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TECHNICAL NOTE

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STR Profiles from DNA Samples with "Undetected" or Low Quantifiler™ Results

ABSTRACT: Screening methods capable of identifying DNA samples that will not yield short tandem repeat (STR) profiles are desired. In the past, quantitation methods have not been sensitive enough for this purpose. In this study, low level DNA samples were used to assess whether Quantifiler™ has a minimum quantitation value below which STR profiles would consistently fail to be detected. Buccal swabs were obtained and the DNA extracted, quantified, and serially diluted to concentrations ranging from 0.002 to 0.250 ng/μL. Samples were analyzed once with Quantifiler™, followed by Profiler Plus™ amplification and capillary electrophoresis analysis. An absolute minimum value below which STR results were unobtainable could not be defined. From the 96 low level samples tested, STR loci (including one full profile) were successfully amplified and detected from 27% of the samples "undetected" by Quantifiler™. However, no STR alleles were detected in 73% of these "undetected" samples, indicating that Quantifiler™ data may be useful for predicting STR typing success.

KEYWORDS: forensic science, DNA typing, quantitative polymerase chain reaction, Quantifiler™, DNA quantitation, low level DNA

Current methods for the forensic analysis of biological samples require polymerase chain reaction (PCR) amplification of short tandem repeat (STR) loci for human identification (1,2). Multiplex and megaplex STR kits have been commercialized for this purpose; however, these kits contribute a large portion of the costs associated with developing a DNA profile. Thus, the use of a human DNA quantitation method as a screening tool would be beneficial, enabling examiners to forego analysis of samples less likely to amplify, saving both time and money.

In the forensic science community, some of the most popular methods for human DNA quantitation include slot blot hybridization, solution hybridization, and real-time quantitative PCR (Q-PCR) (3–6). In the recent past, the most commonly used method for the quantitation of human DNA was the slot blot procedure (6,7), most notably via the QuantiBlot® Human DNA Quantitation Kit (ABI, Foster City, CA). However, forensic advances with real-time PCR have allowed Q-PCR methods to become more widespread, including the acceptance and use of several commercially available kits by the forensic science community (5,6,8). One such kit, Quantifiler™ Human DNA Quantification Kit (ABI), utilizes a primer set that flanks the human telomerase reverse transcriptase (hTERT) locus and a TaqMan® probe equipped with a 5′ fluorescent reporter dye (FAM®) and a 3′ nonfluorescent quencher.

Recent comparisons of quantitation methods have shown differences in their sensitivity, accuracy, and/or precision (5,6,8–10). For example, Kline et al. (6) showed that slot blot methods (including QuantiBlot®) may not detect samples ≤0.16 ng/μL, whereas Q-PCR methods (including Quantifiler™) can detect samples below this range. The manufacturer states that the suggested quantitation range for Quantifiler™ is 0.023–50 ng/μL of human DNA; however, the software can use the standard curve to calculate concentration values (albeit less reliably) much lower than 0.023 ng/μL before samples are determined to be "undetected" (5,10). With regard to accuracy, both methods are accurate enough for use with STR amplification, but the quality of a DNA evidence sample may be unpredictable, altering the performance of the quantitation assay used. For higher quality samples (those lacking PCR inhibitors and degradation), QuantiBlot® and other blotting methods tend to underestimate quantitation values, while Quantifiler™ and other Q-PCR methods tend to generate values closer to or slightly above the expected concentration values (6). However, in the presence of PCR inhibitors, slot blot methods overestimate the amount of amplifiable DNA, whereas Q-PCR methods are able to detect PCR inhibitors, thus indicating the amount of amplifiable DNA—a potential tool for predicting the success of STR amplification (5). Unfortunately, because of the small PCR product sizes targeted, Q-PCR methods are prone to overestimating DNA available for STR amplification when quantifying degraded samples (8). These differences in sensitivity, precision, and accuracy between quantitation methods have been shown to impact PCR amplification and detection of STR loci (11,12).

