Nuclear and mitochondrial DNA quantification of various forensic materials

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Abstract

Due to the different types and quality of forensic evidence materials, their DNA content can vary substantially, and particularly low quantities can impact the results in an identification analysis. In this study, the quantity of mitochondrial and nuclear DNA was determined in a variety of materials using a previously described real-time PCR method. DNA quantification in the roots and distal sections of plucked and shed head hairs revealed large variations in DNA content particularly between the root and the shaft of plucked hairs. Also large intra- and inter-individual variations were found among hairs. In addition, DNA content was estimated in samples collected from fingerprints and accessories. The quantification of DNA on various items also displayed large variations, with some materials containing large amounts of nuclear DNA while no detectable nuclear DNA and only limited amounts of mitochondrial DNA were seen in others. Using this sensitive real-time PCR quantification assay, a better understanding was obtained regarding DNA content and variation in commonly analysed forensic evidence materials and this may guide the forensic scientist as to the best molecular biology approach for analysing various forensic evidence materials.

Keywords: Quantification; Real-time PCR; Forensic materials; Hair; Nuclear DNA; Mitochondrial DNA

1. Introduction

Forensic DNA analyses have been successfully performed on a wide variety of biological evidence materials, such as shed hairs [1–3], saliva stains [3–5], latent fingerprints [6,7] and epithelial cells on various objects [8,9]. However, DNA content found in different biological materials analysed in forensic investigations varies substantially. Furthermore, biological evidence materials found at a crime scene are sometimes degraded or yield insufficient quantities of DNA to enable a successful autosomal STR analysis. These samples are often analysed by sequencing of mitochondrial DNA (mtDNA) due to its high copy number per cell [10] or by low copy number (LCN) typing [11]. Although an mtDNA analysis has a higher success rate compared with autosomal STR analysis, analysis of multiple unlinked autosomal STR markers results in higher discrimination power. A sensitive and accurate DNA quantification assay is therefore essential in order to evaluate
whether a sample contains sufficient DNA for analysis of nuclear DNA. Otherwise, the sample could be consumed such that mtDNA analysis would become ineffective.

The DNA quantification methods used prior to the development of real-time quantification were often not sensitive enough for the trace amounts of DNA present in the types of forensic materials encountered today [12,13]. Consequently, it has not been possible to predict based on quantity the success rate of an analysis of either mtDNA or LCN nuclear DNA, for a particular evidence material. We have previously developed a multiplex quantification assay based on the real-time 5′-exonuclease detection method (TaqMan®) for copy number estimation of both mtDNA and nuclear DNA (nDNA) [14]. This quantification assay can be used to evaluate the DNA content of various common forensic materials and provide guidance of how DNA content varies between different types of biological samples as well as within the same category of materials. Following this, other real-time PCR assays have been developed for use in forensic genetic analysis [15–18].

In this study, we evaluated the DNA content of common types of evidence materials using the previously described quantification assay [14]. Both mtDNA and nDNA copy numbers were determined in the root portion of shed and plucked head hairs while the quantity of mtDNA was estimated in the more distal parts. The quantity of nDNA was determined in body hairs and epithelial cells recovered from accessories (such as rings, watches, necklaces/bracelets, glasses, charms, and earrings), and the quantity of both mtDNA and nDNA was determined in epithelial cells recovered from fingerprints.

2. Materials and methods

2.1. Sample collection and DNA extraction

Shed and plucked head hairs were collected from 24 individuals. Some individuals donated one shed and one plucked hair, while some donated a single hair of either type. In total, 12 shed and 22 plucked hairs were collected. For analysis of intra-individual variation, five additional plucked hairs were collected from two individuals, resulting in a total of 32 plucked hairs. To avoid contamination the hairs were self-collected and placed in an envelope. The hairs were measured for length and thickness using a ruler and a micrometer. Beginning 1 cm from the root end, the hairs were cut into 3 cm pieces along the whole length of the hair. The first part of the hair, including the root, was 1 cm in length. Each hair segment (1 or approximately 3 cm in length) served as a source for DNA. Body hairs were plucked from 17 individuals, and the entire hair was extracted for DNA. From 14 individuals, 2 hairs were taken from the arm, and 2 from the eyebrow. Three additional individuals contributed with one arm hair each. In total 31 arm hairs and 28 eyebrow hairs were analysed. From 6 male donors, 2 beard hairs were taken.

