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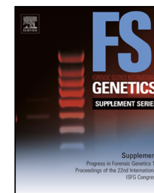

Successful direct STR amplification of hair follicles after nuclear staining

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ABSTRACT

Hairs are commonly encountered at a crime scene through natural shedding (telogen) or trauma such as during an assault (anagen). The forcible removal of anagen hairs results in retention of the root sheath and therefore the potential for DNA evidence. Telogen hairs lack a root sheath and do not provide a good source of DNA. Microscopy may be performed on all hair samples to detect whether there are cells adhering to the proximal tip to determine if there is a chance of success from subsequent DNA profiling. To aid in improving the microscopy, we report of the staining of hair roots using a range of dyes (SYBR® Green I, Diamond™ Nucleic Acid Dye, GelGreen™, EvaGreen™ and Redsafe™) and subsequent results from DNA profiling. Results showed that nuclei were visualized using all the dyes except for GelGreen™. The hairs were then directly amplified and all samples produced an STR profile that met requirements for uploading to a DNA database. The staining procedure conducted before direct amplification had no or little effect on the PCR and electrophoresis of the STR fragments. These results show that these nucleic acid binding dyes can be used as a preliminary assessment as to the viability of the sample (number of nuclei present) for STR analysis.

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1. Introduction

Hairs present at crime scenes are quite common due to people shedding approximately 75–100 hairs per day [1]. The more common type of hair found at a crime scene are telogen hairs or resting hairs which are naturally shed. It has been estimated that 95% of hairs which are collected from a crime scene are identified as telogen hairs [2]. Hairs that are in the active growth stage are known as anagen hairs. These hairs can often contain skin material attached to the root of the hair as they can only be removed by a forceful action. This material is targeted for DNA profiling. Microscopy is the primary technique used for selecting hairs for DNA profiling. Other studies however have stated the usefulness of staining hairs with various dyes to visualize the hair nuclei. These dyes include haematoxylin which binds to chromatin present within DNA and histone complexes and stains the nuclei a dark violet [2,3]. DAPI a minor groove binding dye has also been used to stain hairs to visualize the number of nuclei present to determine viability for STR profiling [4,5]. DAPI is not a highly specific DNA binding dye as it has a fluorescent signal when in the presence of detergents and other compounds. This dye only has approximately a 20 fold increase in fluorescent signal when in the presence of DNA [6,7]. This study looks at using more sensitive dyes to review their ability for staining hairs to visualize nuclei present within the root of the hairs. The dyes used in this study were SYBR® Green I (SG), Diamond™ Nucleic acid dye (DD), RedSafe™ (RS), GelGreen™ (GG) and EvaGreen™ (EG).

2. Methodology

2.1. Nuclear staining

The binding dyes were diluted in a buffer solution down to 20X (1 in 500 dilution) and then an aliquot (1 µL) was applied to the hair shafts (plucked) and viewed under a fluorescence microscope (Nikon Optiphot) using a B2A cube to filter the light.

2.2. STR amplification and analysis

The stained root fragment was placed into a 0.2 mL thin walled tube containing 10 µL of PCR master mix from the NGM SElect™ kit (Life Technologies, Vic., AUS) along with 5 µL of primer mix and 1 µL of AmpliTaq Gold® DNA polymerase. A further 9 µL of sterile water was added to make up a final volume of 25 µL. The

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Table 1
NGM SElect™ profile percentage of plucked hairs stained with DNA binding dyes and the average peak height (RFU) of profile.

	RedSafe™	Diamond™ Dye	GelGreen™	EvaGreen™	SYBR® Green
Number of alleles	30	30	30	30	21
Profile%	100	100	100	100	70
Average Peak Height (RFU)	608.8	2256	3486	2963	232.7

NGM SElect™ contains 15 STR loci plus amelogenin, the number of alleles shown was not including amelogenin.

amplification was conducted using Bio-Rad thermal cycler (Bio-Rad) using the manufacturer's protocol. A standard cycle number of 29 were used throughout the study.

Capillary electrophoresis was performed on an ABI 3130 × *L* Genetic Analyser (Life Technologies) using POP-4 polymer (Life Technologies). An aliquot of 2 μL of the amplified samples was added to a solution containing 0.5 μL of GeneSace-600 LIZ Size Standard and 9.5 μL of Hi-Di Formamide. Samples were denatured at 95 °C for 3 min. Electrophoresis was conducted at 3 kV with a 10 s injection. The data were analyzed using GeneMapper v3.2. The detection threshold was set at 50 RFU.

3. Results and discussion

Table 1 shows the number of alleles amplified from the plucked hair samples that were stained prior to STR amplification. Full profiles were obtained for all stained hairs except for SG where there was allele drop out. The average peak height shows that there was inhibition when using the SG dye as it was substantially lower when compared to the average peak heights from the other stained hairs. When stained with GG the nuclei could not be visualized due to the dye not being cell permeable. Even though a profile was obtained this dye is not suitable for nuclear staining. The other dyes should nuclei staining and therefore could be used as a presumptive test for determining the viability of the sample for DNA analysis.

4. Concluding remarks

Full DNA profiles were obtained after nuclear staining with RS, DD, GG and EG. Ther was allele drop out when staining with SG resulting in only a 70% profile. This would likely be due to the inhibiting nature of SG in the amplification step. This study has shown the ability to stain hair follicles with DNA binding dyes with successful direct amplification of STR loci and the ability for these dyes to be used as a screening methodology prior to STR analysis.

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Conflict of interest

None.

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