J Forensic Sci, July 2008, Vol. 53, No. 4
doi: 10.1111/j.1556-4029.2008.00777.x
Available online at: www.blackwell-synergy.com

TECHNICAL NOTE

Kerry L. Opel,[1] M.A.; Erica L. Fleishaker,[2] B.S.; Janice A. Nicklas,[3] Ph.D.; Eric Buel,[3] Ph.D.; and Bruce R. McCord,[1] Ph.D.

Evaluation and Quantification of Nuclear DNA from Human Telogen Hairs*

ABSTRACT: Nuclear DNA was extracted from human telogen hairs from 60 individuals. Six to nine hairs from each individual were individually extracted. The amount of DNA recovered from each individual varied greatly, and most samples yielded a quantity of 550 pg or less per hair. A selective extraction buffer was used to remove epithelial cell DNA and the amount of exogenous DNA was determined. DNA was also quantified by real time PCR using three different sized amplicons targeting an *Alu* sequence. The results were used to determine the state of degradation of the extracted DNA. Different quantities of sample (<100pg, 100–500pg, >500pg) were amplified with the Miniplex kits to determine the minimum DNA template required for successful amplification. DNA recovered from hair showed degradation; however, partial profiles were obtained for those samples containing at least 60 pg using MiniSTRs.

KEYWORDS: forensic science, telogen, hair, nuclear, DNA, rt-PCR, Miniplex, STR

Shed telogen hairs can be an important form of probative forensic evidence. Such samples are often reserved for mtDNA analysis due to the assumption that little nuclear DNA is present. Unfortunately due to its small size, common matrilineal inheritance, and haploid nature, mtDNA lacks the probative value of nuclear DNA. Several studies have been published on the extraction and amplification of DNA from hair (1–5). While some success has been reported for amplification of DNA in hair, accurate quantification of the extracted DNA has been a problem. Before the use of real time PCR for quantification, DNA analysts relied on methods which lacked the sensitivity required to detect the minute amounts of DNA found in hair. Therefore, information on the actual amount of recoverable nuclear DNA present in hair is scarce. Recently, a method has been developed for qPCR which utilizes multilocus probes and permits quantitation of DNA in the picogram range (6). This procedure is suitable for quantification of the total DNA present in telogen hairs. In order to ensure that the DNA quantified comes from within the hair, methods which can remove exogenous DNA must be utilized. The use of a selective extraction step permits removal of epithelial cells from the surface of the hair and allows for the evaluation of the presence of exogenous DNA (7).

DNA extracted from telogen hair is likely to be degraded due to the keritinization process which occurs during hair growth. Direct evaluation of the state of degradation of DNA can normally be accomplished through agarose gel separation and ethidium bromide staining (8). Chemically degraded DNA presents as a smear on the gel, with a range of fragment sizes (1500 to less than 150 base pairs) (8). However, this method is not applicable when subnanogram amounts of DNA are present (9). In these situations, real time PCR can be used to determine relative level of degradation of the template DNA through amplification with different primers. Comparison of the amounts of large and small amplicons will indicate if degradation is present in the sample (8).

When DNA is degraded, success of amplification of the DNA using standard commercial multiplex kits with large amplicons can be limited. Instead, Miniplex kits specifically designed for use with degraded DNA can provide an alternative for such samples (10). These Miniplex kits produce a reduction in amplicon size for STR loci of up to 191 base pairs when compared to commercial kits and have demonstrated ability to amplify DNA that has been chemically degraded (8,10). Improved amplification and recovery of dropped alleles has also been seen with DNA from naturally degraded skeletal samples (11).

This paper presents a large-scale sampling of DNA from telogen hairs to determine the amount that can be extracted. A method for assessment of the degradation level of DNA from telogen hairs is also presented, as well as the effect of degradation on genotyping.

