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TECHNICAL NOTE

CRIMINALISTICS; PATHOLOGY/BIOLOGY

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A Rapid Wire-Based Sampling Method for DNA Profiling*

ABSTRACT: This paper reports the results of a commission to develop a field deployable rapid short tandem repeat (STR)-based DNA profiling system to enable discrimination between tissues derived from a small number of individuals. Speed was achieved by truncation of sample preparation and field deployability by use of an Agilent 2100 Bioanalyser™. Human blood and tissues were stabbed with heated stainless steel wire and the resulting sample dehydrated with isopropanol prior to direct addition to a PCR. Choice of a polymerase tolerant of tissue residues and cycles of amplification appropriate for the amount of template expected yielded useful profiles with a custom-designed quintuplex primer set suitable for use with the Bioanalyser™. Samples stored on wires remained amplifiable for months, allowing their transportation unrefrigerated from remote locations to a laboratory for analysis using AmpF*l*STR® Profiler Plus® without further processing. The field system meets the requirements for discrimination of samples from small sets and retains access to full STR profiling when required.

KEYWORDS: forensic science, biological sampling, DNA profiling, stainless steel wire, disaster victim identification

Disaster victim identification (DVI) is a multidimensional problem of logistics in which the most efficient approach is dictated by circumstances. Guidance for dealing with genetic analysis in DVI situations has been prepared by the International Society for Forensic Genetics (1). Currently, samples taken at site are transported to a forensic facility for processing. However, in some circumstances, particularly where there are many small fragments to be allocated to separate individuals, a rapid field deployable profiling system would be advantageous. Efforts to develop such systems have focused on highly integrated microfluidics (2,3) and small, potentially discardable capillary electrophoresis chips (4) and are still in development. Approaches that offer a limited level of discrimination may be sufficient when the DVI site involves few victims, particularly if the system is also compatible with laboratory-based high-discrimination analysis when required. This paper reports the development of one such rapid field deployable DNA profiling system for human tissues. In addressing this problem, it examines the dual challenges of simplifying sample preparation and using instrumentation capable of functioning under nonideal conditions which currently limits the resolution that can be achieved in the field.

The canonical stages of DNA profiling can be summarized as: (i) acquisition of a tissue sample of reasonably well-defined size; (ii) preparation of the DNA from that sample to a level of purity acceptable to the PCR to be used; (iii) estimation of the DNA yield and subsampling so an appropriate amount of DNA is presented to the PCR; (iv) PCR amplification; and (v) analysis of the amplification products to determine genotype by using appropriate hardware and software to obtain a profile.

The first three of these stages are both labor-intensive and time-consuming. Existing automated equipment for DNA extraction, while convenient, is expensive, relatively slow and not suitable for use in the field. Current methodologies are hostage to the time taken to solubilize tissue, purify the DNA, estimate the yield, and pipette the appropriate quantity into a PCR. To avoid this bottleneck, a new approach is required to prepare samples for PCR.

The use of FTA™ paper was considered as a means of simplifying sample collection, DNA purification, and presentation of the sample to PCR. Blood samples can be dried onto FTA™ paper (Whatman Inc., Piscataway, NJ), disks punched out and, following washing to remove stabilizing agents, disks or extracted DNA are added to the PCR (5). However, the method is not always well suited to sampling hard tissues such as skin and muscle in the field as they have to be pressed onto the paper with a degree of force. More importantly, while processing is simple, it requires quite long drying times, several minutes for the washes and uses relatively large volumes of processing fluids.

It was considered likely that probing tissue with either cold metallic wire or wire heated to red heat and quenched in a tissue or blood clot would cause adherence of a sufficiently well-defined mass of tissue and DNA to allow direct application of PCR with

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minimal additional processing. In choosing wires to test, it was reasoned that readily corroded wires such as soft iron wires were unlikely to be suitable as the properties of the surface layer might be variable and potentially inhibitory to the PCR. Wires such as copper wires that would release significant amounts of toxic ions were also unlikely to be suitable. Given the potential for development of automated systems, it was also considered important that pieces of wire could be moved by magnetic fields. Stainless steel wire was selected as most likely to satisfy all of the desirable properties and several types and grades of such wire were tested. In the work reported here, it was found that while sampling using a cold wire probe followed by isopropanol fixation often allowed amplification of satisfactory profiles when the wire tip was cut into PCR reagents, sampling using a hot wire with isopropanol fixation was more reliable.