DNA was extracted from the different segments of hair in 200 μl volumes containing 1 × Gene-Amp® PCR Buffer II (Applied Biosystems, Foster City, CA), 0.25–0.5 mg/ml proteinase K (Sigma, St. Louis, MO) and 35 mM DTT (Sigma). The samples were incubated at 56 °C for 1–2 h, followed by proteinase K inactivation at 95 °C for 10 min. The final DNA extracts were stored at −20 °C [3,19].

DNA was extracted from fingerprints deposited on paper by a single individual, of which seven were visualised using magnetic black powder and five using non-magnetic black powder. The area of the fingerprints was measured, and the entire print was cut into pieces for DNA extraction. DNA was collected from different accessories worn by 23 individuals. Among these items were 10 rings, 9 watches, 28 glasses, 5 charms, 8 earrings and 4 necklaces/bracelets. Epithelial cells were collected by swabbing (swabs moistened with 1% SDS) of approximately a 1 cm² area of each item. DNA collected on cotton swabs or paper was extracted using the Wizard® Genomic DNA Extraction Kit (Promega, Madison, WI, USA). Extracted DNA was re-suspended in a volume of 100 μl.

2.2. Nuclear and mitochondrial DNA quantification

The quantification assay was carried out as previously described [14]. In brief, the amplification was performed in a 25 μl volume containing 5 μl DNA extract, 1 × TaqMan Universal PCR Master Mixture (Applied Biosystems), 400 nM of each primer (mt-8294F and mt-8436R or RB1-2672F and RB1-2750R), and 200 nM probe (mt-8345 or RB1-2727). The mtDNA probe (mt-8345) is VIC labelled at the 5′-end, and the nDNA probe (RB1-2727) is FAM labelled. The probes are also labelled with the quencher TAMRA at their 3′-ends. An ABI PRISM 7700 Sequence Detector (Applied Biosystems) was used for amplification (hold for 2 min at 60 °C then 10 min at 95 °C followed by 50 cycles of 95 °C for 15 s and 60 °C for 1 min) and detection. Copy number calculations (threshold always set to 0.1) were performed using the Sequence Detection System software, version 1.6.3 (Applied Biosystems).

For mtDNA quantification, a plasmid clone containing a 613 bp mtDNA insert (8049–8661) was used to generate a quantification standard curve [20]. DNA from the Burkitt lymphoma cell line (Applied Biosystems) was used for the nDNA standard curve. The nDNA quantification standard curve was produced assuming that 1 ng DNA corresponds to 333 genome equivalents. A typical run, conducted in a 96 well format, consisted of a standard serial dilution (ranging from 0.1 to 10⁷ DNA copies) in duplicate. The unknown samples were distributed over the plate to avoid cross contamination and one no template control was analysed for each unknown sample. In total, 18 unknown samples were run in each real-time PCR quantification setup. After analysis, only runs with negative no template controls were evaluated.
To further evaluate the DNA typing success in relation to the quantification data, a subset of hairs were analysed using the HVI/HVI1 mtDNA Linear Array Assay (Roche Applied Science, Indianapolis, IN), as described by Divne et al. 2005 [21].

3. Results

In this study, we utilised a previously described multiplex system for mtDNA and nDNA quantification [14]. The assay can be performed successfully in a multiplex reaction by limiting the primer concentration for the majority species, mtDNA. However, singleplex reactions were used for mtDNA and nDNA quantification of the different materials because maximum PCR efficiency for both targets was a higher priority than preserving DNA material in this study. Quantification of mtDNA was performed along the lengths of shed and plucked head hairs as well as on fingerprint samples. Nuclear DNA quantification was performed on the first centimeter (including the root) of plucked head hairs, the first centimeter of shed head hairs, body hairs and samples collected from fingerprints and accessories.

The mtDNA quantification of 12 shed head hairs revealed that the first centimeter contained on average 45,700 mtDNA copies (S.D. = 44,900). The next two adjacent segments (1–4 and 4–7 cm) contained on average 20,900 and 26,600 mtDNA copies per centimeter (S.D. = 35,600 and 44,000), respectively. The average size, 2.9 and 3.0 cm of the segments (column 3 in Table 1) were used for the calculations of average copy number per centimeter. In the first centimeter of the shed hairs, all hairs contained more than 1000 mtDNA copies and two samples had more than 100,000 copies. In the following two pieces, most samples contained between 10,000 and 100,000 mtDNA copies. None of the 12 shed hairs showed detectable nuclear DNA copies in the first centimeter part, and thus more distal parts were not tested for nDNA content (Table 1).