Materials and Methods

Sample Collection and DNA Extraction

Head hair was collected from 60 volunteers using a comb to remove naturally shed hairs, and subjected to microscopic examination by the analyst to determine the stage of hair growth. Six to nine telogen stage hairs from each individual (510 total hairs) were selected and 3 cm of each hair, including the club end, was sampled.

Comparison DNA samples (to be used to establish full genotypes) were obtained through buccal swabs and were stored either on FTA paper or as dried swabs. FTA stored samples were processed in accordance with the manufacturer's protocols (12), and swab samples were processed in accordance with a previously published protocol (13).

[1]Department of Chemistry and Biochemistry, Florida International University, University Park, 11200 SW 8th Street, Miami, FL 33199.
[2]Department of Chemistry and Biochemistry, Ohio University, 136 Clippinger Laboratories, Athens, OH 45701.
[3]Vermont Forensic Laboratory, 103 South Main Street, Waterbury, VT 05671.

*This project was supported under award 2002-IJ-CX-K007 from the National Institute of Justice.
Portions of this paper were presented at the American Academy of Forensic Sciences Annual Meetings in New Orleans, LA, February 25, 2005 and San Antonio, TX, February 23, 2007.

Received 26 June 2007; and in revised form 23 Nov. 2007; accepted 1 Dec. 2007.

The individual hairs were incubated at 56°C for 2 h in 300 μL of differential extraction solution (100 mM NaCl, Spectrum, Gardena, CA), 10 mM EDTA (Sigma, St. Louis, MO), 0.4% SDS (Spectrum) (7) with 40 μg of proteinase K (Fermentas, Hanover, MD) to remove epithelial cells from the outside of the telogen hairs. The extraction solution was removed and subjected to organic extraction with 300 μL of 70% phenol/chloroform/water (Applied Biosystems, Ventura, CA), and the aqueous layer was purified in YM-30 Microcon® (Millipore, Inc., Danvers, MA) filters according to the manufacturer's protocol (14). The extracted epithelial DNA was collected in 60 μL of deionized H_2O.

The hairs were then rinsed with a 0.9% NaCl solution, then absolute ethanol to ensure complete removal of the extraction solution, transferred to a fresh 600 μL tube and incubated at 56°C for 2 h in 300 μL of hair extraction solution (10 mM Tris-HCl [Sigma], pH 8.0, 100 mM NaCl, 5 mM $CaCl_2$ [Sigma], 2% SDS, 39 mM dithiolthreitol [DTT] [Sigma] [2]) (modified) with 80 μg of proteinase K added to extract the DNA from within the telogen hairs. The DNA was separated and extracted using 300 μL of 70% phenol/chloroform/water, and the aqueous layer containing the DNA was purified in YM-30 Microcon® filters according to the manufacturer's protocol (aqueous layer centrifuged down to dryness in the filter, washed with H_2O, and centrifuged to dryness again) (14). The purified DNA was collected in 60 μL of deionized H_2O (Sigma).

All human samples were obtained in accordance with the institutional review boards of Ohio University and Florida International University.

Quantification

Both epithelial DNA and internal hair DNA extracts were quantified by real time PCR using the Corbett Rotor-Gene 3000 (Corbett Research, Sydney, Australia), primers for the 82 base pair *Alu* amplicon, and SYBR Green I (Invitrogen, Carlsbad, CA) dye using a previously published protocol (6). Hairs yielding high concentrations (>25 pg/μL) of template DNA (n = 9) were selected for fragment size comparison, and were quantified, along with nondegraded control DNA (Cell line 9948; Applied Biosystems) (with three replicates of each sample) by primers for an 82 bp amplicon, a 124 bp amplicon, and a 201 bp amplicon in separate reactions. Primer sequences for these amplicons are shown in Table 1.

