signifies no detection.
[a] STR amplification results are shown in Fig. 3.

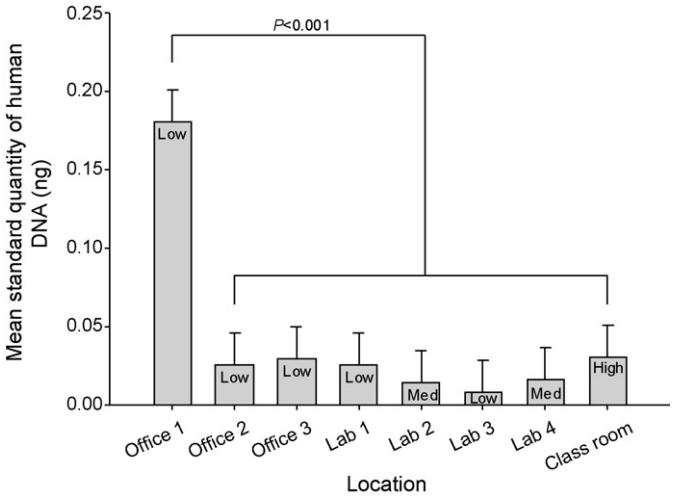

Fig. 1. Differences among quantities of human DNA detected in indoor dust. Effect of location (horizontal axis) was significant in Experiment 1 for one location *vs*. all other locations (significance labeled). Traffic level is marked on bars for reference.

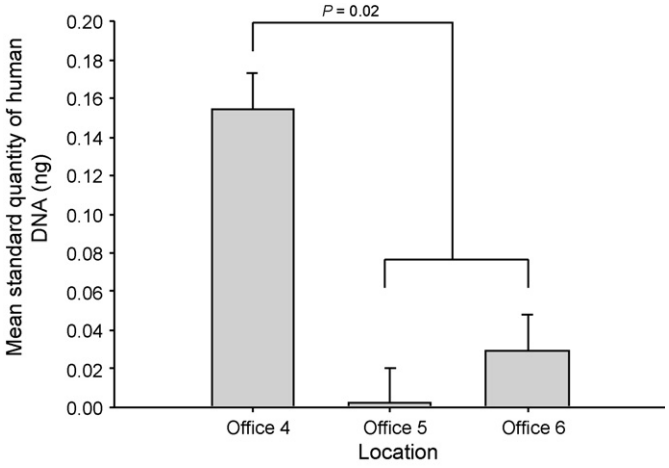

Fig. 2. Human DNA detected from medium deposition dusty surfaces in low-traffic locations (Experiment 2). One office was found to have significantly more human DNA than two other offices.

3.3. STR analysis

Input human DNA ranged from 38.6 pg to 1.8 ng per AmpF*ℓ*STR® Profiler Plus™ reaction. Of 34 total dust samples assayed with Profiler Plus™, 22 yielded positive allele calls for at least one locus and 21 exhibited an X allele at the Amelogenin locus (Table 3). Ten of the 34 total reactions (29%) had <0.1 ng input human DNA, and of these, only three exhibited amplification at the Amelogenin or STR loci. One of these three reactions generated alleles at two STR loci, and one generated alleles at five loci. Fourteen reactions had input human DNA levels of 0.1–0.5 ng and of these, eight generated alleles for one or more loci, with an average of 2 (±2) loci per sample. Of the six reactions with input human DNA levels ranging from 0.5 to 1.0 ng, all generated alleles at three or more loci, with an average of 4 (±1) loci per sample. Of the four reactions that had more than 1.0 ng of input human DNA, all generated alleles at three or more loci, with an average of 5 (±1) loci per sample (Table 3). Genotype recovery did not vary among locations when categorized by dust level ($P = 0.143$) or traffic level ($P = 0.907$).

