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TECHNICAL REPORT

Successful direct amplification of nuclear markers from a single hair follicle

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Abstract We report on successful amplification of DNA profiles from a single hair. Direct amplification was used on the root tip of both anagen and telogen hairs using a kit to amplify 15 STR loci. All 30 anagen hairs tested from five different people gave full DNA profiles after 29 cycles with no allelic drop-in or heterozygous imbalance. Six of the 30 telogen hairs tested resulted in a full DNA profile, and a further four telogen hair samples tested produced a DNA profile of five or more complete loci that could be uploaded to the National DNA Database (Australia). A full DNA profile was also obtained from the shaft of an anagen hair. Current practice for many laboratories is that a single hair may not be subjected to DNA testing as there is little chance of success, hence this 100 % success rate from anagen hairs is a significant advancement. A full DNA profile was obtained from a 5 year-old single hair illustrating the success when using direct PCR rather than attempting an extraction prior to the amplification step. The process described deliberately uses current DNA profiling methods with no increase in cycle number, such that the methodology can be incorporated readily into operational practice. For the first time in the field of human identification, single hairs can be analyzed with confidence that a meaningful DNA profile will be generated and the data accepted by the criminal justice system.

Keywords Hair · DNA · PCR · Identification · Profile

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Introduction

Direct PCR of samples has gained much interest in a forensic context due to the potential saving in time [1–4], increase in sensitivity, and minimizing of steps open to error or contamination [5]. Much of the focus has been on direct amplification from a stain [6] with an emphasis also on the speed of the DNA typing [7] and the types of DNA polymerase used. A previous report illustrated the potential benefit of direct amplification from fibers [5] and touch DNA [8]. To date there has been no testing of human hairs with direct PCR despite the prevalence of hair sampling during forensic examination [9]. The potential to generate a profile from hair follicles is dependent on the presence of nucleated cells such as within a follicular tag, as such hairs in the growth phase (anagen) being more likely to generate a result than hairs in the dormant phase (telogen). In many forensic laboratories, nuclear DNA profiling is not attempted on hairs when there is no indication of a root sheath at the proximal tip; leading to a potential loss of information. Mitochondrial DNA testing is possible from single hairs for animal testing [10] and human identification [11–13] but requires specialist equipment and skills. If nuclear DNA testing is required then there may be a need to use low template DNA typing methods [14]. Trace amounts of nuclear DNA limit the possibility of generating meaningful DNA profiles from single hairs or hairs in the telogen state. Current practice at Forensic Science South Australia (FSSA) is that hairs are examined by microscopy for the presence of sheath material. If there is no sheath material the hair is not submitted for DNA analysis as there is a low probability of obtaining an interpretable DNA profile. While the majority of hairs on the scalp are in the anagen phase, it is telogen hairs that are found more frequently in forensic investigations as these are the hairs that

are shed naturally, thus the ability to obtain nuclear DNA profiles from these samples would be of great benefit.

We report on the first use of direct amplification of a DNA from single hair in the anagen and telogen growth phases. As a single hair is used in the analysis it is assumed that any resulting DNA profile will be from a single source. The aim was to develop a simple, operational method that could be used routinely in forensic science casework with no further modification and a greater DNA profiling success rate than standard extraction methods on this type of sample. For ease of implementation, the process should also adhere to standard methods with no increase in the cycle number and also generate DNA profiles from hairs of unknown age.

Materials and methods

Samples

A buccal swab and scalp hairs were collected from both male and female donors working at FSSA. DNA was extracted from the buccal swabs using the QIAGEN (Doncaster, Victoria, Australia) Mini DNA extraction kit following the manufacturer's protocol. The resulting DNA profiles were used in subsequent comparisons.

Donors were asked to pluck a number of hairs from their scalp; as well as collect loosely/naturally shed hairs. Each hair was examined microscopically to determine its growth phase. Hairs were categorized as either anagen or telogen (with one example of catagen). A total of 30 hairs in the anagen growth phase and also 30 hairs in the telogen growth phase were analyzed. Two examples of body limb, pubic, and eyebrow hair samples (six in total), that had been stored in sealed plastic bags and kept at room temperature since 2007, were also supplied by FSSA. The shaft of three anagen hairs from one individual was also analyzed.