Materials and Methods

Human Samples

Anticoagulant-free human blood was collected from a healthy volunteer, aliquoted, and allowed to clot naturally. Purified DNA was prepared from blood from the same individual using protease K and phenol by standard methods (6). Solid human tissues were obtained from the Joint Flinders Medical Centre (FMC)/Repatriation General Hospital Tissue Bank and the Ray Last Anatomy Laboratory (School of Medical Sciences, University of Adelaide). Ethics approval was from the FMC Clinic Research Ethics Committee. All tissues were stored at −80°C.

Stainless Steel Wire

Stainless steel wire (single strand, solid 0.5 mm diameter from B2K Co. Ltd, Pusan, Korea; Analysis %: C = 0.07; Si = 0.37; Mn = 1.28; $p = 0.028$; Ni = 8.16; Cr = 18.18; Fe = remainder) was sonicated (Soniclean™ Adelab Scientific, Thebarton, SA, Australia) for 15 min in 0.1% Pyroneg™ (an alkaline cleaning agent containing sodium carbonate, benzenesulfonic acid, 4-C10-13-sec-alkyl derivatives and ethylene glycol, from Johnson Diversey, Auckland, New Zealand), washed in triple deionized water, and cut into 4 cm lengths. Cleaned wires, other nonsterile materials, and equipment including the wire-cutter, PCR tubes, and filter paper were sterilized for 30 min in a 6-L stainless steel container fitted with two 14 W high-ozone UV lamps (GPH287T5VH/4; Ultraviolet Products Pty Ltd, Keswick, SA, Australia).

Sampling Protocols

For blood clots, the end of a length of wire was flamed to red heat for ~5 sec using a portable butane burner (Labogaz 206; Aktivlab Pty. Ltd., Mount Barker, SA, Australia) and then inserted ~5 mm into the clot just as the tip lost incandescence and rotated gently by rolling between the fingers for ~5 sec. The wire was withdrawn from the clot and deposited tip-first into a PCR tube containing at least 100 µL of isopropanol, sufficient to cover the tissue-loaded section of the wire.

To aid wire penetration, a site on thawed solid tissue a few millimeters wide was softened by jabbing several times with the tip of a size 11 triangular surgical blade (Livingstone International Pty Ltd, Rosebery, NSW, Australia) prior to sampling with hot wire as described earlier. For frozen tissue, hot wire was inserted directly into the tissue with gentle rotation, removed, and immersed in isopropanol as described earlier.

Sample wires were fixed in isopropanol for at least 40 sec, and 2 mm of the wire tip coated with blood or tissue sample was cut, while still wet with isopropanol, into a clean UTW™ (ultrathin wall) PCR tube (Finnzymes, Inc., Woburn, MA) with a wire-cutter (Electronic-pliers, precision range, tungsten carbide side-cutters; Carl Kammerling International Ltd, Pwllheli, U.K.). As a precaution against sample carry-over, the wire-cutter was cleaned between cuts by clipping a double layer of sterile 3 MM filter paper (Whatman Ltd, Kent, England) at least five times. The sample-coated wire tips were incubated at room temperature for no less than 5 min to ensure complete evaporation of the residual isopropanol prior to the addition of PCR reagents. The sample tips were stored in PCR tubes at room temperature for future use (Fig. 1*A*).