The mtDNA quantification of 32 plucked head hairs revealed that the first centimeter contained on average 45,700 mtDNA copies (S.D. = 44,900). The next two adjacent segments (1–4 and 4–7 cm) contained on average 20,900 and 26,600 mtDNA copies per centimeter (S.D. = 35,600 and 44,000), respectively. The average size, 2.9 and 3.0 cm of the segments (column 3 in Table 1) were used for the calculations of average copy number per centimeter. In the first centimeter of the shed hairs, all hairs contained more than 1000 mtDNA copies and two samples had more than 100,000 copies. In the following two pieces, most samples contained between 10,000 and 100,000 mtDNA copies. None of the 12 shed hairs showed detectable nuclear DNA copies in the first centimeter part, and thus more distal parts were not tested for nDNA content (Table 1).

As expected, the mtDNA content in plucked head hairs was higher than in shed hairs, both when comparing hair fragments and complete hairs. Nuclear DNA quantification revealed that the first centimeter (the root part) contained on average 25,800 nDNA copies and 50% of the hairs (11/22) contained more than 10,000 nDNA copies (Table 1).

To further investigate inter-individual variation, a comparison of mtDNA content in seven shed and seven plucked hairs from different individuals was made. The first centimeter of the shed hairs contained between 5100 and 96,500 mtDNA copies (mean = 30,025, S.D. = 32,000) while plucked hairs showed between 212,400 and 15,501,400 mtDNA copies (mean = 3,715,300, S.D. = 5,459,700). Thus, the variation of mtDNA copies contained within the first centimeter of shed hairs from different individuals ranges more than one order of magnitude, while the range in plucked hairs is almost two orders of magnitude. In the next two adjacent segments (1–7 cm), the mtDNA copies per centimeter varied between 1000 and 30,700 (mean = 10,700, S.D. = 8,700) in shed hairs and between 2600 and 43,200 (mean = 21,100, S.D. = 12,400) in plucked hairs. This indicates that a large variation of mtDNA content exists among the hairs of different individuals, and the variation in mtDNA content among both hairtyypes spans three orders of magnitude (Fig. 1).

Intra-individual variation was evaluated by comparison of mtDNA content in five plucked hairs from each of two individuals (A and B). In the first centimeter of the hair, mtDNA copy numbers were measured between 435,700 and 5,283,600 (mean = 1,811,900, S.D. = 1,985,400) for individual A, and for individual B between 834,800 and 27,295,800 (mean = 10,056,800, S.D. = 12,694,500). Individual B showed a slightly higher average mtDNA copy number in the first centimeter of the hairs but lower average copy number in the distal parts (three segments), compared to individual A. The variation in the first centimeter of the hair of individual A is 12-fold, while the variation in shaft parts (7–10 cm) is between 5- and 8-fold. The variation for individual B is 33-fold in the first centimeter and 2- to 4-fold in the following parts (Fig. 2). To further illustrate the variation seen among a larger number of hairs, a comparison of the measured mtDNA content in the three first segments (0–1, 1–4 and 4–7 cm) of all shed and plucked head hairs is shown in Fig. 3. Even though there are large differences within the groups, a consistent trend of a decrease in DNA copy number towards more distal parts of the hair is observed. In general, a higher average mtDNA copy number is observed in plucked compared to shed head hairs, but a few plucked hairs did contain less mtDNA than any of the shed hairs at the corresponding length.