Direct PCR amplification and conditions

Direct PCR was conducted by removing approximately 5 mm of the proximal tip using sterile scissors and tweezers. For the shaft samples, once the proximal tip had been cut for use, the next 5 mm was cut for use. The hair fragment was placed into a 0.2 mL thin walled tube containing 10 µL of PCR master mix from either the AmpFℓSTR® NGM™ or NGM SElect™ kit (Life Technologies, Victoria, Australia) along with 5 µL of the primer mix and 1 µL of AmpliTaq Gold® DNA polymerase. A further 9 µL of sterile H_2O were added to make the final volume 25 µL. The amplification was conducted in a GeneAmp® System 9600 thermal cycler (Life Technologies) using the manufacturer's recommended conditions. The standard 29 cycles was used for all reactions. There was no alteration to the methodology of amplification deliberately so as to ensure that the process could be adopted readily by the forensic science community. The NGM™ and NGM Select™ kits amplify 15 STR loci plus the amelogenin locus.

Capillary electrophoresis

Capillary electrophoresis was performed on an ABI 3130*xl* Genetic Analyser (Life Technologies) using POP-4™ polymer (Applied Biosystems). An aliquot of either 1 µL or 1 µL of a 1 in 50 dilution into H_2O of the PCR sample was added to a solution of 0.5 µL of ABI GeneScan-600 LIZ® Size Standard and 9.5 µL of Hi-Di™ Formamide. Samples were then 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 relative fluorescence units (RFU).

Data analysis

The data were tabulated based on the number and percentage of alleles generated from the 15 STR loci amplified by the NGM™ and NGM SElect™ kits. Currently FSSA use Profiler Plus (Life Technologies), which amplifies 9 STR loci plus amelogenin. Five complete STR loci are required to up-load to the Australian National DNA Database, therefore a profile that can be uploaded if there are greater than five complete loci whereas an incomplete profile is when less than five loci are generated.

Results and discussion

Anagen and catagen hairs

A total of 30 hairs (29 anagen and 1 catagen) from five individuals were amplified directly using the NGM™ and NGM SElect™ kits and the data analyzed. A full profile, comprising all 15 STR loci and the amelogenin locus, was obtained for every individual hair sample (Fig. 1). In 30 instances, the DNA profile generated from the anagen hair section matched the DNA profile of the donor with no additional alleles and no allelic drop-out. Initially, most of the samples yielded DNA profiles with RFU values above 10,000 for some loci; this is typical of over amplification. We recommend a dilution of the final PCR product for anagen hair samples. For example, a 1 in 50 dilution of the anagen samples decreased the average RFUs from 4,217 to 1,240 (Table 1). Allele pull-up was not observed in the

diluted samples, and background noise was also greatly decreased. The average heterozygous peak balance (defined as the lower intensity peak divided by the higher intensity peak) was observed to be 84.8 % for neat samples and 86.6 % for diluted samples. There was no increase in stochastic effects compared to DNA profiles generated using the standard extraction methods. Heterozygous balance was good (with the two peak heights being within 80 % of each other in all direct amplification experiments) and dropout occurred in the expected range.

Telogen hairs

A total of 30 telogen hairs from five individuals were amplified directly and the data analyzed. Full DNA profiles were obtained from six samples (Fig. 2), and up-loadable profiles (with five or more complete loci) were observed in a further four samples. The remaining twenty samples yielded profiles containing eleven alleles or less. In all 30 instances, the DNA profile generated from the telogen hair section matched the DNA profile of the donor with no additional alleles. In total, the 30 samples had an average RFU value of 1,202 and heterozygous peak balance of 82.4 %.

Aged hairs

Two hairs of different somatic origin (pubic, eyebrow, and body limb) were amplified directly using the NGM™ kit and the data analyzed. The growth stages of the hairs were not identified prior to amplification to avoid the risk of transferring and losing any DNA onto the microscope slides. The hairs had been stored in sealed plastic bags for use in microscopic hair training without taking measures to preserve the DNA at the root. As the objective was to establish if any meaningful DNA profiles could be obtained via direct PCR, a comparison to a reference profile was not required. The complete 15 STR loci were observed for each of the six samples and the two samples from each hair type revealed identical profiles as each other. In total the six samples had an average RFU value of 2,929 and heterozygous peak balance of 82.9 %.

Anagen hair shaft

Standard procedure at FSSA for anagen hair extraction includes using a section of the hair shaft as the negative control. The first 5 mm from the proximal tip is removed