Primers

Three groups of genetic markers were used in the study: (i) single locus, a DQα locus (for preliminary studies only) or the amelogenin AMG locus (for preliminary studies and subsequent testing); (ii) a quintuplex short tandem repeat (STR) system (Table 1) targeting the AMG, D21S11, D18S51, and FIBRA loci using the primer sequences from Miscicka-Sliwka et al. (7) and the D5S818 primer sequences from Kupfer et al. (8); (iii) the commercial AmpF*l*STR®

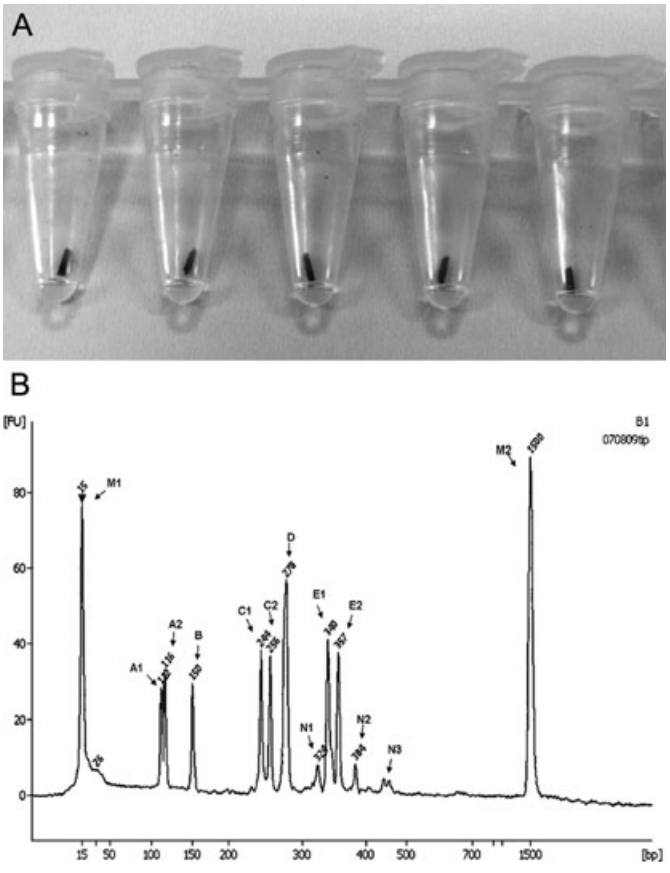

FIG. 1—*Samples taken on wire ready for PCR and a profile from a blood clot obtained by capillary electrophoresis using the field deployable system. Samples were taken with hot wire, fixed in isopropanol and (A) 2 mm sections clipped into PCR tubes, amplified using the quintuplex primers and (B) analyzed on an Agilent 2100 Bioanalyser™. Vertical axis: DNA fluorescence units, horizontal axis: nominal length in base-pairs. Loci amplified: A1: AMG-X; A2: AMG-Y; B: D5S818; C1 & C2: D21S11; D: D18S51; E1 & E2: FGA. N1, N2 & N3 are artifacts. M1 and M2 are size markers. Fragment sizes reported vary from known lengths, reflecting the standardization method used by the instrument.*

TABLE 1—*Primers used in this study.*

Locus	Product Length (bp)	Primer Sequences (5′–3′)
DQα	~250	F: GTGCTGCAGGTGTAAACTTGTACCAG R: CACGGATCCGGTAGCAGCGGTAGAGTTG
AMGx/y	106 and 112	F: CCCTGGGCTCTGTAAAGAATAGT R: ATCAGAGCTTAAACTGGGAAGCTG
D5S818	123–155	F: GGTGATTTTCCTCCTTGGTATCC R: AGCCACAGTTTACAACATTTGTATCT
D21S11	205–249	F: ATATGTGAGTCAATTCCCCAAG R: TGTATTAAGTCAATGTTCTCCAG
D18S51	275–323	F: CAAACCCGACTACCAGCAAC R: GAGCCATGTTCATGCCAATG
FIBRA	350–400	F: GGACAATCTTAACTGGCATTCA R: TGCGCTTCAAGGACTTCA

Profiler Plus® (Applied Biosystems, Carlsbad, CA) with nine loci. All primers for genetic markers in groups 1 and 2 were synthesized by GeneWorks Pty Ltd (Hindmarsh, SA, Australia).