The body hairs were plucked and DNA was extracted from their entire lengths. In the arm hairs (N = 31, length = 6–24 mm) an average of 13,700 nDNA copies (S.D. = 18,700) were measured. Eyebrow hairs (N = 28, length = 2.5–17 mm) contained an average of 38,100 nDNA copies (S.D. = 68,700), while beard hairs (N = 12,
Table 1
Summary of mtDNA and nDNA quantification results of a variety of forensic evidence materials

<table>
<thead>
<tr>
<th>Type</th>
<th>Sample</th>
<th>Size (average)</th>
<th>Mitochondrial DNA copy number per sample</th>
<th>Nuclear DNA copy number per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0–1000 – 10000 – 100000 – 1000000 – 10000000</td>
<td>0–1000 – 10000 – 100000 – &gt;100000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average</td>
<td>S.D.</td>
</tr>
<tr>
<td>Shed head hair&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0–1 cm</td>
<td>10 mm</td>
<td>3 7 2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>1–4 cm</td>
<td>29 mm</td>
<td>11 1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>4–7 cm</td>
<td>30 mm</td>
<td>1 4 1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>7–10 cm</td>
<td>30 mm</td>
<td>2 3</td>
<td>5</td>
</tr>
<tr>
<td>Plucked head hair&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0–1 cm</td>
<td>10 mm</td>
<td>1 5 9 13 4</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>1–4 cm</td>
<td>31 mm</td>
<td>1 15</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>4–7 cm</td>
<td>30 mm</td>
<td>2 12 9</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>7–10 cm</td>
<td>33 mm</td>
<td>13 6</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>10–13 cm</td>
<td>35 mm</td>
<td>4 2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>13–16 cm</td>
<td>30 mm</td>
<td>2 1 3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>16–19 cm</td>
<td>30 mm</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>19–22 cm</td>
<td>30 mm</td>
<td>2</td>
<td>2</td>
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<tr>
<td></td>
<td>22–25 cm</td>
<td>30 mm</td>
<td>2</td>
<td>2</td>
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<tr>
<td></td>
<td>25–28 cm</td>
<td>30 mm</td>
<td>1</td>
<td>1</td>
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<tr>
<td></td>
<td>28–31 cm</td>
<td>30 mm</td>
<td>1</td>
<td>1</td>
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<tr>
<td></td>
<td>31–34 cm</td>
<td>30 mm</td>
<td>1</td>
<td>1</td>
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<tr>
<td></td>
<td>34–37 cm</td>
<td>30 mm</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Body hairs&lt;sup&gt;e&lt;/sup&gt; (plucked)</td>
<td>Arm hair</td>
<td>14 mm</td>
<td>3 10 12</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Eyebrow</td>
<td>7 mm</td>
<td>5 6 12 3</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Beard</td>
<td>5.5 mm</td>
<td>1 8 3</td>
<td>12</td>
</tr>
<tr>
<td>Fingerprints&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Magnet powder</td>
<td>3 cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1 5 1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Black powder</td>
<td>2 cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3 2</td>
<td>5</td>
</tr>
<tr>
<td>Accessories</td>
<td>Rings</td>
<td>1 cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6 3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Watches</td>
<td>1 cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1 6 2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Necklaces/Bracelets</td>
<td>1 cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Glasses</td>
<td>1 cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1 5 20 3</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Charms</td>
<td>1 cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3 2 6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Earrings</td>
<td>1 cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1 2 1 2 2</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> The last segment of each hair was not exactly 3 cm resulting in variable average lengths.
<sup>b</sup> The 32 plucked head hairs, 0–1 cm, consist of samples from 22 different individuals and 2 additional individuals from the intra-individual study (5 × 2 hairs).
<sup>c</sup> Only the 0–1 cm segment was quantified for nDNA.
<sup>d</sup> Note that this value is based on 10 mm long hairs compared to the other segments of approximately 30 mm for shed and 31 mm for plucked head hairs.
<sup>e</sup> Fingerprints and body hairs were quantified in duplicates.
length = 3–11 mm) had 78,000 nDNA copies (S.D. = 112,600) on average. Thus, the beard hairs contain more DNA than the first centimeter of plucked head hairs (mean = 25,800 nDNA copies for plucked head hairs) despite their shorter average length (5.5 mm). Eyebrow hair DNA content was comparable, and in contrast, the arm hairs contain less nDNA compared to the first centimeter of plucked head hairs (Table 1).

The fingerprint samples were tested for both mtDNA and nDNA content. The magnetic black powder treated fingerprints (N = 7) contained an average of 7300 mtDNA and 90 nDNA copies (S.D. = 6200 and 80, respectively). The black powder treated prints (N = 5) revealed on average 12,000 mtDNA and 170 nDNA copies (S.D. = 10,700 and 290, respectively). Thus, in this study fingerprints visualised using black powder contained somewhat more DNA in comparison to prints visualised by magnetic black powder (Table 1).