PCR Amplification

Selected samples with the highest concentrations of DNA (>25 pg/μL in 55 μL) were amplified according to published protocols (10) with all three Miniplex kits (Miniplex 2, Miniplex 4, and Big Miniplex) (primer sequences listed in Table 2) and the PowerPlex® 16 commercial kit using 5 μL of the sample. Nonacetylated bovine serum albumin (BSA) (Sigma) (0.5 μg) was added to the reaction mix. Low concentration samples (<25 pg/uL) were concentrated using YM-30 Microcons® (extract was added to the filter and centrifuged to dryness), collected in a smaller volume (10 μL) and quantified again. Samples with less than 100 pg total DNA were amplified with Miniplex 2, samples with 100–550 pg were amplified with Miniplex 2 and Miniplex 4, and samples with more than 550 pg were amplified with all three kits. The full volume of recovered DNA (5–7 μL) was used for amplification. BSA (0.5 μg) was added to the reaction mix. PCR was performed in the GeneAmp® PCR System 9700 (Applied Biosystems). The thermal cycling parameters for the Miniplexes were: 11 min at 94°C soak, followed by 33 cycles of 95°C for 1 min denaturing, 55°C for 1 min annealing, and 72°C primer extension. The final two soak steps were at 60°C for 45 min and 25°C forever. Published protocols (15) for Powerplex® 16 kits (Powerplex Corporation, Madison, WI) were used for amplification of the samples analyzed with the Powerplex® kit.

TABLE 1—*Primer sequences for the three ALU amplicons. All three use the same forward primer.*

Primer	Sequence (5′–3′)
ALU forward	GTCAGGAGATCGAGACCATCCC
82 bp reverse	CCACTACGCCCGGCTAATTT
124 bp reverse	TCCTGCCTCAGCCTCCCAAG
201 bp reverse	GCTCTGTCGCCCAGGCTGGAGT

TABLE 2—*Primer sequences for Miniplex loci.*

THO1 F	6FAM –CCTGTTCCTCCCTTATTTCCC
THO1 R	GTTTCTTGGGAACACAGACTCCATGGTG
CSF1PO F	VIC-ACAGTAACTGCCTTCATAGATAG
CSF1PO R	GTGTCAGACCCTGTTCTAAGTA
TPOX F	NED-CTTAGGGAACCCTCACTGAATG
TPOX R	GTTTCTTGTCCTTGTCAGCGTTTATTTGC
FGA F	6FAM-AAATAAAATTAGGCATATTTACAAGC
FGA R	GCTGAGTGATTTGTCTGTAATTG
D21S11 F	VIC-ATTCCCCAAGTGAATTGC
D21S11 R	GGTAGATAGACTGGATAGATAGACGA
D7S820 F	NED-GAACACTTGTCATAGTTTAGAACGAAC
D7S820 R	GTTTCTTTCATTGACAGAATTGCACCA
D5S818 F	6FAM-GGGTGATTTTCCTCTTTGGT
D5S818 R	AACATTTGTATCTTTATCTGTATCCTTATTTAT
D8S1179 F	VIC-TTTGTATTTCATGTGTACATTCGTATC
D8S1179 R	ACCTATCCTGTAGATTATTTTCACTGTG
D16S539 F	NED-ATACAGACAGACAGACAGGTG
D16S539 R	GCATGTATCTATCATCCATCTCT
vWA F	6FAM-AATAATCAGTATGTGACTTGGATTGA
vWA R	ATAGGATGGATGGATAGATGGA
D18S51 F	VIC-TGAGTGACAAATTGAGACCTT
D18S51 R	GTCTTACAATAACAGTTGCTACTATT
D13S317 F	NED-TCTGACCCATCTAACGCCTA
D13S317 R	GTTTCTTCAGACAGAAAGATAGATAGATGATTGA

Separation and Analysis

Samples were separated on the ABI Prism® 310 genetic analyzer (Applied Biosystems) using POP-4 polymer and GS ROX® 500 (Applied Biosystems) internal standard in a 47 cm 50 μm ID capillary. The samples were injected at 5 kV with 15 kV separation and a run time of 24 min. The data were analyzed with GeneScan® and Genotyper® software (Applied Biosystems) using in-house macros for genotyping.