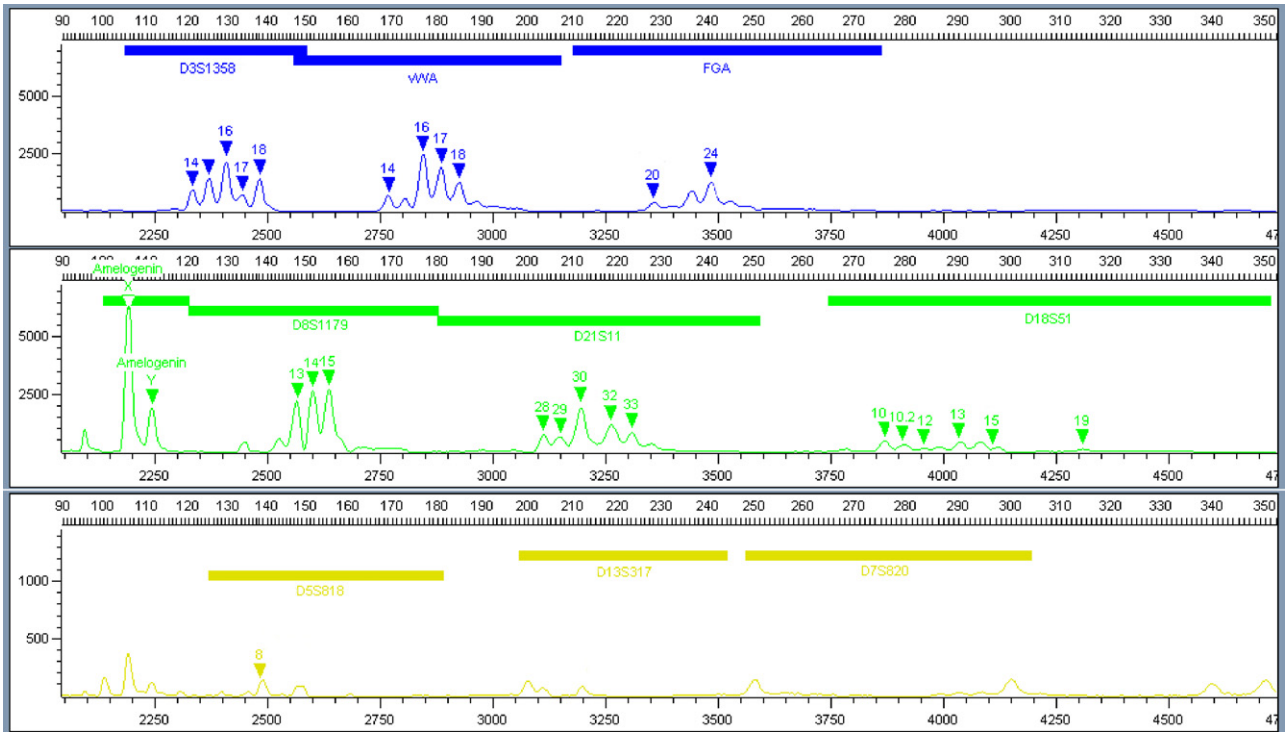

Fig. 3. Electropherogram of human STR alleles generated through analysis of a typical dust sample (Classroom 3, high traffic, medium dust). Vertical axis is RFU. Lower horizontal axis is scan number; upper axis is size in base pairs. Allele calls included for reference are shown atop allele peaks.