DNA samples collected from accessories worn by different individuals were tested for nDNA content. Samples collected from rings contained on average 180 nDNA molecules (S.D. = 400, 580 and 110, respectively). Greater DNA quantities were detected in samples from watches and glasses with an average of 1800 and 4200 nDNA molecules (S.D. = 2900 and 6000), respectively. Finally, the highest DNA amounts were observed in samples from earrings with an average of 144,400 nDNA copies (S.D. = 336,800) (Table 1).

To evaluate mtDNA typing success in relation to the quantification data, a subset of hairs were analysed using the HVI/HVII mtDNA Linear Array Assay [21]. One hair was mtDNA typed for all segments through the entire 34 cm long hair (Fig. 4a). Four other hairs (two shed and two plucked) were typed for the three segments between 1 and 10 cm of the hair (Fig. 4b). In general, a decreased signal intensity from the positive probes were observed in the most distal hair segments, in agreement with lower amount of DNA.

4. Discussion

In this study, real-time DNA quantification of mtDNA and nDNA copy number was performed on various materials that are often subject to forensic DNA analysis. Shed hairs

![Fig. 1](image1.png)  
Inter-individual hair analysis. Comparison of mtDNA content at different lengths in seven shed (−) and seven plucked (●) head hairs from different individuals.

![Fig. 2](image2.png)  
Intra-individual hair analysis. Comparison of mtDNA content at different lengths in five plucked head hairs from individual A (−) and five plucked head hairs from individual B (●).

![Fig. 3](image3.png)  
Illustration of variation in mtDNA content between shed and plucked head hairs at different lengths.
(usually in telogen phase) are most often, and plucked hairs are occasionally, found at a crime scene [2]. As hairs are frequently used in forensic DNA analysis, the study herein focused primarily on evaluating the DNA content in shed and plucked head hairs as well as other types of hairs. In addition, the amount of DNA isolated from a few other categories of evidence samples was evaluated.

Although slightly less intra-individual variation was observed compared to inter-individual variation, the overall variation of hair DNA content was found to be large. The difference in the average mtDNA content in the first centimeter between plucked and shed hairs was 77-fold. The first centimeter of plucked hairs contained an average of 25,800 nDNA copies while no nDNA copies were detected in the first centimeter of shed hairs. Variation in mtDNA content was further evaluated along the length of the hairs. Overall, a decrease in DNA content was seen in more distal hair segments. However, slightly higher average mtDNA content was detected in the third segment (4–7 cm) for shed head hairs, and the seventh (16–19 cm) for plucked hairs. This result is probably due to a larger impact of inter-individual variation with decreasing number of quantified segments. The decrease in average mtDNA content per centimeter was 2-fold between the first centimeter and the following 1–4 cm part of shed hairs. In contrast, an 87-fold decrease in the average mtDNA content per centimeter was observed between the first centimeter and the following 1–4 cm of plucked hairs. Thus, most of the DNA content difference between shed and plucked hairs

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**Fig. 4. HVI/HVII mtDNA Linear Array Assay of mtDNA quantified hairs.** The quantification data reflect the number of input mtDNA copies to PCR. (a) Analysis of a 34 cm long plucked hair, divided in 12 segments. The first segment is 1 cm and includes the root. The following segments are 3 cm long each. The presence of polymorphisms resulting in “blank” results for some regions was confirmed by Sanges sequencing (b) Analysis of hairs from four different individuals, two shed (S) and two plucked (P) head hairs. Three segments, ranging from 1 to 10 cm, have been analysed for each individual.
is observed in the first centimeter. The first centimeter of shed hairs contain approximately equal average mtDNA amounts per centimeter as the second segment (1–4 cm) of plucked head hairs (45,700 and 40,200 mtDNA copies per centimeter, respectively). The large difference between DNA content in shed and plucked hairs is likely to be a consequence of the different growth phases of hair. Hair growth stages are divided into anagen, catagen and telogen phases, characterised by different number of living cells in the bulb of the root. While most plucked hairs are in anagen or catagen phase and are rich in living cells, naturally shed hairs are in most cases in the telogen resting phase in which breakdown of DNA is progressing and contain few if any follicular sheath cells [22,23]. Also the large inter- and intra-individual variation, especially in the first centimeter of plucked hairs, is likely to reflect the different growth phases of the analysed hairs. Plucked hairs could be near the telogen phase, offering a possible explanation for negative results obtained in nDNA quantification of the root part of plucked hairs. Of the different types of body hairs evaluated, beard contained on average 2- and 6-fold more nDNA compared to eyebrows and arm hair, respectively.