PCR Amplification

Analyses using amelogenin primers or the quintuplex primer set were performed in 25-μL reactions comprising Phusion GC buffer (750 mM Tris–HCl, pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) Tween® 20), 200 μM of each dNTP (New England Biolabs, Inc., Ipswich, MA), 0.5 U of Phusion™ high-fidelity DNA polymerase (Finnzymes, Inc.), 0.14 μM of each AMG primer, 0.2 μM of each D5S818 primer, 0.4 μM of each D21S11 primer, 0.16 μM of each D18S51 primer, and 0.14 μM of each FIBRA primer. After a hot start at 98°C for 30 sec, there were 34 cycles of 10 sec at 98°C, 13 sec at 59°C, and 11 sec at 72°C with a final 5 min extension at 72°C on a Piko™ Thermal Cycler (Finnzymes, Inc.). Analyses using AmpF*l*STR® Profiler Plus® were performed by Forensic Science South Australia using an Applied Biosystems GeneAmp® PCR System 9700 in 25 μL volumes for 28 and 32 cycles with the manufacturer's PCR protocol.

Detection Systems

Two capillary electrophoresis systems were used in this study (i) an Agilent 2100 Bioanalyser™ system (Agilent Technologies, Waldbronn, Germany) with the DNA 1000 kit (Agilent Technologies) for detecting the quintuplex STR system PCR products and (ii) an AB 3130*xl* Genetic Analyzer (Applied Biosystems) with the 3130*xl* Genetic Analyzer Data Collection software v3.0 (Applied Biosystems) for detecting AmpF*l*STR® Profiler Plus® PCR products. For both systems, all operations were performed according to manufacturer's instructions.

DNA Estimation

The concentration of purified human DNA was measured using a NANODROP 1000 spectrophotometer (Thermo Scientific, Waltham, MA). The amount of DNA obtained by wire sampling was estimated from the sum of fluorescence peaks on the Agilent 2100 Bioanalyser™ following amplification for 34 cycles using the quintuplex primer set by comparison to a standard curve primed with purified human DNA.

Results and Discussion

Initial experiments exploring sampling methods, choice of enzymes, cycling protocols and tolerance to biological debris for simplicity employed single loci for the analyses, initially DQα and later amelogenin which proved more robust. During these searches, it was found that heat-denatured tissue improved tolerance of PCR amplification to potential inhibitors present in samples, suggesting that sampling with hot wire would be a viable way of obtaining samples that could be used directly in PCRs (data not shown). The initial testing of wire sampling used single locus amplifications to avoid difficulties in interpretation because of the potential for imbalances in multiplex amplifications. These initial observations laid the foundations for development of robust multiplexes (Fig. 1*B*) suitable for use in the field deployable Bioanalyser™ for discrimination among small sets and, in addition, demonstrated that samples taken on wire can be transported to a forensic laboratory for high resolution analysis with AmpF*l*STR® Profiler Plus®.

The Choice of Hot Wire for Acquisition of Samples

In pilot experiments using DQα primers, hot wire was found to bind more DNA than cold wire (data not shown). For example, when sampling blood clots, which are a relatively poor source of template (Table 2), 28 cycles of amplification did yield an observable product with hot wire, but not with cold wire. However, product could be detected when samples taken with cold wire were subjected to further rounds of amplification, showing that heating is not essential for adherence of DNA template to stainless steel wire. The better performance of hot wire is presumably a consequence of denaturation of proteins from the tissue creating a gel that helps adherence of tissue and DNA to the wire. The use of hot wire has the additional advantage of ensuring the wire is free of contamination at the moment of sampling and also facilitates the direct sampling of unthawed tissues.

