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Research article

The visualization and quantification of cell nuclei in telogen hair roots by fluorescence microscopy, as a pre-DNA analysis assessment

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Abstract

Although nuclear DNA-profiling of human hairs is a well-known technique in forensic investigations, its success rate is quite low. Because the extracted nuclear DNA (nuDNA) is scarce and often degraded, a simple and effective method was developed to estimate the number of cell nuclei in telogen roots. DAPI, a fluorescent, non-destructive DNA-stain, allows visualizing nuclear DNA and does not interfere with subsequent PCR analyses. After staining 3242 roots from 27 volunteers and subsequent STR-profiling of a selection of roots, we show that the amount of analysable nuDNA can be predicted. This screening method allows the genetic laboratory to analyse only the most promising hair roots.
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Keywords: Telogen hair; DAPI; Fluorescence microscopy; Short tandem repeat

1. Introduction

For more than 10 years our lab has gained experience in hair analysis for forensic purposes. Since morphological hair analyses are sometimes not discriminating enough [1,2], a subsequent DNA typing is usually performed. The success rate of STR-profiling is low [3,4], especially with telogen hair roots, so an efficient pre-DNA assessment would be useful. We developed a method to improve the selection of interesting hairs for STR-analysis. Using DAPI, a fluorescent molecule binding to double-stranded DNA, cell nuclei present in hair roots were visualized. The amount of analysable nuDNA in the root could be predicted. This new information will support the choice between nuclear or mitochondrial DNA analysis.

2. Methods

Twenty-seven volunteers collected naturally shed hairs, of which 3242 telogen hairs were isolated for this study. With the informed consent of 14 volunteers, 134 of the selected hairs with visible nuclei were afterwards submitted to nuclear DNA analysis. Their STR-profile, needed as a reference, was obtained from a buccal scrape.

2.1. Old screening method

The hair roots are examined under a bright light stereomicroscope at 20–80× magnification and divided according to their development stage. The telogen roots are subdivided in three types: (1) without germinal tissue (2572 hairs), (2) with a small amount of germinal tissue (309 hairs), and (3) with a large amount of germinal tissue (361 hairs) [5]. Assuming that only the germinal leftovers around the telogen roots contain analysable nuDNA, only these hairs have been submitted to the laboratory for genetic analysis. The type 1 telogen roots (most common) have not been submitted to STR-profiling, because no nuDNA was thought to be present and subsequent nuclear analysis would be negative [6].

2.2. New screening method

After the root stage determination (Section 2.1), the 3242 hair roots are incubated overnight at room temperature in the DAPI reagent: 160 μg DAPI (4′-6-diamidino-2-phenylindole) and 224 mg DABCO (1,4-diazabicyclo(2.2.2)octane) in 1 ml Tris–HCl 0.20 M, pH 7.4 mixed with 9 ml glycerol. The cell

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Table 1
Distribution of hair roots (three types) for amount of visible cell nuclei

Visible cell nuclei	Without germinal tissue	With small amount of germinal tissue	With germinal tissue	Total
0–10	2341 (55)[a]	248 (49)[a]	234 (57)[a]	2828 (161)[a]
11–30	32	26	56	114
31–50	37	22	26	85
>50	162 (85)[b]	13	40	215 (85)[b]

[a] Number of hair with 1–10 visible cell nuclei.
[b] Number of epithelial sacs.

nuclei are counted using a fluorescence microscope ZEISS Axioplan-II with DAPI filter at 100–200× magnification. The DAPI molecule, after binding on DNA and being excited with UV light of 372 nm, emits a bleu coloured light of 456 nm [7]. The hair roots are then divided in four groups according to the number of visible cell nuclei (Table 1). The first group consists of hair with 10 or less visible cell nuclei in the root (mostly 0). Group 2 has between 11 and 30 visible cell nuclei, this is just below the theoretical detection limit for non-degraded DNA in our laboratory. The third group has between 31 and 50 cell nuclei, an in between group. Finally there is a group with more than 50 visible cell nuclei, theoretically enough for STR-typing (Fig. 1).

2.3. DNA typing

One hundred and thirty-four of the roots with visible nuclei were submitted to the genetic analysis laboratory. The non-rinsed samples, after organic extraction [8] were amplified for 28 cycles with the ProfilerPlus™ kit and injected for 66 s at 1 kV on an ABI 3100 sequencer. Eight DAPI negative roots without visible cell nuclei were used as blanks. Also the used DAPI solution was tested for contamination.

3. Results

The new method is fast and does not interfere with DNA extraction and amplification Contamination is a major concern, as analysis involves an extra step but can be handled. The results (Table 1) of the DAPI screening show surprisingly that 57% of the 670 telogen roots with some germinal tissue remnants did not show any fluorescent nuclei. And 7.7% of the 2572 telogen roots without any germinal tissue remnants do contain at least 30 visible nuclei.

A big increase in the DNA success rate is shown for hairs with more than 50 visible nuclei. When epithelial sacs [9] were present around the hair root, the success rate increases up to 82%, as lots of cell nuclei are present. Only one hair root of this group showed no profile. In the group with 31–50 cell nuclei, 42% shows whole or partial (at least half of the expected alleles) STR-profiles. When less than 30 visible nuclei were present in the hair roots, no profiles were obtained (4 analyses). All profiles were from the corresponding volunteer and all visible cell nuclei were present on the hair root and the tissue surrounding the root area.

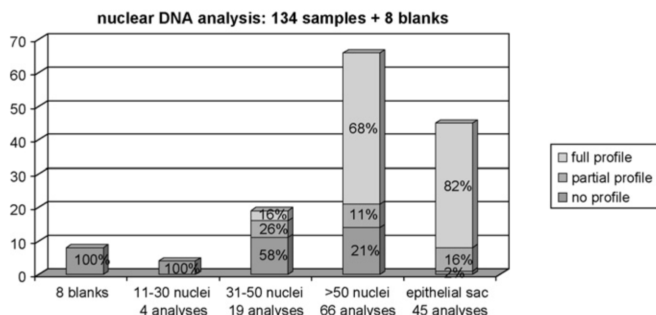

Fig. 1. Success rate of STR-analysis of telogen hair roots.

4. Discussion

Using DAPI to count the observed cell nuclei allows predicting a positive STR-analysis. This non-destructive, quick and inexpensive method will help to control or even decrease judicial costs. Our new selection step should contribute to the efficiency of nuDNA analysis of human hair. The DNA success rate could even increase with the use of shorter STR [8]. More tests are necessary to determine if cell nuclei presence is donor dependant and/or hair type dependent. For now we use the technique to select human hairs for STR-analysis, casework results are promising and analyses costs have decreased. Further development is necessary as more microtraces could be microscopically tested before DNA-analysis e.g. microscopic blood stains, fingerprints.

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