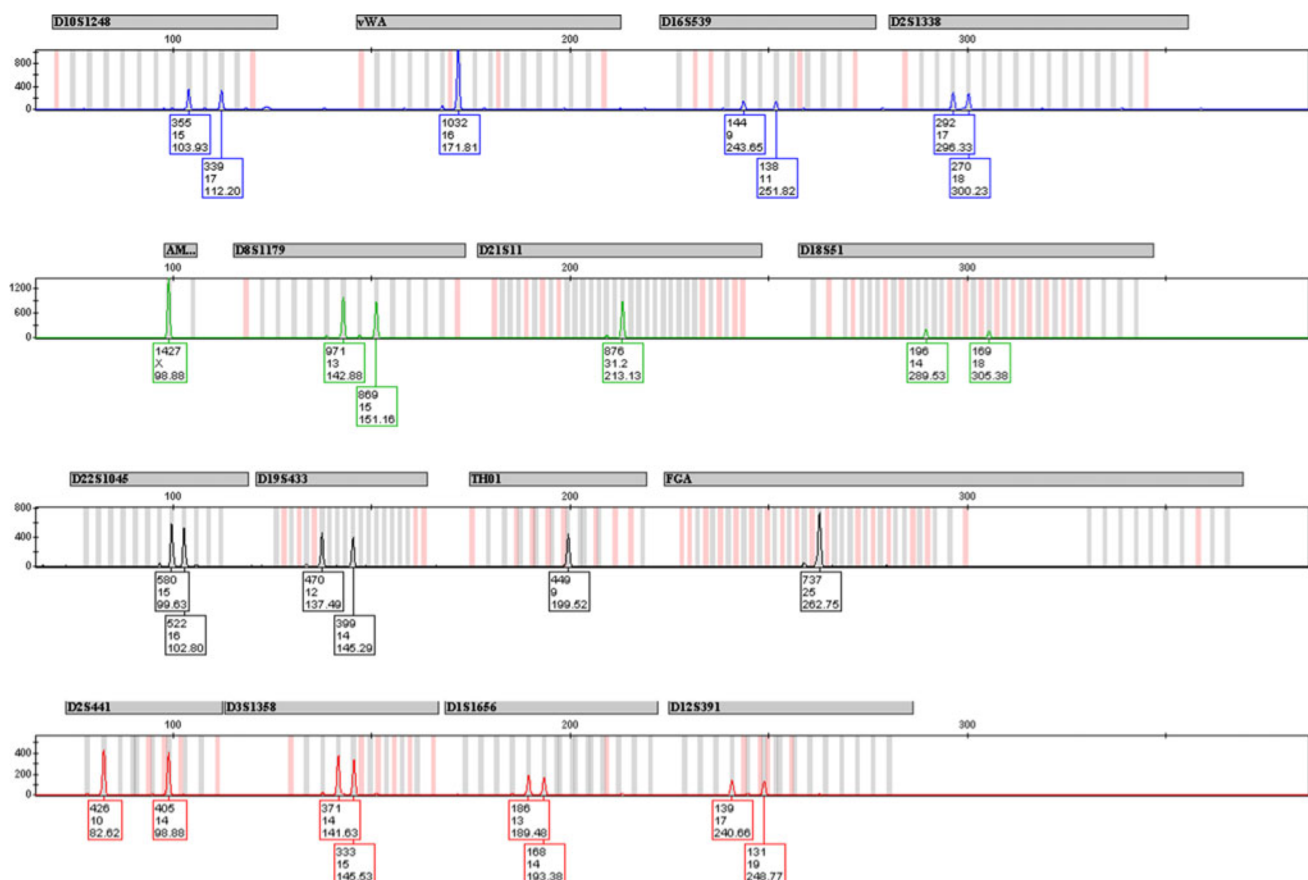

Fig. 1 Chromatogram of nuclear DNA from a single anagen hair root, amplified using AmpFℓSTR® NGM™ kit at 29 cycles on a GeneAmp® System 9600 thermal cycler. Sample was injected on an Applied Biosystems 3130*xl* Genetic Analyser at 3 kV for 10 s

Table 1 Comparison of DNA profiles obtained from different growth phases of hair, showing the success rate (out of 30 STR alleles for NGM and 18 STR alleles for Profiler Plus)

Hair type	NGM profile type (%)			Average RFU
	Complete	Incomplete and up-loadable	Incomplete and not up-loadable	
Anagen	100	0	0	4,217 (1,240 diluted)
Telogen	20	13.3	66.7	1,202
Aged	100	0	0	2,929
Hair type	**Profiler plus profile type (%)**			
	Complete	Incomplete but up-loadable	Incomplete and not up-loadable	
Anagen	53	12.5	34.5	
Telogen	10	6	84	

An incomplete and up-loadable profile is when less than all the alleles, but more than 5 complete loci, were generated. The average RFU values for hairs tested using direct PCR are also shown