In forensic casework, the best-case scenario would involve obtaining a high quality, balanced, full profile from an evidence sample. However, this is often not possible when case samples yield low level DNA, severely degraded DNA, or no DNA at all. Unfortunately, in these cases it is difficult to determine at what point an examiner can comfortably end the analysis of a sample. For example, full and partial STR profiles have been obtained from blood stains that had tested negative with confirmatory blood tests

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(13); this has also been observed in samples that were "undetected" for human DNA with QuantiBlot® (14). However, the use of a new quantitation method (e.g., Quantifiler™) that is more sensitive than previous methods carries with it the hope of finding a minimum value at or below which examiners can reliably report "insufficient DNA" without proceeding through STR amplification and detection. This could have a tremendous impact on a DNA laboratory. Not having to pursue samples containing insufficient DNA would save time and significant reagent costs, allowing examiners to more efficiently attack casework backlogs.

This study intends to evaluate the Quantifiler™ Human DNA Quantification Kit to determine whether or not a minimum quantitation value exists for STR typing—below which STR profiles would consistently not be detected by capillary electrophoresis (CE).

Materials and Methods

Sample Collection

Buccal swabs were obtained from each of 12 volunteers (11 female and one male, ranging in age from 22 to 38 years) using sterile cotton swabs. Buccal swabs were chosen for this study because they are convenient, commonly used in human identity testing, and they typically generate full STR profiles without having to adjust for PCR inhibition or degradation (T.D. Cruz, personal observation).

DNA Extractions

One entire buccal swab from each volunteer was used for DNA extraction with the QIAamp® DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's recommendations and modifications made by Greenspoon et al. (15). Extracted DNA was eluted in a final volume of 100 μL of Qiagen Buffer AE elution buffer.

Quantitation and Dilution

DNA extractions were quantitated by real-time PCR using the Quantifiler™ Human DNA Quantification Kit according to the manufacturer's recommendations and as reported by Green et al. (5). The ABI Prism® 7000 Sequence Detection System was used for analysis. The internal positive control was examined for each sample for possible indications of PCR inhibition. Ninety-six low level samples were prepared from QIAamp® extractions from 12 individuals via a serial dilution (to concentrations of: 0.250, 0.125, 0.063, 0.031, 0.016, 0.008, 0.004, and 0.002 ng/μL). These low level samples and a negative control (2 μL of TE^{-4}) were then analyzed a single time with Quantifiler™. The value obtained was used to concentrate each sample to the target DNA concentration for multiplex STR amplification (1.5 ng in a volume of 5 μL of TE^{-4}) using Microcon® YM-10 concentrators (Millipore Corporation, Bedford, MA). If there was an insufficient quantity of DNA in the sample dilution to obtain the target amount, the entire sample dilution (50–150 μL) was concentrated to 5 μL in TE^{-4}.

STR Amplification and CE Analysis

Nine STR loci and the gender marker, Amelogenin, were PCR amplified from extracted DNA in a multiplex reaction using the AmpF*l*STR® Profiler Plus™ PCR Amplification Kit (ABI). Each sample was amplified using 5.7 μL of AmpF*l*STR® PCR Reaction Mix, 2.0 μL of AmpF*l*STR® Profiler Plus™ Primer Set, 2.10 μL of TE^{-4}, and 0.2 μL of AmpliTaq Gold Polymerase (5 U/μL). Amplifications were performed using the GeneAmp PCR System 9600 thermalcycler (Perkin-Elmer Corporation, Norwalk, CT) according to the manufacturer's recommendations (16), with a modified final extension time of 90 min and a final hold of 4°C.