Results and Discussion

Quantification results for the 82 bp amplicon showed that 30% of individuals had on average (n = 6–9 hairs per person) less than 100 pg of recoverable DNA per hair, while 41.6% had on average between 100 and 550 pg of recoverable DNA per hair. Only 28.4% of individuals possessed more than an average of 550 pg of recoverable DNA (Fig. 1) per shed telogen hair. Quantification of individual hairs yielded similar results: recovered DNA per hair ranged from 0 to 9200 pg (n = 510). For all hairs, 33.1% yielded less than 100 pg of DNA (12.2% of the hairs [62 hairs] yielded no DNA), 45.6% of hairs yielded 100–550 pg of DNA, and 21.3% yielded more than 550 pg of DNA. We found no correlation between the amount of extracted DNA and the age, sex, ancestry, or hair color of the donor. We did not examine the effect of

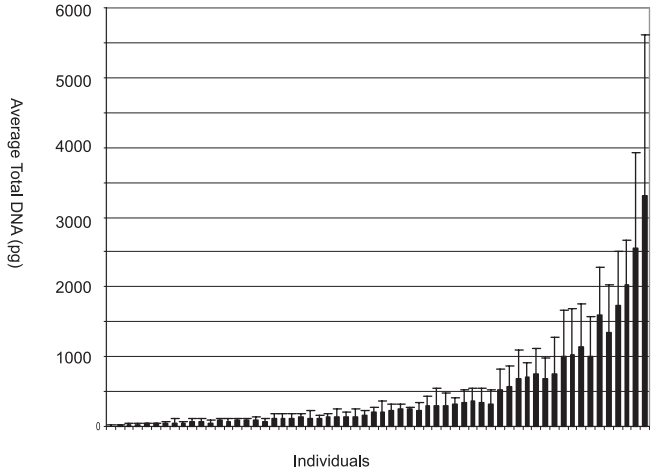

FIG. 1—*Average total DNA in a single hair for the 60 individuals sampled. The errors bars represent two standard deviations from the average (95% confidence).*

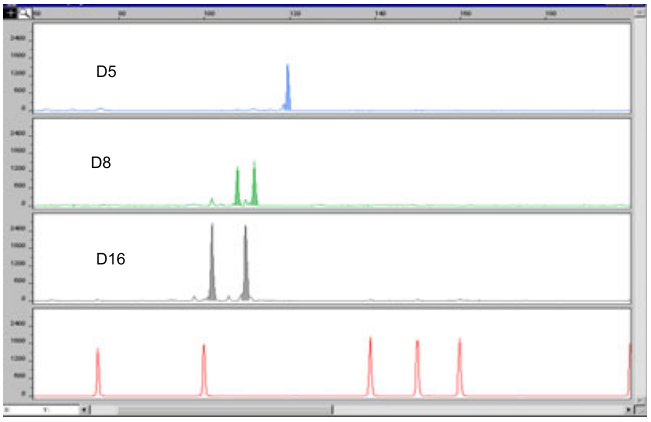

FIG. 2—*Amplification of differential extract with the Miniplex 2 kit (D5, D8, and D16 loci). No discernible minor contributor is present.*

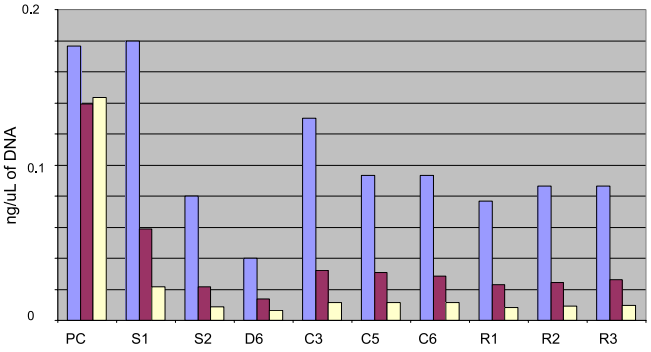

FIG. 3—*Comparison of quantification of telogen hair extracts using three different sizes of Alu amplicons: 82 bp, 124 bp, and 201 bp. The reduced amount of the larger amplicons in the hair samples indicates that the DNA in hair is degraded.*

chemical treatment, which may have some affect on recoverable DNA. We will be investigating this aspect in the future.