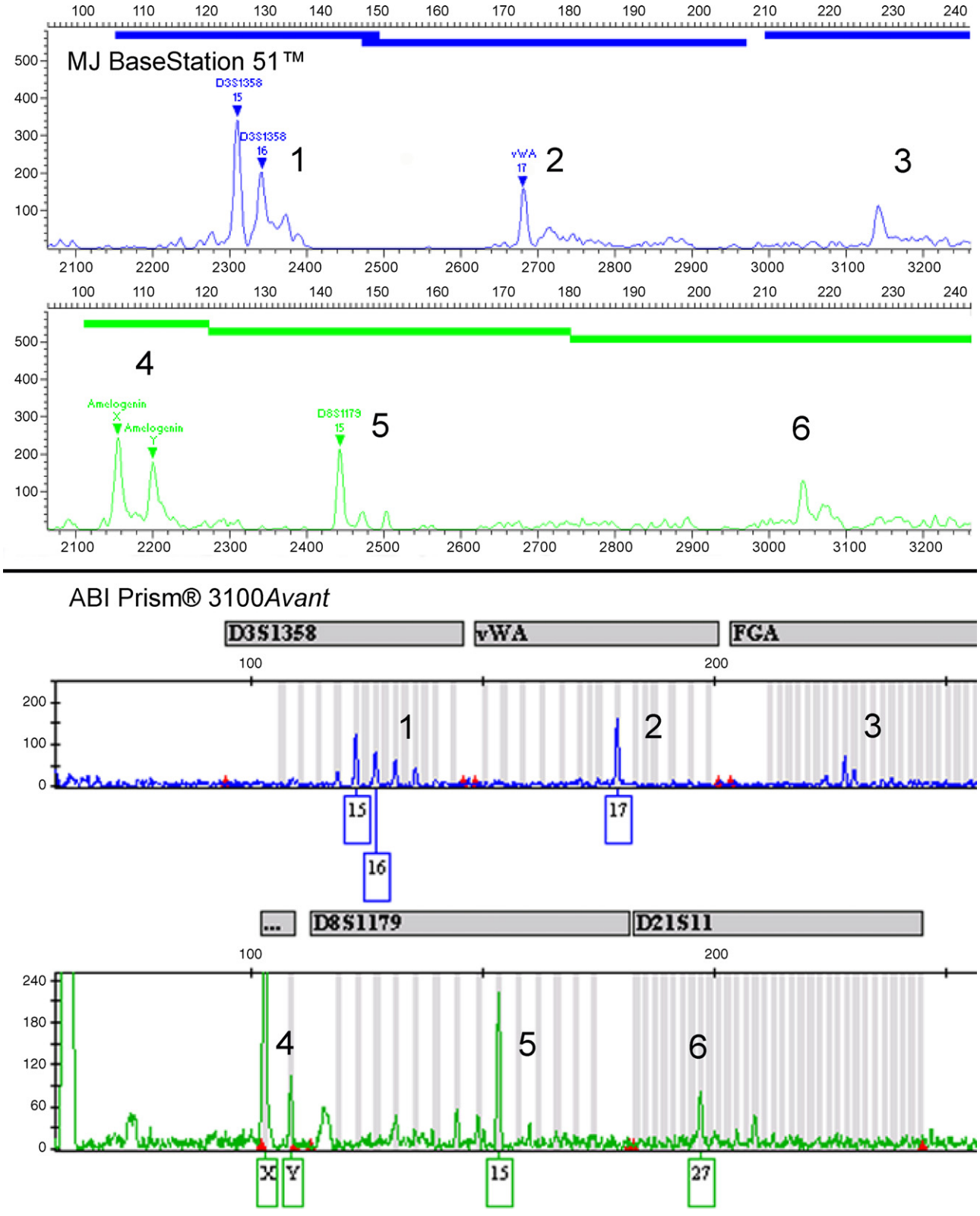

Fig. 4. Comparison of one AmpFℓSTR® Profiler Plus™ reaction (Lab 3, low traffic, medium dust) run on two different platforms. Vertical axis is RFU. Lower horizontal axis is scan number; upper axis is size in base pairs. Corresponding loci on both platforms are labeled (1) D3S1358, (2) vWA, (3) FGA, (4) Amelogenin, (5) D8S1179 and (6) D21S11.

Table 3
Allelic data for 34 environmental samples assayed using AmpFℓSTR® Profiler Plus™ and resolved on MJ BaseStation 51™