Complete STR profiles were obtained for each reference sample used in this study by amplifying 1.5 ng of each extract. These profiles were compared to the STR results from the 96 low level samples. For the 96 low level samples, a target of 1.5 ng of DNA was amplified, when available. If less than 1.5 ng of DNA was available, the entire low level sample was consumed in order to obtain as close to 1.5 ng as possible. Although forensic laboratories differ in their policies regarding evidence consumption, this research study intended to provide the best-case scenario by consuming the entire sample for amplification (when necessary).

Following amplification, STR products were detected via capillary electrophoresis using the ABI Prism® 3100-*Avant* Genetic Analyzer accompanied by the Data Collection Software, version 2.0 and analyzed with GeneMapper® ID, version 3.2 (ABI). For detection, 1.2 μL of STR amplification product was added to 0.5 μL of GeneScan™-500 ROX™ (ABI) and 12.0 μL of Hi-Di Formamide (ABI). Samples were denatured for 5 min at 95°C, followed by a snap freeze on ice for 5 min. Samples were electrokinetically injected for 10 sec and 3 kV into an internally uncoated, 36 cm capillary (ABI) and size-separated using Performance Optimized Polymer-4 (POP-4™) (ABI) at 15 kV and a temperature of 60°C. Peak threshold was set to our laboratory's validated minimum of 75 relative fluorescence units (rfu), a common threshold used among forensic labs using the 3100-*Avant* and/or 3100, but slightly above the default setting (50 rfu) suggested by the GeneMapper® ID software (17).

Data Analysis

STR profiles were analyzed using GeneMapper® ID, version 3.2. Profiles obtained from the 96 low level samples were compared to the reference profiles to determine if a partial or complete profile was detected at each of the nine STR loci. The number of typed loci were counted and compared to the Quantifiler™ quantitation values of the 96 low level samples. From these, five arbitrary quantitation ranges were identified ("undetected," <0.010, 0.010–0.030, 0.030–0.100, and >0.100 ng/μL), and the average number of typable loci from each range was tabulated. A locus was considered "complete" if the expected homozygote allele typed or if both expected alleles of a heterozygote pair were typed. Note: Values <0.010 ng/μL and those in the range of 0.010–0.030 ng/μL are below the range of detection suggested for the Quantifiler™ kit, whereas those in the ranges of >0.100 and 0.030–0.100 ng/μL represent values well within the detection range and standard curve of the Quantifiler™ kit.

Results

Quantitation values from the 96 low level samples (target concentrations of 0.002–0.250 ng/μL) ranged from "undetected" (i.e., no human DNA detected) up to 0.225 ng/μL upon Quantifiler™ analysis. Overall, from all low level samples tested, full profiles were obtained from 13.5% of the samples, partial profiles were obtained from 54%, and no typable loci were obtained from the remaining 32%. All low level samples tested provided STR profiles consistent with the expected reference profiles; no instances of contamination or allele drop-in were noted in the samples or negative controls. A summary of the average number of typable

TABLE 1—*Average number of typable loci from low level DNA samples.*

Quantifiler™ Reading (ng/μL)	No. Samples	Average No. Complete Loci (of 9)
"Undetected"	33	0.8 ± 2.2
<0.010	18	2.3 ± 3.1
0.010–0.030	15	5.5 ± 3.1
0.030–0.100	17	6.1 ± 2.9
>0.100	13	7.5 ± 1.9

loci at various quantitation ranges can be found in Table 1. In general, as the Quantifiler™ quantitation value decreases, the average number of typable loci also tends to decrease. Samples that were reported as "undetected" by Quantifiler™ resulted in an average of <1 STR locus per sample analyzed. However, it should be noted that, in this study, Quantifiler™ was used to detect concentrations that often fell below the manufacturer's reported optimal detection limit (0.023 ng/μL); therefore, such quantitation values may not be entirely accurate, resulting in the observed high standard deviations.