Quantification of the samples which yielded both epithelial and internal DNA showed varied results. Of the analyzed samples (n = 20), 45% of the samples contained less DNA on their surface as they did inside the hair, 25% of the hairs contained more DNA present on their surface as they did inside the hair, and 30% were roughly equivalent in DNA concentration between the hairs and their surface. The small number of hair surface extraction samples which contained sufficient DNA for amplification (n = 3) were tested with the Miniplex kits for contamination (Fig. 2). No minor contributor was evident in any of this small number of samples, and the STR profile matched the hair donor. Since these samples were collected in a controlled environment (directly from the donor), other samples collected in a manner similar to forensic samples were also tested. Samples from three individuals (n = 9 per person) were collected indirectly (removed from household surfaces and placed in sterile containers) and subjected to the same differential extraction procedure. The surface of the simulated forensic samples (for which sufficient DNA for amplification was extracted) also showed no minor contributor for all loci and the profile matched the donor.

The purpose of this particular study was first to determine if contamination would be present, and second to determine if it would be advisable to skip the first (“decontaminating”) extraction step in order to get more DNA from the hairs. Since there was such a variation in the amounts found in the epithelial extract, no strong conclusion could be reached for this question. However, we recommend using the epithelial extraction step to eliminate the chance of contamination, particularly since the examiner would be dealing with low copy DNA.

Degradation of the DNA from the hair was assessed through the use of real time quantitative PCR (qPCR) and three different size amplicons (82, 124, and 201 bp). Since the degradation process breaks the DNA into smaller fragments, the smaller amplicons produced in real time PCR by one set of primers should be more prevalent in the sample than the larger amplicons produced by another set when compared to nondegraded (control DNA). The control DNA (9948 cell line) gave consistent quantification results with the larger two amplicons, while the DNA extracted from telogen hairs (n = 8) showed a decrease in amplification of the larger size amplicons (Fig. 3). This loss in amplification of larger sized DNA amplicons can be compared to a similar loss of larger DNA fragments seen when larger quantities of degraded genomic DNA are separated on a yield gel (8).

Of those individual hair samples which contained sufficient DNA (greater than 25 pg/μL) and were successfully amplified (peaks detected above the detection threshold of 150 RFUs) (n = 10), amplification of at least 9 of the 12 Miniplex loci was observed. The three largest amplicons (FGA, D21S11, D7S820) most commonly failed to produce amplification. This is likely due to the effect of degradation (Fig. 4). Amplification of these samples with the Powerplex® 16 kit produced amplification of fewer loci (2–9 of 12), particularly in the larger sizes, and these results are consistent with our previous work on degraded DNA from skeletal remains (11). Because such low concentrations of DNA were produced from the telogen hair samples, we examined the capability of the commercial kit to amplify DNA at lower levels, and found that the concentration of the DNA template did not affect the amplification success (Fig. 5). This indicates that the problem with the telogen hair samples is likely an issue with degradation or inhibition and not sensitivity. One must be cognizant that the quantitation is based on the amplification of an 82bp fragment. In the extreme, if all of the DNAs were in 83bp pieces, the quantitation would not be affected but no amplification of any STR larger than 82 bp would be possible no matter how much DNA is added.