Sample name	Replicate	Traffic level	Dust level	Input DNA (ng)	Locus									
					Amelogenin	FGA	vWA	D3 S1358	D5 S818	D7 S820	D8 S1179	D13 S317	D18 S51	D21 S11
Classroom 1	1	High	Unapp	0.1645	–	–	–	–	–	–	–	–	–	–
Classroom 1	2	High	High	0.3341	–	–	–	–	–	–	–	–	–	–
Classroom 1	3	High	Low	0.0845	–	–	–	–	–	–	–	–	–	–
Classroom 2	1	High	Medium	N/A	X,Y	–	–	–	–	–	–	–	–	–
Classroom 2	2	High	Medium	0.7827	X,Y	–	–	–	–	–	(6)	–	(7)	(10)
Classroom 2	3	High	Medium	1.8534	X,Y	4	7	5	1	–	4	–	–	8
Classroom 3[a]	1	High	Medium	1.5514	X,Y	1	5	5	–	–	3	–	(6)	5
Classroom 3	2	High	Medium	0.2611	(X,Y)	–	(5)	–	(10)	–	(10)	–	(6)	(11)
Classroom 3	3	High	Medium	0.7219	X,Y	6	6	5	–	–	3	–	–	(9)
Classroom 4	1	High	Medium	0.3648	X	–	(3)	1	2	–	–	–	(12)	(9)
Classroom 4	2	High	Medium	0.7283	X,Y	–	3	5	–	–	6	–	–	(7)
Classroom 4	3	High	Medium	0.6541	X,Y	–	1	3	–	–	10	–	–	–
Office 1	1	Low	High	0.1427	X,Y	–	2	3	–	–	2	–	–	–
Office 1	2	Low	Low	0.051	–	–	–	–	–	–	–	–	–	–
Office 1	3	Low	High	0.2944	X,Y	–	1	–	–	–	1	–	–	–
Office 2	1	Low	High	0.7616	X	7	1	5	1	–	3	–	4	6
Office 2	2	Low	High	1.7856	X,Y	4	4	6	–	–	3	–	2	5
Office 2	3	Low	Low	0.928	X,Y	–	1	4	–	–	2	–	–	6
Office 3	1	Low	Unapp	0.0386	–	–	–	–	–	–	–	–	–	–
Office 3	3	Low	Low	0.4698	X	–	3	1	4	–	2	–	–	–
Office 3	2	Low	Unapp	0.0557	–	–	–	–	–	–	–	–	–	–
Office 4	–	Low	Medium	1.5821	X,Y	–	5	4	–	–	5	–	–	–
Office 5	–	Low	Medium	0.3034	X	–	1	2	–	–	1	–	–	–
Office 6	–	Low	Medium	0.0198	–	–	–	–	–	–	–	–	–	–
Lab 1	1	Low	Medium	0.2106	–	–	–	–	–	–	–	–	–	–
Lab 1	2	Low	High	0.1555	–	–	–	–	–	–	–	–	–	–
Lab 1	3	Low	High	0.1242	–	–	–	–	–	–	–	–	–	–
Lab 2	1	Medium	Low	0.0826	–	–	–	–	–	–	–	–	–	–
Lab 2	2	Medium	Low	0.0826	X,Y	–	–	–	–	–	–	–	–	–
Lab 2	3	Medium	Low	0.1094	X,Y	–	1	–	–	–	–	–	–	–
Lab 3	2	Low	Low	0.0845	X	–	1	2	–	–	–	–	–	–
Lab 3	3	Low	Medium	0.0646	X,Y	–	1	3	–	–	1	–	–	–
Lab 4	1	Medium	Low	0.1741	X	–	–	1	–	–	–	–	–	–
Lab 4	3	Medium	Medium	0.1318	X	–	2	2	–	–	2	–	3	2

Two samples were excluded due to absence of quantifiable human DNA in replicate Quantifiler™ assays. Numbers represent the count of alleles present at each locus. Allele calls displayed in parentheses indicate distinct peaks that occurred at low RFUs below the baseline threshold. Dashes signify absence of alleles for a locus.
[a] STR amplification results are shown in Fig. 3.

Based on the STR data (Table 3), it is clear that an expected correlation exists in these samples between quantity of input DNA and success of STR amplification. Comparison of results from the MJ BaseStation 51™ with results from the ABI Prism® 3100-Avant (Table 4), reveled that the ABI platform recognized fewer peaks for each locus than the MJ BaseStation platform. Due to the differences in threshold, the profiles were similar but not perfectly concordant (Fig. 4). Nevertheless, both platforms showed the presence of human DNA from multiple contributors. Six of the nine samples compared across the two platforms had at least one locus with matching allele calls, four samples had two or more loci with matching ranges of alleles, and one sample had six loci with matching allele calls. The minimal differences observed in allele calls were primarily attributable to differences in allele binning rules across the two software platforms.