For all 96 low level samples tested, allele/locus dropout was counted and compared to product size to determine if preferential amplification was occurring. Partial profiles were detected in 54% of samples tested with allele dropout ranging from 1 to 6 instances per sample, regardless of the quantitation value. One sample tested (Quantifiler™ concentration of 0.00865 ng/μL) suffered from allele dropout at six of nine loci, with two additional loci that fell below threshold and failed to be detected altogether (data not shown). As expected, locus and/or allele dropout occurred more frequently at STR loci with larger PCR product sizes, with the exception of the FGA locus (Fig. 1).

Of the 33 samples that were "undetected" by Quantifiler™, 24 (73%) were truly undetected and did not result in any typable STR loci after amplification and CE analysis. However, of the remaining nine "undetected" samples, a full profile was obtained from a single sample (Fig. 2), and a second "undetected" sample fell short of a complete profile by a single allele, due to allele dropout at a single heterozygous locus (data not shown). Although the remaining seven "undetected" samples did display partial profiles, they generally did not yield enough typable loci to provide useful information for identification (Table 1), but could be valuable for elimination purposes. Given these observations, it is not possible to define an absolute minimum Quantifiler™ value below which STR results are not obtained. However, the data indicate that Quantifiler™ may be a useful tool for predicting the success of STR amplification.

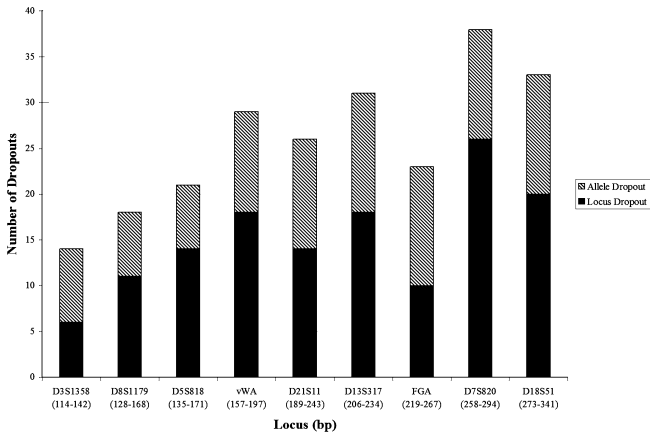

FIG. 1—*Locus and allele dropout from 96 low level DNA samples. Partial profiles were detected in 54% of the low level samples (n = 96), in which a total of 233 of 864 loci experienced allele dropout (including instances in which the entire locus dropped out). In general, loci with larger STR amplification products failed to amplify and/or suffered from allele dropout at heterozygous loci more than loci with smaller STR products.*

Discussion

Finding a minimum quantitation value that would predict STR success could save forensic examiners a great deal of time and money. This would assure that attempting to amplify STR loci from DNA with quantitation measurements at or below the minimum value would consistently be unsuccessful and would not result in any typable STR loci upon subsequent CE analysis. Examiners could then reliably choose to stop analysis of any sample that has a quantitation value at or below the minimum value. In this study, Quantifiler™ accurately predicted complete STR amplification failure (i.e., no alleles were amplified) after a single quantitation reading in 73% of "undetected" samples tested. However, given that one low level "undetected" sample gave a full profile and several "undetected" samples yielded partial profiles (average of 1.3 ± 2.2 loci), the data suggest that there is not an absolute true minimum value with the Quantifiler™ method using the ABI Prism® 7000 Sequence Detection System. Although our laboratory's internal validation of Quantifiler™ does show run to run variability in quantitation results from a single sample, it is common practice for forensic laboratories to quantify a sample only one time before amplifying. Thus, our study was purposefully based on a single quantitation attempt in order to depict actual forensic laboratory practice. However, it should be noted that samples whose quantitation values fall below the limit of detection for this method may give an inaccurate quantitation result that is not reproducible upon a second analysis. This could explain the occurrence of a full STR profile from an "undetected" sample, as well as the lack of any STR loci from several detected samples.

