Forensic Science International 263 (2016) 132–138

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Technical note

Comparison of Quantifiler® Trio and InnoQuant™ human DNA quantification kits for detection of DNA degradation in developed and aged fingerprints

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ARTICLE INFO

Article history:
Received 29 November 2015
Received in revised form 18 March 2016
Accepted 7 April 2016
Available online 22 April 2016

Keywords:
Latent fingerprints
Nanotechnology
DNA
Quantifiler® Trio
InnoQuant™
Degradation ratio

ABSTRACT

The development techniques employed to visualize fingerprints collected from crime scenes as well as post-development ageing may result in the degradation of the DNA present in low quantities in such evidence samples. Amplification of the DNA samples with short tandem repeat (STR) amplification kits may result in partial DNA profiles. A comparative study of two commercially available quantification kits, Quantifiler® Trio and InnoQuant™, was performed on latent fingerprint samples that were either (i) developed using one of three different techniques and then aged in ambient conditions or (ii) undeveloped and then aged in ambient conditions. The three fingerprint development techniques used were: cyanoacrylate fuming, dusting with black powder, and the columnar-thin-film (CTF) technique. In order to determine the differences between the expected quantities and actual quantities of DNA, manually degraded samples generated by controlled exposure of DNA standards to ultraviolet radiation were also analyzed. A total of 144 fingerprint and 42 manually degraded DNA samples were processed in this study.

The results indicate that the InnoQuant™ kit is capable of producing higher degradation ratios compared to the Quantifiler® Trio kit. This was an expected result since the degradation ratio is a relative value specific for a kit based on the length and extent of amplification of the two amplicons that vary from one kit to the other. Additionally, samples with lower concentrations of DNA yielded non-linear relationships of degradation ratio with the duration of aging, whereas samples with higher concentrations of DNA yielded quasi-linear relationships. None of the three development techniques produced a noticeably different degradation pattern when compared to undeveloped fingerprints, and therefore do not impede downstream DNA analysis.

1. Introduction

The modest objective of the work reported here was to compare the efficacy of the recently developed Quantifiler® Trio (Thermo Fisher Scientific, Oyster Point, CA, USA) and InnoQuant™ (InnoGenomics, New Orleans, LA, USA) kits for measuring the extent of degradation of DNA in forensic samples. Samples chosen for the comparative study included latent fingerprints that were first developed for enhancement of visualization and then aged in ambient conditions. As reference, DNA was also quantified in latent fingerprints that were not developed but still aged.

Both fingerprint examination and DNA profiling are widely used methods of identification. Application of two different identification techniques on one evidence sample can strengthen the validity of evidence [1]. Latent fingerprints are developed and compared with fingerprint databases as well as against fingerprints harvested from suspects and other individuals at crime scenes. Genomic DNA from fingerprints has been shown to yield successful short tandem repeat (STR) profiles, and mitochondrial DNA sequencing has been performed from palm prints on paper [2,3]. Thus, analysis of DNA from fingerprints is valuable in resolving criminal cases.

A significant difficulty in processing these samples for both enhanced visualization and DNA analysis is that the fingerprint often contains low amounts of DNA. In outdoor crime scenes, exposure to excessive heat or cold as well as to humidity or aridity can preferentially degrade higher-molecular-weight DNA [2].

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http://dx.doi.org/10.1016/j.forsciint.2016.04.009
0379-0738/

In addition, peculiar DNA damages are caused by UV radiation [4]. Environmental insults may result in allelic drop-outs, yielding partial DNA profiles, commonly observed when attempting amplification of low-template or degraded DNA with STR kits [5–7].

In forensic laboratories, samples containing small amounts of DNA may be stored for long periods of time. Assessment of degradation in DNA-containing samples is becoming increasingly commonplace [8]. Hence, it is now known that while environmental factors such as high/low temperature and humidity/aridity play major roles in degrading DNA, aging at ambient conditions can also incur severe degradation [4].

Traditional fingerprint-development techniques to enhance visualization for forensic examiners may be detrimental to the preservation of DNA within the fingerprint residue. For example, black powder may degrade DNA [2,3], Crowles double stain and Hungarian red may reduce DNA amplification efficiency [9], and cyanoacrylate may reduce specific products of the polymerase chain reaction (PCR) [10]. Furthermore, airflow and exposure to UV radiation can degrade biological initiators of cyanoacrylate polymerization [11]. Thus, many traditional fingerprint-development techniques have the potential to inhibit downstream DNA analysis [2,9,11,12]. In contrast, the columnar-thin-film (CTF) technique borrowed from nanotechnology involves the deposition of a 50–1000-nm-thick CTF conformally on a latent fingerprint [13,14]. The CTF entombs the fingerprint residue and may thereby preserve DNA in the residue. As CTF deposition occurs in a low-pressure chamber the biological material may be altered or depleted [15]. However, recent study indicates that biological material such as blood and saliva are not degraded by the CTF deposition [16].