4. Discussion

4.1. Presence/absence of human DNA in dust

This investigation was designed to characterize human DNA present in indoor environmental samples composed primarily of dust and to test the potential utility of this source of DNA for analysis of inhabitants within a defined location. Our results demonstrate unequivocally that measurable levels of human DNA are present in indoor dust. Quantitation of the human DNA from multiple dust samples indicated that although the human DNA component is minute, it constitutes a measurable portion of the total DNA. In samples with very small quantities of human DNA, mtDNA was detected more often than nuclear DNA, which is not surprising considering differences in the nature of the two molecules. Nuclear DNA is a long linear molecule consisting of two copies per cell, whereas mitochondrial DNA is a small, circular molecule, potentially existing as hundreds to thousands of copies in a cell [14]. Long linear DNA is more easily damaged than short circular DNA by shearing, biological and chemical degradation, and UV exposure. These facts are generally accepted as the basis for the contention that mitochondrial DNA is easier to recover from degraded samples than nuclear DNA [15]. In the present study, samples that tested positive for mtDNA and negative for nuclear DNA probably contained very old or degraded human DNA (i.e., older dust deposition), whereas those that tested positive for both types of DNA most likely contained more recently sloughed human

Table 4
Comparison of alleles present per locus for nine AmpFℓSTR® Profiler Plus™ reactions analyzed on both an ABI Prism® 3100-Avant and MJ BaseStation 51™

Sample	Replicate	Platform	Locus									
			Amelogenin	FGA	vWa	D3 S1358	D5 S818	D7 S820	D8 S1179	D13 S317	D18 S51	D21 S11
Classroom 2	2	ABI	X, Y	2	1	3	2	–	3	–	–	1
		MJ	X, Y	–	–	–	–	–	(6)	–	(7)	(11)
Classroom 2	3	ABI	X, Y	5	4	4	2	–	3	2	–	3
		MJ	X, Y	4	7	5	1	–	4	–	–	8
Classroom 3[a]	1	ABI	X, Y	–	–	4	2	–	2	–	–	–
		MJ	X, Y	2	5	5	1	–	3	1	6	5
Classroom 4	1	ABI	X	–	–	1	1	–	–	–	–	1
		MJ	X	–	(3)	1	2	–	–	–	(12)	(9)
Office 2	1	ABI	X	–	–	–	–	–	–	–	–	–
		MJ	X	6	1	5	1	–	3	–	4	6
Office 4	–	ABI	X, Y	–	2	4	1	–	1	–	–	–
		MJ	X, Y	–	5	4	–	–	5	–	–	–
Office 5	–	ABI	–	–	–	–	–	–	–	–	–	–
		MJ	–	–	1	2	–	–	1	–	–	–
Lab 3[b]	3	ABI	X, Y	–	1	2	1	–	1	–	–	1
		MJ	X, Y	–	1	3	–	–	1	–	–	–
Lab 4	3	ABI	–	–	–	–	–	–	–	–	–	–
		MJ	X	–	2	2	–	–	2	–	3	2

[a] STR amplification results are shown in Fig. 3.
[b] Visual comparison of STR electropherograms across platforms shown in Fig. 4.

keratinocytes. The implication of these results is that a simple PCR screen of the types of human DNA present may offer useful information about the nature or quality of human DNA in an environmental dust sample.

4.2. Quantitation

This study demonstrates that human DNA is present in indoor dust in sufficient quantity to amplify with qPCR and to yield human STR genotypes. The ABI Quantifiler™ kit has been validated to consistently quantify human DNA to levels as low as 10 pg per reaction and to routinely quantify as little as 6 pg in casework samples [16]. The choice not to validate the kit for amounts lower than 10 pg has been related to the possibility of standard breakdown—a term used to describe the breakdown of quantitation reliability due to ever higher dilutions of human control DNA. The higher the dilution, the more likely that sampling will be inconsistent and correlation of duplicate standards will fail [16]. This is a concern in the current study because of the extremely small amounts of DNA measured in some dust samples. The lowest quantity of control human DNA employed to create standard curves was 23 ng/μl; therefore, samples with the lowest concentrations of human DNA, calculated by extrapolation beyond the lowest point on the standard curve, were below the limit validated for this kit. Although this is a concern, the very high correlation of duplicate standards substantiates the reliability of estimates taken from the lower bounds of the curve. Furthermore, the fact that those same low concentration specimens yielded genotypes in AmpFℓSTR® Profiler Plus™ reactions illustrates that despite potentially imprecise quantitation, human DNA was indeed present and could affect downstream analysis. Given the range of human DNA detected in these experiments, it is advisable for future low-copy DNA studies to include triplicate standards (as opposed to duplicate) and standard dilutions to levels of 5 pg and below.