Amplification was tested on a limited number of low template samples (n = 30). Prior to these tests, the effect of input quantity of

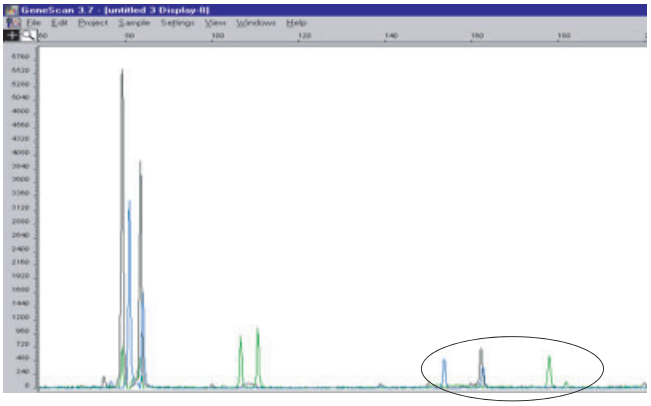

FIG. 4—*Amplification of hair extract with Miniplex kit (all three kits on one graph). The larger loci of Big Mini (FGA, D21, and D7, circled) show much lower intensity than the other loci, evidence of degradation and possible inhibition in the DNA sample.*

TABLE 3—*Relative percent success for each locus for all low concentration samples. D5S818, D8S1179, and D16S539: n = 30; vWA, D18S51, and D13S317: n = 20; TH01 – D7S820: n = 10.*

Locus	Percent Success	Kit
D5S818	93	Miniplex 2
D8S1179	50	Miniplex 2
D16S539	83	Miniplex 2
vWA	55	Miniplex 4
D18S51	25	Miniplex 4
D13S317	70	Miniplex 4
TH01	60	Big Miniplex
CSF1PO	10	Big Miniplex
TPOX	50	Big Miniplex
FGA	10	Big Miniplex
D21S11	10	Big Miniplex
D7S820	10	Big Miniplex

telogen template hair DNA was tested. A large number of hairs (n = 35) from a single individual (who had previously yielded large amounts of DNA from shed hairs) were extracted and the extracts were combined. The total sample was quantified, and different volumes of the sample corresponding to known amounts of DNA were tested (n = 3 replicates for each concentration). Miniplex 2 produced full profiles with 120 pg of DNA, and Miniplex 4 produced full profiles with 150 pg of DNA. Partial profiles (two loci) for these kits were obtained with 60 pg of DNA. However, with the Big Miniplex (six loci) kit, only two loci produced results for all amounts of template tested (30–500 pg). These two loci, TH01 and TPOX, have previously demonstrated lower susceptibility to PCR inhibition (16), and therefore an inhibition problem was suspected for the tested sample. Two possible methods of relieving inhibition were tested: addition of BSA and addition of larger amounts of Taq polymerase. Normally, 0.5 μg of BSA is included in the amplification mix for noncontrol samples, as this amount has been shown to remove inhibition for the Miniplex sets (16). A range of BSA amounts were tested with the control hair sample (0–5 μg) with no improvement in amplification success. One to four times the normal amount of Taq (2–8 U) was also tested with the control sample. One locus (CSF1PO) produced detectable amplification with 8 U of Taq for some of the samples, but no improvement was seen in the other three loci (FGA, D21S11, and D7S820). We are currently testing a variety of methods in further efforts to reduce this problem.

A limited number of low template samples (n = 30) were re-concentrated in smaller volumes and amplified with one or more of the kits. For these samples, the success of amplification varied. The samples with less than 100 pg total DNA produced profiles for two of the three loci in Miniplex 2 in 50% of samples tested (n = 10). The samples with 100–550 pg of total DNA produced full (six loci