In order to overcome difficulties in typing degraded, inhibited, and low-template DNA, often referred as low-copy-number (LCN) DNA, amplification kits such as the AmpFlSTR® MiniFiler™ PCR Amplification kit have been developed [7]. With these kits which generate amplicons of less than 270 base pairs (bp), it is possible to amplify extracts that may contain degraded DNA as well as inhibitors of the polymerase chain reaction (PCR). The results obtained from partial DNA profiles can be combined with those obtained with miniSTRs for better discrimination. However, when samples contain very low amounts of DNA, amplification of extracts in duplicates can consume the samples, leaving no possibilities for opposing counsel to repeat the tests. Also, in forensic laboratories every amplification kit used on casework samples must be validated, which leads to budgetary constraints and loss of valuable time. Recent research has employed the use of a standardized degraded DNA sample to help in determining critical parameters for comparing different kits [17].

As mentioned at the beginning of this section, the main objective of this research was to compare the efficacy of the two recently developed DNA quantification systems: Quantifiler® Trio and InnoQuant™ kits. Both of these kits, which use a quantitative PCR (qPCR) assay, can assess the amount of human DNA and can also determine the level of DNA degradation, thus providing guidelines for more adequate downstream STR analysis [18,19]. Most of the samples chosen for the comparative study were latent fingerprints that were either (i) developed using one of three different techniques and then aged in ambient conditions or (ii) undeveloped but still aged. Furthermore, in order to properly evaluate the differences in their performance, manually degraded samples were produced by controlled exposure of DNA standards to UV radiation.

The extent of degradation is determined by comparing the amount of a short strand of amplified DNA with the amount of a long strand of amplified DNA to obtain a DNA degradation ratio. Some researchers have shown that the use of one short DNA target and one long DNA target demonstrates a positive relationship between an increasing DNA degradation ratio and a loss in longer STR alleles [20–23]. Others have implemented this technique with the use of an internal positive control or Y chromosome target [20,21]. Different quantification kits available in the forensic community utilize different targets for the same assessment [18,19,24]. The use of different DNA targets in different qPCR systems may lead to different results for the sensitivity of DNA quantitation, the extent of DNA degradation, and the level of inhibitors present. The differences in the origin of these targets in the human genome may lead to slight differences in the values obtained by the quantification procedure. Since these quantification kits are used for comparison of the amounts of the short amplicon with that of the long amplicon, differences in the length of the long amplicon may lead to different degradation ratios.

2. Materials and methods

2.1. Samples degraded manually by UV exposure

2.1.1. DNA standards

Standard samples used for the manual-degradation study included 9947a, control DNA in the amplification kits from Life Technologies (Oyster Point, CA, USA), concentration 0.10 ng/μL and 2800 M (Promega Corporation, Madison, WI, USA), concentration 10 ng/μL. These two DNA standards with different concentrations, one low and other high, were chosen in order to examine the degradation patterns when DNA is present either as low-template DNA (such as in fingerprints) or in larger quantities (such as in bloodstains or saliva samples).

2.1.2. Manual DNA degradation

Manually degraded DNA samples were generated using a PCR workstation (CBS Scientific Co., Del Mar, CA, USA). The UV source installed in the workstation was a germicidal 15-W lamp (G15T8, General Electric, Schenectady, NY, USA) with a 4.9-W ultraviolet (254 nm) output. The workstation was sterilized by having the interior surface exposed to UV radiation for 2 h prior to starting the experiments.

A defined volume of each standard (10 μL) was transferred into a sterile 0.5 mL centrifuge tube, exposed to UV radiation, and taken out of the hood in 10-min intervals from 0 min to 60 min. The lids of the tubes were left open during the exposure and the tubes were placed so that the samples were facing upright when they were sitting in the rack. There was no obstruction between the bulb and the sample. Exposure to UV radiation was not performed in an ice bath. However, the sample volumes were measured before and after exposure, and it was determined that no considerable evaporation took place. The distance between the UV lamp and the samples of DNA standards was approximately 2 ft. The process was repeated three times with both DNA standards, resulting in a total of 42 samples. Samples designated as "0-min" were not exposed to UV radiation.

2.1.3. DNA quantification and assessment of DNA degradation

The Quantifiler® Trio and InnoQuant™ kits were used for quantitation of human DNA and for determining the extent of DNA degradation. The Quantifiler® Trio kit is a four-target system amplifying 80 bp (short) and 214 bp (long) amplicons specific for human DNA, a 75 bp amplicon specific for human male DNA, and a 130 bp amplicon from synthetic template as a positive control [25]. The InnoQuant™ kit, on the other hand, is a three-target system comprising 80 bp (short) and 207 bp (long) amplicons specific for human DNA and 172 bp amplicon from synthetic template as a positive control [26]. While both kits use multi-copy elements, the InnoQuant™ kit uses mobile elements Yb8 Alu and