4.3. STR analysis

Typical human STR analysis involves identifying an individual based on the allele profile at multiple STR loci. However, because our sampling locations were regularly occupied by many people, identification of a single individual was not an objective of our study. Thus, a novel approach to the interpretation of these data was necessary for STR analyses. The electropherograms were analyzed based on signal from dust samples as compared to the appearance of fluorescent background detected in the negative control reactions. Alleles were designated typeable at a specific locus analyzed on the MJ BaseStation 51™ if the peaks present exceeded the validated minimum threshold (200 RFUs). The consistent absence of alleles at two of the nine Profiler Plus© loci (D13S317 and D7S820) was not surprising due to the extremely small starting quantities of human DNA in these reactions. The largest DNA repeats are found at these loci, and therefore would be more likely to be degraded. It has been documented that allelic and complete locus dropout occur in low-copy-number (LCN) and degraded samples, and that even with pre-amplification of samples, it can be difficult or impossible to recover these alleles [17,18].

5. Conclusion

Human DNA is present in indoor environmental samples containing dust, and at levels high enough to permit detailed molecular analyses. The quantity and quality of human DNA in dust samples did not vary statistically among locations with different human traffic or dust levels. However, the direct relationship of traffic and dust levels with the amount and quality of human DNA is difficult to generalize due to the potential effects of other factors not controlled or considered in this investigation. Environmental variables, including light, heat, and moisture can degrade DNA [19]. Cleaning agent residues (e.g., bleach) can destroy DNA. Finally, ventilation systems can serve as vehicle for transferring dust among rooms, and introducing DNA that does not originate from past or present occupants of a particular room.

This study introduces the potential use of dust samples for detecting human habitation and possibly for identification of the inhabitants of a certain space. Here we have demonstrated the ability to gain at least partial profiles for numerous people inhabiting one location, without pre-amplification of DNA in the samples. An eloquent survey of two whole genome amplification (WGA) kits using Φ-29 phage DNA polymerase to pre-amplify LCN and degraded human DNA samples [17] found that whole genome amplification of both LCN and lab-degraded samples effectively increased not only the quantity of human DNA, but also restored the presence of restriction enzyme-digested alleles in STR analysis. Future studies gauging the success of WGA on environmental dust samples may yield similar results. Finally, if dust samples are pre-amplified and successful STR analysis is performed, our current data indicate that deconvolution of the alleles derived from numerous individuals would be necessary. This has been studied [20,21] and different methods have been reported for interpretation which are most notably effective when the DNA of only two or three individuals is present in the mixture.

Results of this study have implications regarding the processing of forensic samples. First, the presence of genotypeable human DNA in dust illustrates a significant potential contamination source in forensic investigations. Twenty-five of 36 samples contained sufficient input human DNA for STR analysis using the AmpFℓSTR® Profiler Plus™ assay (∼1.0 ng), and 36% (including low-input samples) produced alleles at multiple loci. These results demonstrate that even though anti-contamination measures may be in place at a crime scene and in the laboratory, trace DNA derived from dust in the vicinity of other evidence is capable of producing signals higher than background noise in STR analyses. Therefore, where stains, fingerprints, or other trace evidence are collected for DNA analysis, care must be taken to avoid sampling surrounding areas with visible dust, as confounding alleles will almost certainly be present. Ultimately, common sense on the part of the investigator, careful collection, and use of serology to confirm boundaries of biological specimens will prevent confounding evidence with the ubiquitous ambient human DNA documented here.