Effects of Stainless Steel Wire on PCR

The presence of metal oxides on the wire surface and potential changes in the heating and cooling rates of the amplification mix are expected sources of interference when stainless steel wire is present in a PCR. To examine these possibilities, the effect of varying the quantity of wire present in a PCR was examined. Although a 2 mm length of 0.5-mm stainless steel wire does have a measurable effect on PCR amplification, it is acceptably small (Fig. 2). In a 25-μL PCR, each millimeter of wire reduces the amplification rate by approximately 12%, irrespective of whether the template DNA was clean control DNA (Fig. 2*A*) or from hot wire stabbed into a blood clot (Fig. 2*B*). As the effects are approximately linear, this implies an approximately 25% decrement to the yield expected from a 25-μL reaction mix when it contains the 2-mm tip of an 0.5-mm-diameter sampling wire. This inhibition is the price to pay

TABLE 2—*Amount of DNA obtained from human tissues on a 2-mm wire tip.*

Tissue	Number of Tips	Mean DNA Amount (±SE), ng
Bladder	8	1.44 ± 0.94
Blood	40	0.51 ± 0.09
Kidney	15	10.27 ± 2.71
Liver	6	0.69 ± 0.46
Lung	6	1.69 ± 0.48
Muscle	6	0.25 ± 0.08
Pancreas	6	9.06 ± 6.75
Prostate	13	0.35 ± 0.16
Skin	15	2.59 ± 1.38

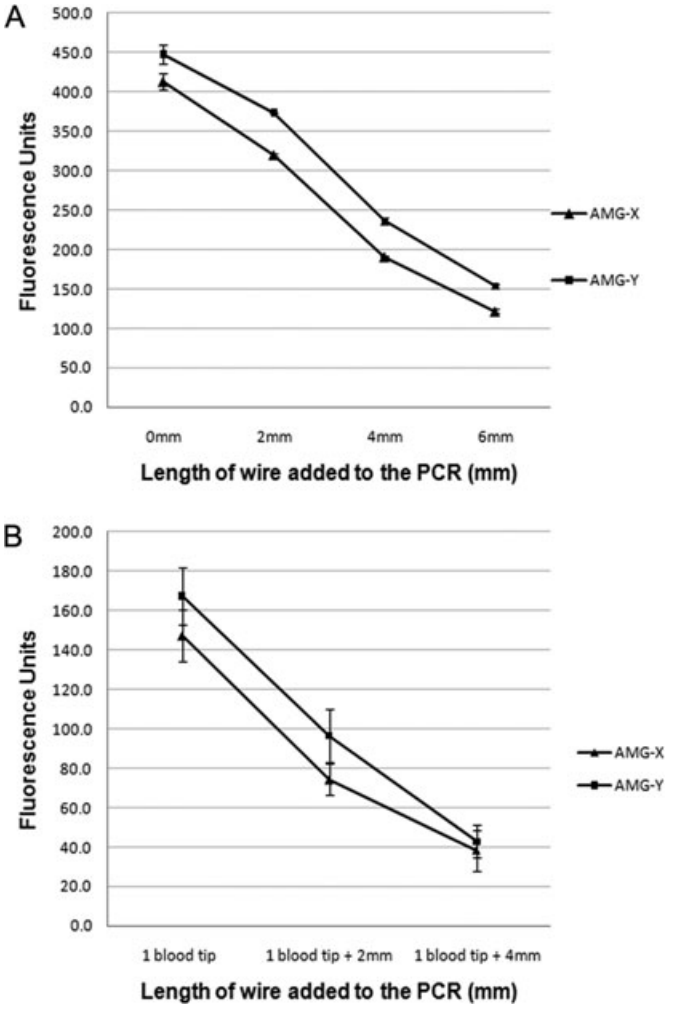

FIG. 2—*The effect of stainless steel wire on PCR amplification. (A) Purified human DNA ~18 ng per reaction, a gross excess to ensure template was not rate limiting or (B) 2 mm blood-coated tips were used as template and amplified with the AMG-X and AMG-Y primer pairs. The indicated lengths of stainless steel wire heated to red heat then cooled were added prior to amplification. The amplification products were analyzed on an Agilent 2100 Bioanalyser™. Each datapoint represents the mean (±SD) of the combined fluorescence values of peaks in each of a set of four replicates.*