Although an absolute minimum value for predicting STR success does not seem to exist for this method, our data do clearly suggest that most "undetected" samples from Quantifiler™ analysis will likely generate no profile or a partial profile that would likely contain so few typable loci that the information would not be useful for human identification purposes. The parameters of this study were designed to provide a best-case scenario through the use of buccal swabs (reference samples) and the consumption of the entire sample and extract (when necessary) to amplify as close to 1.5 ng of DNA as possible. Given that 73% of the "undetected" samples failed to amplify at any of the tested STR loci, it is even less likely that an "undetected" lower quality forensic sample, which typically cannot be consumed, would yield any typable STR loci. Nonetheless, a single typed locus could provide enough data to provide exclusionary results in a case. In this study, 27% of "undetected" samples tested (9 of 33) provided results at ≥1 STR locus. This data should be useful for forensic laboratory directors and examiners seeking to define laboratory policies regarding further analysis of case samples that are "undetected" for human DNA by Quantifiler™.

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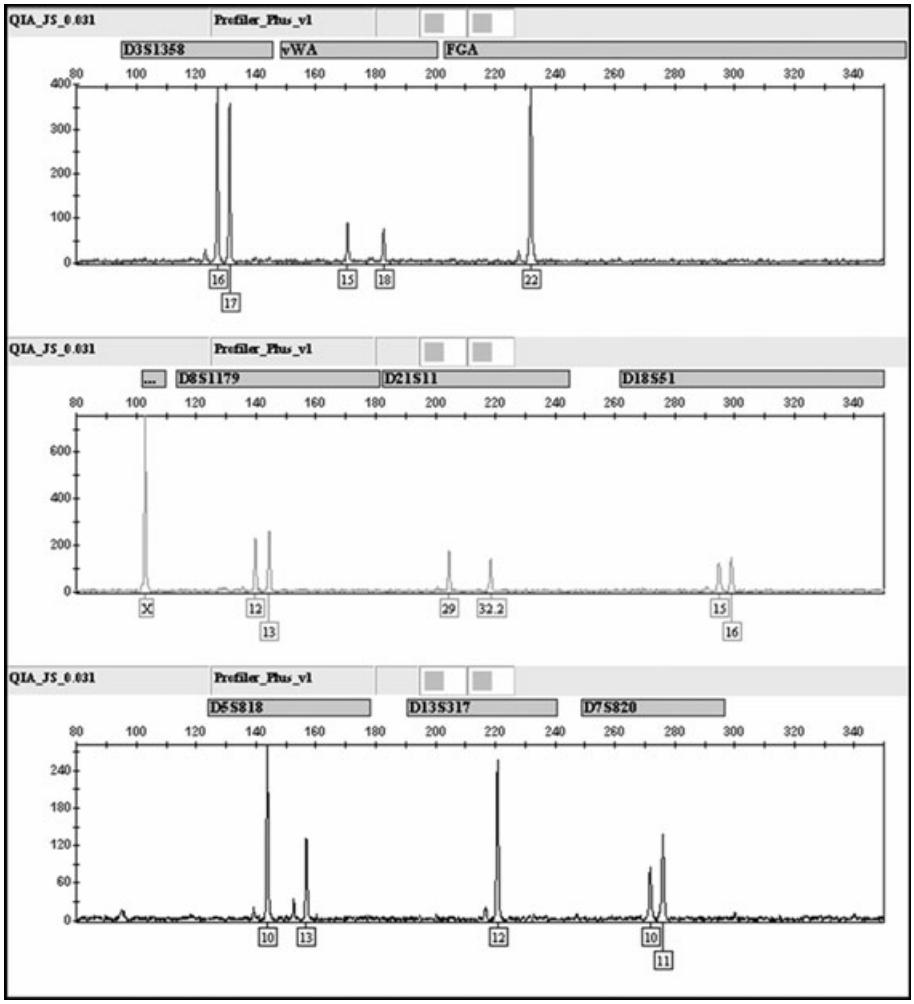

FIG. 2—*Full profile from a sample "undetected" with Quantifiler™.*

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