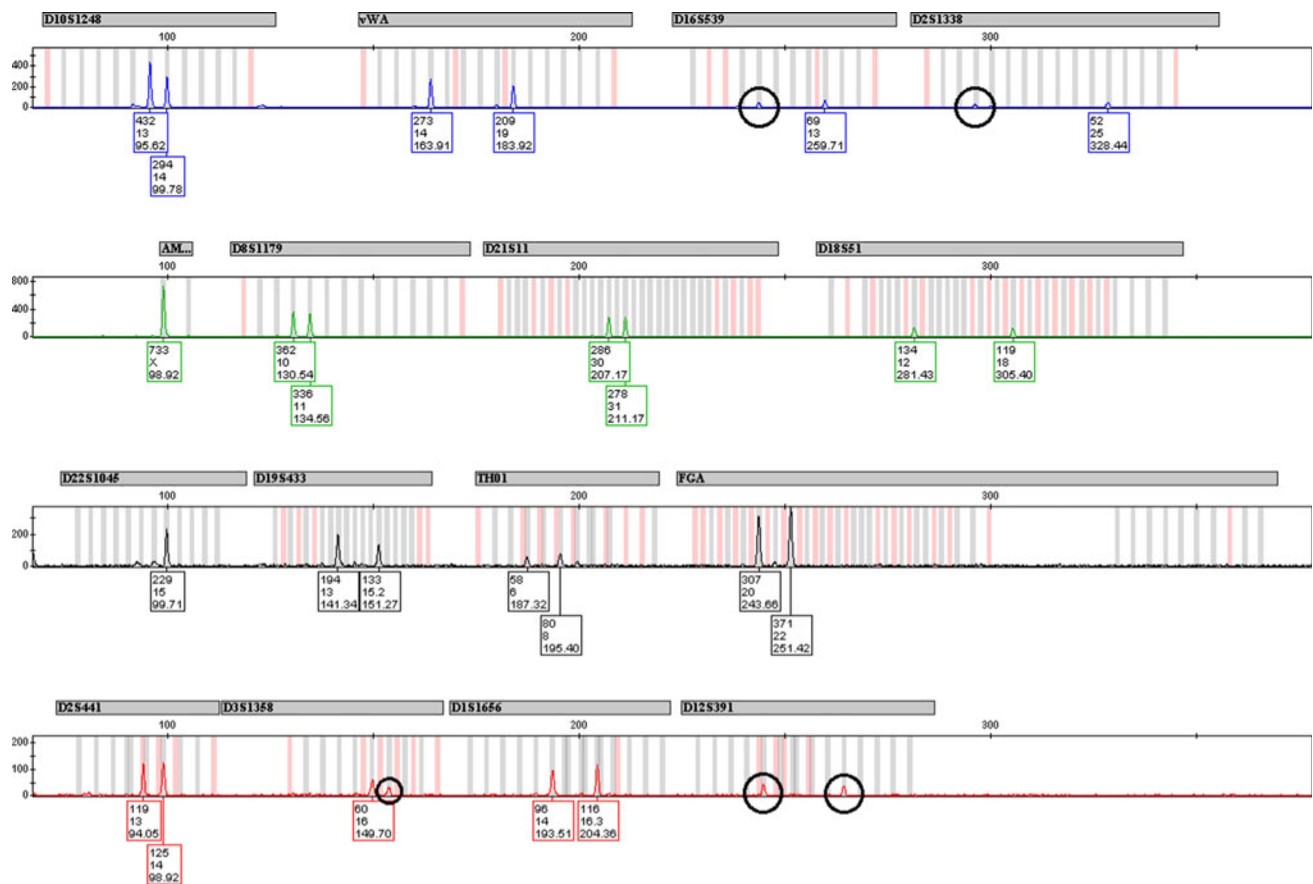

Fig. 2 Chromatogram of nuclear DNA from a single telogen hair root, amplified using AmpFℓSTR® NGM™ kit at 29 cycles on a GeneAmp® System 9600 thermal cycler. Sample was injected on an Applied Biosystems 3130*xl* Genetic Analyser at 3 kV for 10 s. Allelic drop out can be observed at the *circled* loci D16S539, D2S1338, D3S1358, and D12S391

for extraction, and the following 5 mm is used as the negative control. This method was adapted using direct PCR for three anagen hair shaft samples. A full profile belonging to the individual was obtained from one hair sample. The remaining two shaft samples yielded eight alleles each.

FSSA comparison

FSSA have previously conducted an in-house study to compare the growth phase of a hair sample against the DNA profile obtained in order to determine which category of hair yields the most complete DNA profile. The hair type and percentage of profile obtained can be observed in Table 1. A profile of value (up-loadable to the National DNA Database, Australia) was obtained in 33.3 % of telogen samples in this study compared to 16 % by FSSA. Similarly, the success for obtaining meaningful profiles from anagen samples has increased from 65.5 to 100 % in this study. However, telogen hairs in forensic practice are typically not processed for DNA due to the low success rate. For example, based on the FSSA standard operating procedure for hair analysis, a telogen hair would not be submitted for further DNA analysis. There is no standard operating procedure nationwide for hair analysis; however there are numerous studies detailing the analysis on single telogen hairs for forensic purposes resulting in limited success and inconsistent results [9, 15].