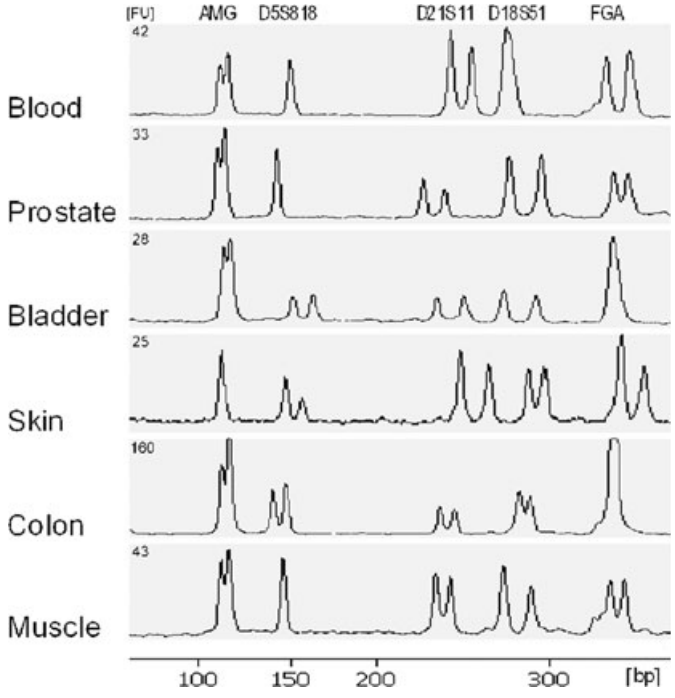

FIG. 3—*Quintuplex profiles from various human tissues from six different individuals. Profiles obtained using an Agilent 2100 Bioanalyser™ from human tissues sampled with hot wire and amplified using the quintuplex primer set. Frozen blood clots, prostate, bladder and colon were thawed before sampling. Frozen skin and muscle were sampled directly without thawing. Vertical axes: DNA fluorescence units (FU) of the highest peak; horizontal axis: nominal length in base-pairs.*

for the gain in speed of sample preparation by avoiding both DNA purification and estimation but can be managed by choice of sample size. How much of the interference with amplification is from the metals and metal oxides on the wire surfaces or from physical effects such as thermal inertia influencing amplification conditions was not determined.

Applicability to Various Tissues

Even though the quantity of DNA obtained on the wire tip varied from one tissue to another (Table 2), sampling with hot wire yielded full profiles from each of blood clots, prostate, bladder, skin, colon, and muscle using the quintuplex primer set and the Agilent 2100 Bioanalyser™ (Fig. 3). Full profiles were also obtained from liver, lung, kidney, pancreas, and from dried blood moistened with sterile DNA-free water (data not shown). In generating a primer set for field analysis that was compatible with the resolution obtainable with the Agilent 2100 Bioanalyser™, some combinations of the Miscicka-Sliwka et al. (7) primers and the D5S818 primers from Kupfer et al. (8) were trialed. The quintuplex primer set was the most tolerant to variation in DNA amount and discriminated between one set of 50 randomly chosen human tissue samples (data not shown).

Samples on Wire are Stable for Extended Periods

To test the stability of samples taken with wire, hot wires were dipped into blood clots, fixed with isopropanol, dried, and kept at room temperature for periods up to 180 days (6 months). Periodically, loaded wires were amplified using the quintuplex primer set and the products analyzed on the Agilent 2100 Bioanalyser™. Samples remained amplifiable for 6 months (Fig. 4). The variation in yield over this period was random rather than time dependent, suggesting safe storage times are likely to be considerably longer. Thus, samples taken on wire can be returned to the laboratory for profiling in cases where field analysis is impracticable or where it is required for quality control or for any other reason and can be archived for significant periods (at least for 6 months as tested in this study).