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References

[1] N. Petraco, The occurrence of trace evidence in one examiner's casework, J. Forensic Sci. 30 (1984) 485–493.
[2] E. Locard, The analysis of dust traces, Part I, Am. J. Police Sci. 276 (1930) 276–298.
[3] N. Petraco, A guide to the rapid screening, identification, and comparison of synthetic fibers in dust samples, J. Forensic Sci. 32 (1986) 768–777.
[4] D.W. Griffin, V.H. Garrison, J.R. Herman, E.A. Shinn, African dust in the Caribbean atmosphere: microbiology and public health, Aerobiologia 17 (2001) 203–213.
[5] J. Cleave, L. Ingram, E. Barber, P. Willson, Airborne dust, endotoxin, and DNA downwind from swine barns. Sask Pork Final Report, 2002 (accessed online April 15, 2006). http://www.extension.iastate.edu/airquality/reports/saskpork.pdf.
[6] S.R. Roy, A.M. Schiltz, A. Marotta, Y. Shen, A.H. Liu, Bacterial DNA in house and farm barn dust, J. Allergy Clin. Immunol. 112 (2003) 571–578.
[7] R.A.H. van Oorschot, M.K. Jones, DNA fingerprints from fingerprints, Nature 387 (1997) 767.
[8] R.A.H. van Oorschot, S. Treadwell, J. Beaurepaire, N.L. Holding, R.J. Mitchell, Beware of the possibility of fingerprinting techniques transferring DNA, J. Forensic Sci. 50 (2005) 1417–1422.
[9] P. Gill, J. Whitaker, C. Flaxman, N. Brown, J. Buckleton, An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA, Forensic Sci. Int. 112 (2000) 17–40.
[10] S. Stouder, K.J. Reubush, D.L. Hobson, J.L. Smith, Trace evidence scrapings: a valuable source of DNA? Forensic Sci. Commun. (Online), 2001 <http://www.fbi.gov/hq/lab/fsc/backissu/oct2001/stouder.htm> accessed 8/3/06.
[11] R.A. Wickenheiser, Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact, J. Forensic Sci. 47 (2002) 442–450.
[12] S. García-Obregón, M.A. Alfonso-Sánchez, A.M. Pérez-Miranda, C. Vidales, D. Arroyo, J.A. Peña, Genetic position of Valencia (Spain) in the Mediterranean Basin according to *Alu* insertions, Am. J. Hum. Biol. 18 (2006) 187–195.
[13] S. Luciani, G. Fornaciari, O. Rickards, C. Martínez Labarga, F. Rollo, Molecular characterization of a Pre-Columbian mummy and *in situ* coprolite, Am. J. Phys. Anthropol. 129 (2006) 620–629.
[14] D. Bogenhagen, D.A. Clayton, The number of mitochondrial deoxyribonucleic acid genomes in mouse L and human HeLa cells, J. Biol. Chem. 249 (1974) 7991–7995.
[15] M.C. Kline, P.M. Vallone, J.W. Redman, D.L. Duewer, C.D. Calloway, J.M. Butler, Mitochondrial DNA typing screens with control region and coding region SNPs, J. Forensic Sci. 50 (2005) 377–385.
[16] R.L. Green, I.C. Roinestad, C. Boland, L.K. Hennessy, Developmental validation of the Quantifiler™ real-time PCR kits for the quantification of human nuclear DNA samples, J. Forensic Sci. 50 (2005) 809–825.
[17] K.N. Ballantyne, R.A.H. van Oorschot, R.J. Mitchell, Comparison of two whole genome amplification methods for STR genotyping of LCN and degraded DNA samples, Forensic Sci. Int. 166 (2006) 35–41.
[18] P.M. Schneider, K. Balogh, N. Naveran, M. Bogus, K. Bender, M. Lareu, A. Carracedo, Whole genome amplification—the solution for a common problem in forensic casework? Int. Congr. Ser. 1261 (2004) 24–26.
[19] I. Marota, C. Basile, M. Ubaldi, F. Rollo, DNA decay rate in papyri and human remains from Egyptian archaeological sites, Am. J. Phys. Anthropol. 117 (2002) 310–318.
[20] I.W. Evett, P.D. Gill, J.A. Lambert, Taking account of peak areas when interpreting mixed DNA profiles, J. Forensic Sci. 43 (1998) 62–69.
[21] J.M. Curran, C.M. Triggs, J. Buckleton, B.S. Weir, Interpreting DNA mixtures in structured populations, J. Forensic Sci. 44 (1999) 987–995.