In the quantification analysis of DNA content of fingerprints visualised by two different methods, the black powder treated prints contained slightly more mtDNA and nDNA compared to magnetic black powder treated prints. The results illustrate that some prints contain up to 700 nDNA copies and should be sufficient for autosomal STR typing. Moreover, the majority of the prints contain a sufficient amount of DNA for mtDNA analysis. DNA extracted from fingerprints can therefore be used for DNA analysis where the prints are smeared or partial, thereby preventing identification by traditional fingerprint analysis. The quantification analysis of DNA collected from accessories showed that earrings contained the most recoverable DNA, at 144,400 nDNA copies on average, while samples taken from rings, necklaces/bracelets contained the lowest amounts of DNA at 80–300 nDNA copies on average. Large variations were seen in DNA content between samples taken on the same category of item as well as between different categories. The DNA content in these types of samples is likely to be dependent on where on the body the item has been worn and for how long it has been in contact with the skin. However, the DNA content deposited on these types of materials, as well as in fingerprints, is also likely to be influenced by the donor depending on if he/she is a good or a poor shedder of DNA [24].

It is important to keep in mind that the obtained quantification results are highly dependent on the PCR efficiency in the assay. In this study, all measurements were performed using the TaqMan Universal PCR Master Mixture (Applied Biosystems) without any efficiency enhancing additives. However, when quantifying a subset of samples included in this study with the addition of the inhibitor counteracting agent BSA (0.16 mg/ml), a 2- to 60-fold increase in copy numbers was seen. It is therefore possible that nDNA copies may be detected in the first centimeter of shed hairs under alternate extraction or PCR conditions. In fact, it has been shown that STR typing of DNA from telogen shed hairs is possible using short PCR amplicons [25]. Reducing the amplicon size for STR analysis has proven that correct genotypes are obtained in all replicates (10/10) containing 100 pg (33 copies), and a majority of replicates (9/10) containing 50 pg of DNA [26]. Furthermore, an analysis of a subset of samples using the Platinum® Quantitative PCR SuperMix-UDG (Invitrogen, Carlsbad, CA) showed no difference in DNA copy numbers compared with the use of TaqMan Universal PCR Master Mixture (Applied Biosystems). In a forensic casework investigation, it is important to understand the methodology used to quantify the DNA samples to make the most judicious selection of DNA typing approach. The Taqman system is PCR-based and thus approximates the amplification phases of mtDNA and nDNA typing targets. However, one should bear in mind that the efficiency of the amplification site (primers and target) may vary as well.

Using the AmpF/STR® Identifier® PCR Amplification Kit and PowerPlex® 16 System kit (Applied Biosystems, Promega), autosomal STR typing can be performed in routine forensic DNA analysis with at least 0.25 ng of genomic DNA, corresponding to approximately 85 genome equivalents. Using this threshold for guaranteed successful analysis, 95% (21/22) of the plucked head hair roots from different individuals, none of the 12 shed head hairs, 80% (57/71) of the body hairs, 77% (49/64) of the accessories and 42% (5/12) of the fingerprints in this study would be suitable for routine STR analysis. Amplification of less genomic DNA than required for a routine STR analysis is possible with LCN approaches, in which typically a higher number of PCR cycles in combination with careful interpretation of results are applied. The limit of LCN analysis is 25–50 pg of genomic DNA [11], corresponding to approximately 8–16 genome equivalents. While the stochastic effects are greater, under these circumstances, an additional six samples (three body hairs, two fingerprints and one accessory) in this study could possibly have been analysed. Of all samples quantified for nDNA in this study, 24% (43/181) did not contain any detectable copies and would thus not be suitable for nDNA analysis. The quantification of mtDNA revealed that 92% (11/12) of the fingerprints contained more than 1000 mtDNA copies. The majority (33/35) of the shed hair segments contained more than 1000 mtDNA copies in the different segments of a hair shaft between 0 and 10 cm. Also, the majority (99/104) of the plucked hair segments contained more than 1000 mtDNA copies between 1 and 37 cm. Thus, most of these shed and plucked hair segments have sufficient DNA to yield successful mtDNA results. A high success rate in mtDNA analysis was further confirmed by analysis of a subset of hairs using the HV1/HV1 mtDNA Linear Array Assay. In previous studies, the success rate of mtDNA analysis in telogen hairs has been reported to be around 70% and it has been suggested that melanin could have an impact as PCR inhibitor [27–29]. As all the different
segments of most of the shed hairs contain substantial amounts of mtDNA, dividing longer hairs in half preserves a second portion of the hair sufficient for re-testing. Balancing the probability of obtaining a profile as well as conserving as much sample as possible is an important consideration in mtDNA analysis [30]. The analysis of a subset of hairs with the HV1/HVII mtDNA Linear Array Assay illustrated results in concordance with the real-time PCR quantification data in this study.