Conclusion

We demonstrated a 100 % success rate for the generation of complete profiles (15 STR loci and amelogenin) from a single hair in the anagen growth phase. This is a significant increase compared to the process of performing standard DNA extraction then amplification of the extract. It is noted that some of the differences between the results from the standard extraction (using Profiler Plus) and the direct amplification (using NGM) could be attributed to improvements in the kit chemistry, although the commercial amplification kit used in the direct amplification is not designed primarily for this application.

A complete DNA profile at all fifteen STR loci were also obtained from hairs that have been stored for 5 years. It was found that direct PCR of single anagen hairs produced overloaded DNA profiles after the standard 29 amplification cycles and we recommend a dilution of the product prior to electrophoresis of the sample. The heterozygote balance was typically greater than 80 % and in no case was below 60 %. The profiles indicated no stochastic effects beyond what is expected from standard DNA profiling techniques. No allelic drop-in or any indication of contamination was noted in any of the 66 samples tested.

Direct PCR produced DNA profiles that could be uploaded to the National DNA Database (Australia) from a single hair in the telogen growth phase in ten of the 30 samples tested. Twelve telogen hairs produced partial profiles with eleven alleles or less, and the remaining eight samples produced no alleles. In all cases the profiles obtained were more complete and of greater quality than profiles obtained using standard extraction methods.

The generation of DNA profiles from telogen hairs was not always dependent on initial visualization of a follicular sheath. It may be that free-DNA on the surface of the hair, or associated loosely with the hair shaft, is that source of the DNA. Free-DNA has been suggested as a source of DNA from a variety of sources previously such as fabrics [5] and sweat [16].

There is the possibility of detecting a DNA profile from a hair that is not from the donor of the hair. The introduction of non-donor DNA to a hair can occur prior to the hair being examined in the laboratory and secondly during the laboratory process. The first is due to extraneous DNA from another individual being deposited on the hair while the second can be more formally referred to as contamination of the hair during laboratory procedures.

The deposition of a second individual's DNA onto a hair could be determined readily if the resultant profile is from more than one individual. This indicates multiple donors to the hair and would readily be assessed by the analyst. The potential risk is that a single source profile is obtained, which has not originated from the DNA of the hair donor, and would not be flagged by an analyst. This type of result from a single hair is potentially possible, however as it is proposed that nuclear DNA on the surface of the hair is the predominant source of DNA, then a second contributor (i.e. not from the hair donor) is only possible if a source of DNA comes into contact with the hair surface and no profile is obtained from the donor of the hair. We believe this possibility to be unlikely. Alternatively this may result from the hair being contacted with a biological fluid such as semen or blood. Prior microscopic examination of a hair by a trained analyst would determine the presence of a biological fluid on the hair shaft or bulb.

We propose that contamination of a single hair during the laboratory process is less likely to occur from this direct approach compared to standard extraction procedures. Standard DNA extraction procedures involve numerous wash methods and tube changes, each being a possible step where contamination may occur before the amplification of the DNA. It is also these same steps where the initial DNA can be lost, either via washing or retained on the silica membrane. The method described in this paper avoids the extraction process and thus greatly reduces DNA loss and potential contamination prior to amplification.

This simple process of direct PCR from single hairs can be readily adopted for use into forensic DNA practice and we demonstrate that the process has the capability of generating full DNA profiles from anagen hairs, aged hairs, and partial profiles from single telogen hairs. Hairs that would otherwise not be tested, as there was little chance of gaining a meaningful profile, can now be profiled.

Key Points

1. A successful and novel method for obtaining DNA profiles from single hairs using direct PCR.
2. The DNA extraction process was completely circumvented. This allows for a greater yield of DNA from hair, as DNA is not lost via wash steps or tube changes.
3. Full profiles were obtained in 100 % of anagen hair samples. Profiles 'up-loadable' to the Australian National DNA Database were obtained in 33.3 % of the telogen hair samples.
4. Allelic drop-in or contamination was not observed in any of the 66 hair samples tested.
5. Standard protocol for DNA amplification from the NGM^{TM} and NGM SElect^{TM} STR typing kits was not modified, allowing for easy and quick implementation into forensic laboratories.
6. This method is cost-effective and time saving in forensic casework, as a lengthy extraction process is not required.

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