Samples on Wire can be Genotyped with AmpFlSTR® Profiler Plus®

The feasibility of obtaining profiles from samples taken with wire using megaplex profiling systems was tested with AmpF*l*STR® Profiler Plus®, which is used in many laboratories worldwide for profiling human DNA. Blood clots were chosen as the source of template because their relatively low yield of DNA (Table 2) would test the limits of sensitivity and potential interference by contaminants in the sample. Although amplification for 28 cycles gave

incomplete profiles, extending the amplification to 32 cycles gave useful profiles on an AB 3130*xl* Genetic Analyzer in every case with triplicate trials of each of 1-mm (Fig. 5), 2-mm, and 3-mm tips cut from the sample wire. Although DNA yields varied considerably because of the interplay of template and inhibitor amounts, including the length of wire immersed in the PCR mix, the locus imbalance was not abnormal for 32-cycle amplifications using AmpF*l*STR® Profiler Plus®. However, it was noted that the fluorescence signal for D7S820 was commonly low and in one case was below the calling limit (50 RFU).

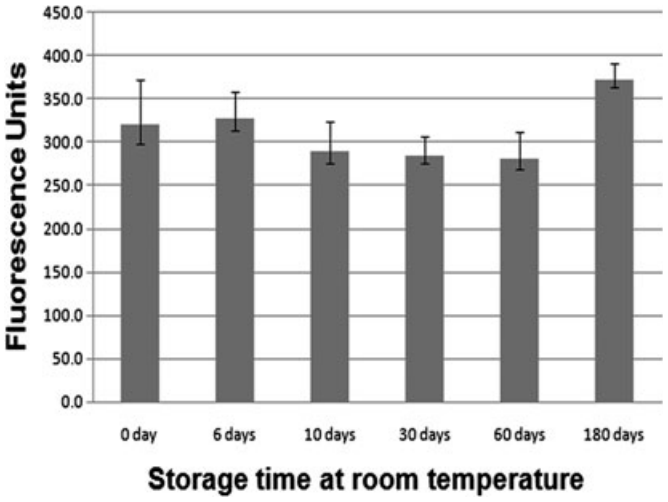

FIG. 4—*Storage of samples on wire. Samples were taken from a blood clot with hot wire, fixed in isopropanol, dried and stored at room temperature for periods up to 180 days prior to analysis of six replicates using the quintuplex primer set and Agilent 2100 Bioanalyser™. The Y-axis is the pooled peak heights, in arbitrary fluorescent units, of all five loci.*

Conclusions

This paper describes a rapid, simple, and robust way of collecting template from tissue and blood clots suitable for use in the field and on frozen cadavers. Samples can be added directly to PCRs avoiding the time normally taken for DNA extraction and estimation. The method works well with single locus amplifications and multiplexes of five and nine loci, including AmpF*l*STR® Profiler Plus® widely used in the forensic industry. Although the amount of template obtained from any one tissue is repeatable, it varies from tissue to tissue. Under- or overloading a PCR with template can be controlled by varying the length of wire clipped into the tube. Replication of sampling is trivial, so many samples can be taken without significant commitment of resources and because the template is stable on wires, it is readily transported or stored enabling choice of field profiling at low resolution or full genotyping in a laboratory, or both. It was noted that wire provides a practicable and convenient sample-acquisition technology with flow