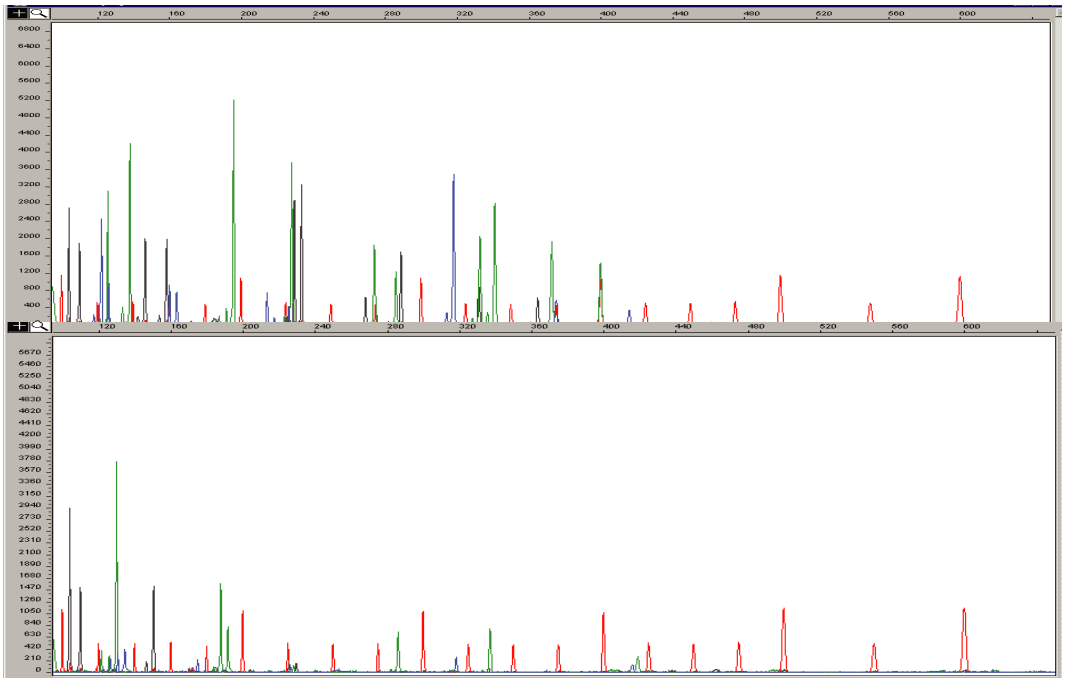

FIG. 5—*Amplification of 100 pg of Control DNA with Powerplex® 16 kit shows sufficient amplification for typing of all loci. The same amount of DNA from telogen hairs results in much lower intensity of the larger loci, evidence of degradation and possible inhibition in the DNA sample.*

Miniplex 2 and Miniplex 4) profiles for 40% of the samples, and at least five loci amplified for 60% of the samples (n = 10). The samples with greater than 550 pg total DNA produced six loci profiles (from Miniplex 2, Miniplex 4, and Big Mini) for 50% of the samples, but only 30% produced 8–12 loci profiles (n = 10). Some loci are much more robust, indicated by the percent success for each locus presented in Table 3.

Conclusions

In our efforts to determine the amount of telogen DNA that can be extracted from shed telogen hairs of consistent length, we were able to determine that very low concentrations of DNA can be extracted, and that the amount extracted varies from person to person. Samples taken of epithelial DNA present on telogen hairs produced the same type as the hair donor, and the amount also varied greatly. DNA from telogen hairs shows a high level of degradation, and this degradation reduced the probability of obtaining a full profile. For samples with recoverable nuclear DNA, the Miniplex kits with their reduced-size amplicons can provide improved results from telogen hair over the commercial kit although loss of larger-sized amplicons is still a problem. Telogen hairs can be an important form of forensic evidence, both for morphological and DNA studies. Since the amount of nuclear DNA that can be extracted may be very low as well as highly degraded, for many samples mtDNA analysis may provide a better chance of obtaining a profile. However, based on these results, if sufficient DNA can be recovered from hair (>60 pg), partial profiles may be produced when using the Miniplex STR systems. For larger amounts of DNA (>550 pg), more loci can be obtained; however, full profiles are rarely recovered due to the extensive degradation that is present. In addition to degradation, inhibition was found to be a problem for some samples. Additional BSA and Taq polymerase did not significantly reduce this problem, which may indicate the source of inhibition in these samples involves damage to the DNA template through chemical modification.