Recently, Walker et al. described a multiplex quantification assay for autosomal, Y chromosome, and mtDNA [31]. They criticised other quantification assays because they were not human specific and our method in particular for the possible cross reactivity with mtDNA pseudogenes residing in the nuclear genome. At the time our quantification assay was designed this particular chromosome 1 sequence was not available. In the second release of the human consensus sequence, an mtDNA homologous region with the target used in our assay was discovered on chromosome 1 (gi[29791381] and gi[51511461]). Such a requirement and concern of no cross-reactivity with nuclear pseudogenes for an mtDNA quantification assay is fallacious. First, there has been continuous transfer of mtDNA genes to the nuclear genome since the inception of the eukaryote. Thus, it is likely that at least one copy of a portion of every region of the human mtDNA genome resides as a pseudogene in the nuclear genome [32,33]. Therefore, the criterion for no cross-reactivity would likely reject all mtDNA quantification assays and for no apparently good reasons. Assuming that each cell contains 1000 mtDNA copies and that the target site for a quantification assay has complete homology with a pseudogene, the impact would be detection of 1002 rather than 1000 mtDNA copies per cell [10]. Moreover, for some tissues the nuclear pseudogene is even less relevant. The data herein show that little or no nuclear DNA is detected in hair shafts. For hairs, the miniscule two extra nuclear copies do not apply. Thus, this phenomenon does not have an effect on the copy number estimation in all but the most contrived of forensic evidence scenarios. The assumption, however, was that the primer binding and probe target sites of the real-time PCR assay have complete homology. While in our quantification assay there is complete homology for both primers and the probe, for most assays, such as that reported by Walker et al., a few sites will differ. There typically are less selective constraints on pseudogenes than on functional sites. Thus, it is likely that the efficiency of amplification is less on a copy-by-copy basis for many pseudogenes. Regardless, there is no basis for concern for the practical consequences when quantifying mtDNA because of the possible presence of a nuclear pseudogene.

In general, the growing sequence information in genomic databases is likely to reveal similar scenarios with target or species specificity in other developed assays. An example of greater consequence is illustrated by the mtDNA quantification system described by Walker et al. [31]. The ultimate 3' position in the Walker et al. forward PCR primer is position 8473. An analysis of a large collection of mtDNA coding region sequences (N = 2064) revealed that position 8473 is polymorphic (www.genpat.uu.se/mtDB). In 51 out of 2064 individuals, a C nucleotide is present at position 8473. As this polymorphism is located at the very 3' end of the primer the amplification efficiency and consequently the quantification results are likely to be affected in samples with this polymorphism. This position was also used to demonstrate human specificity. There will always be some variation in the population regarding any target in the mtDNA, and this is unavoidable. But, performing population surveys can be useful in avoiding regions where variation is such that a notable percentage of samples would fail to yield a result even though sufficient DNA was present. To resolve their deficiency, Walker et al. might consider moving the primer a base or two 5' distal to the current position or add a redundant primer.

In conclusion, the use of real-time DNA quantification in this study has revealed several important insights regarding DNA content in various forensic materials. Information regarding inter- and intra- individual variation, variation in DNA content within plucked and shed hairs at different lengths, the average DNA content in different types of hairs as well as in other materials is highly informative for intelligent design in forensic applications.

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References