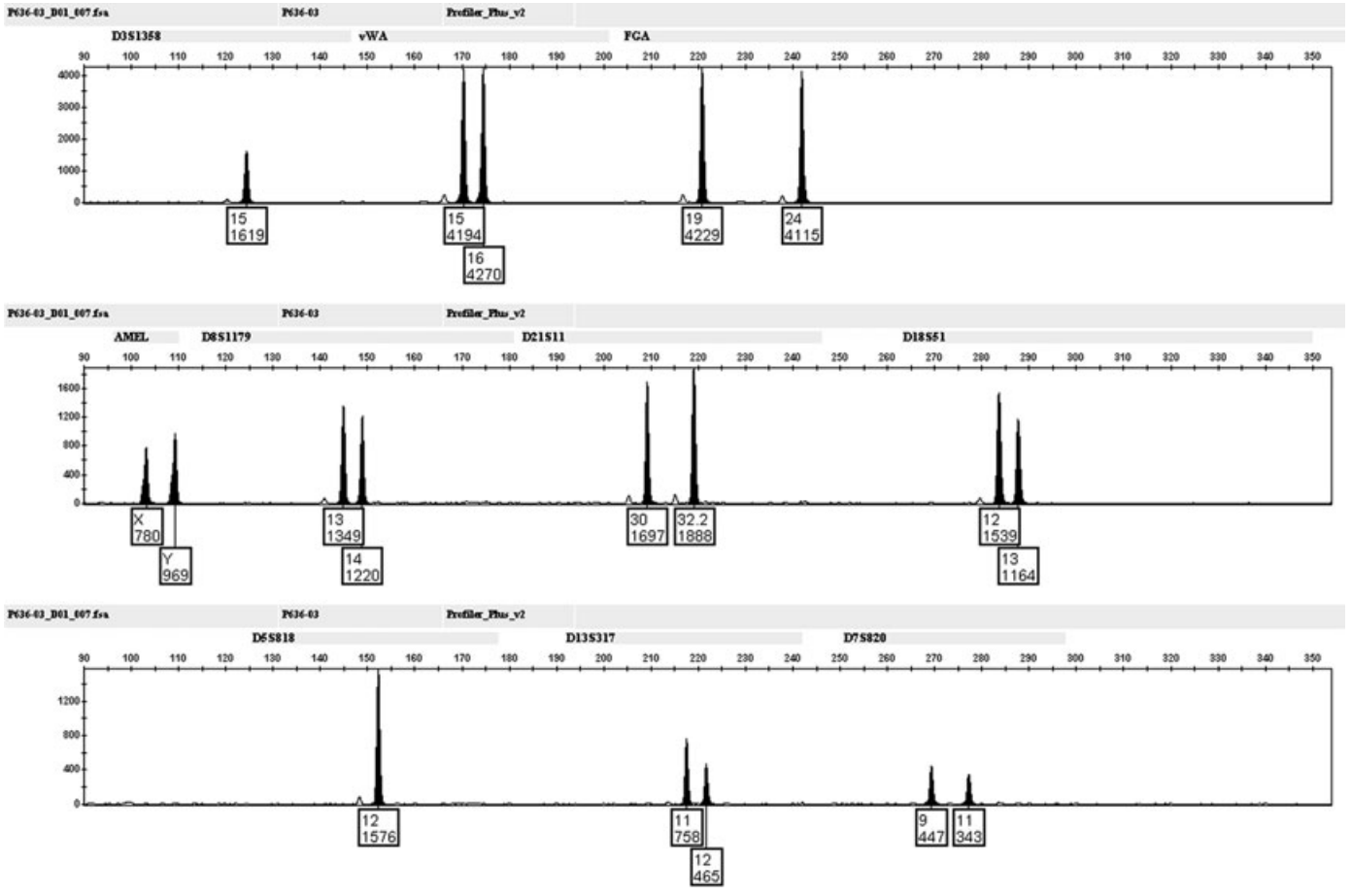

FIG. 5—*An example of the profiles obtained with AmpF*l*STR® Profiler Plus® from samples taken with wire. Samples were taken from a blood clot with hot wire, fixed in isopropanol, dried and a 1-mm length clipped directly into AmpF*l*STR® Profiler Plus® amplification reaction and amplified for 32 cycles. One microliter samples were analyzed by capillary electrophoresis on an AB 3130xl Genetic Analyzer. The profile shown is representative of data from triplicate amplifications for each of 1, 2, and 3 mm lengths clipped from the sample wire.*

on applications for the development of field deployable genotyping tools. Wires might be sculptured to provide breakpoints to improve the standardization of sample size and the resultant bullet handled magnetically in a profiling chip.

Acknowledgments

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