Acknowledgments

Points of view in the document are those of the authors and do not necessarily represent the official view of the U.S. Department of Justice. We would like to gratefully acknowledge Dr. John Butler from the National Institute from Standards and Technology for his technical support with the Miniplex primer sets, Wes Garber from Ohio University, Albert Araluce and Sarah Hughes from Florida International University, and to all of our volunteer hair donors.

References

1. Angelini G, Mantovani V, Pelotti S, Pappalardo G, Barboni F. Simultaneous DNA analysis of HLA-DPB and -DQB loci from single hairs: a forensic case report. Hum Immunol 1991;32:9.
2. Hellmann A, Rohleder U, Schmitter H, Wittig M. STR typing of human telogen hairs—a new approach. Int J Legal Med 2005;114:269–73.
3. Nozawa H, Yamamoto T, Uchihi R, Yoshimoto T, Tamaki K, Hayashi S, et al. Purification of nuclear DNA from single hair shafts for DNA analysis in forensic sciences. Leg Med 1999;1:61–7.
4. Schreiber A, Eberhard A, Storch V, Sauer G. The extraction of high-molecular-mass DNA from hair shafts. FEBS Lett 1988;230:209–11.
5. Takayanagi K, Asamura H, Tsukada K, Ota M, Saito S, Fukushima H. Investigation of DNA extraction from hair shafts. Int Congr Ser 2003;1239:759–64.
6. Nicklas JA, Buel E. Development of an *Alu*-based, real-time PCR method for quantitation of human DNA in forensic samples. J Forensic Sci 2003;48:936–44.
7. Jehaes E, Gilissen A, Cassiman J, Decorte R. Evaluation of a decontamination protocol for hair shafts before mtDNA sequencing. Forensic Sci Int 1998;94:65–71.
8. Chung DT, Drabek J, Opel KL, Butler JM, McCord BR. A study on the effects of degradation and template concentration on the amplification efficiency of the STR Miniplex primer sets. J Forensic Sci 2004;49:733–40.
9. Bourzac KM, LaVine LJ, Rice MS. Analysis of DAPI and SYBR Green I as alternatives to ethidium bromide for nucleic acid staining in agarose gel electrophoresis. J Chem Educ 2003;80:1292–6.
10. Butler JM, Shen Y, McCord BR. The development of reduced size STR amplicons as tools for analysis of degraded DNA. J Forensic Sci 2003;48(5):1054–64.
11. Opel KL, Chung DT, Drabek J, Tatarek NE, Meadows-Jantz L, McCord BR. The application of Miniplex primer sets in the analysis of degraded DNA from human skeletal remains. J Forensic Sci 2005;51:351–6.
12. Whatman Biosciences. FTA technology manual. Cambridge: Whatman Biosciences Ltd., 2000.
13. Comey CT, Koons BW, Preseley KW, Smerick JB, Sobieralski CA, Stanley DM, Baechtel FS. DNA extraction strategies for amplified fragment length polymorphism analysis. J Forensic Sci 1994;39:1254–69.
14. http://www.millipore.com/userguides.nsf/a73664f9f981af8c852569b9005-b4eee/e525b3933d55175a85256b250071786b/$FILE/99394.pdf. Accessed May 13, 2008.
15. Promega Corporation. GenePrint® PowerPlex™ 16 system technical manual, Part # TMD012 (4/00). Madison, WI: Promega Corporation, 2000.
16. Chung DT. The development of novel STR primer sets for the analysis of degraded and compromised DNA samples [dissertation]. Athens, OH: Ohio University, 2004.

Additional information and reprint requests:
Bruce R. McCord, Ph.D.
Department of Chemistry and Biochemistry
Florida International University
University Park
11200 SW 8th Street
Miami, FL 33199
E-mail: mccordb@fiu.edu