Biol. Chem., Vol. 380, pp. 1329–1331, November 1999 · Copyright © by Walter de Gruyter · Berlin · New York

Short Communication

An Evaluation of Techniques for the Extraction and Amplification of DNA from Naturally Shed Hairs

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Hair can be a valuable source of DNA for the noninvasive study of human and nonhuman populations. However, hairs contain extremely small quantities of DNA, making the method used to extract the DNA of paramount importance. This study compares the effectiveness of 4 different methods of DNA extraction from shed chimpanzee hair, as measured by the ability to amplify mtDNA targets using PCR. The most successful method is also the simplest, requiring only digestion of the root end in a buffer compatible with subsequent PCR without a prior purification or extraction step. Strategies to non-specifically preamplify the template are not successful with DNA from stored shed hairs.

Key words: Chimpanzees / Extraction / Hair / Noninvasive / PCR / Preamplification.

The DNA obtained from shed or plucked hairs is useful for genetic analysis in forensic investigations (Pascali *et al.*, 1994; Allen *et al.*, 1998; Menotti-Raymond *et al.*, 1997; Savolainen and Lundeberg, 1999) and for questions of the population structure and molecular evolution of a variety of species (Vigilant *et al.*, 1989; Morin *et al.*, 1994; Garner and Ryder, 1996; Taberlet *et al.*, 1997; Goossens *et al.*, 1998; Lum *et al.*, 1998; Gagneux *et al.*, 1999). Many studies have examined only the more abundant mtDNA because nuclear DNA targets are more difficult to amplify, particularly from shed rather than plucked specimens (Goldberg and Ruvolo, 1997; Saltonstall *et al.*, 1998). A possible solution to the problem of low starting amounts of DNA would be the use of a preliminary PCR procedure employing degenerate primers to non-specifically increase the amount of template prior to locus-specific amplification (Zhang *et al.*, 1992; Cheung and Nelson, 1996). As part of a long-term project investigating social behavior and reproductive strategies in natural populations (Boesch, 1997; Gagneux *et al.*, 1999), I evaluated the relative success of a variety of methods of extraction and amplification of DNA using shed chimpanzee (*Pan troglodytes verus*) hairs. Hairs used were collected in 1994 from wild chimpanzee nests, placed individually in glassine envelopes and kept dessicated at room temperature for several weeks prior to subsequent long-term storage at –80 °C (Gagneux, 1998).

Individual hair root segments approximately 3 mm in length were treated by four different methods: (1) organic extraction subsequent to proteinase K digestion (Higuchi *et al.*, 1988; Vigilant *et al.*, 1989); (2) Qiagen tissue kit purification, which is a protease digestion followed by DNA purification and isolation using a spin column with a silica-gel membrane as per the manufacturer's instructions; (3) Chelex (Biorad) ionic bead resin treatment, with and without accompanying proteinase K digestion (Walsh *et al.*, 1991); and (4) proteinase K digestion in a PCR-compatible buffer (Allen *et al.*, 1998). The results of these comparisons are presented in Table 1. The most successful methods are also the simplest. Extraction of DNA using Chelex resin is the method most commonly used with field-collected hair (Taberlet *et al.*, 1997; Field *et al.*, 1998; Gagneux *et al.*, 1999). Although in this study it was possible to amplify an mtDNA target from 70% of the samples, reported success rates using Chelex are often substantially lower (Goldberg, 1996; Saltonstall *et al.*, 1998). The other simple method of enzymatic digestion without further purification, was more successful (85%), although the difference was not statistically significant. It has been observed that

Table 1 Comparison of Methods for the Isolation of DNA from Single Hairs.

Method	Time[a]	Success[b] (%)
1. Organic	O/N + 3 h	12/25 (48)
2. Tissue kit	1 h	11/26 (42)
3. Chelex	O/N + 20 min	19/27 (70)
4. Buffer digest	O/N + 20 min	22/26 (85)

[a] Approximate amount of time required to process up to 12 samples, O/N (overnight) digests with proteinase K were performed for 14–18 h.

[b] Number of extracts that successfully amplify a 286 bp segment of the mtDNA control region using 0.2 μM each of primers L15996 (Vigilant *et al.*, 1989) and H16242v (5′-GTGTCGTTG-GAGTTGTGT-3′) as well as 1 U Amplitaq Gold (Perkin Elmer) polymerase, 1 × PCR Buffer II (Perkin Elmer), 2 mM $MgCl_2$, 10 mM dNTPs, 25 μg BSA (Boehringer Mannheim) in a Perkin Elmer 9600 thermocycler with conditions of denaturation of 5 min at 95 °C, 50 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. One μl of 200 μl total extract was used per reaction. Success of the PCR was determined by the presence of a visible band of the correct size after electrophoresis of 1/5th of the product through a 3% NuSieve (FMC) agarose gel.

components that co-purify with the Chelex-extracted DNA can inhibit subsequent analyses and possibly cause DNA degradation during storage (Prager *et al.*, 1998; Steffens and Roy, 1998), making the identification of an easy alternative in the form of enzymatic digestion without further purification particularly useful.

It is possible that reactions may fail not due to the low amount of initial template presence, but due to the presence of inhibitors that persist through the DNA isolation procedure. To check for this effect, aliquots of unsuccessful reactions from all methods were added at five times the amount normally used to 50 ng of control DNA. These mixtures did subsequently amplify, suggesting that the original failures are due to low template concentration. This is also supported by a comparison of the ability to amplify mtDNA segments of various lengths, in which a clear tendency for reduced success with increasing target size was seen. In tests of the 26 samples extracted by the buffer digest method, segments up to 300 bp long are amplified with an efficiency of more than 80%, but the chance of amplifying a 400 bp segment decreases to only about 60%. Finally, only about 15% of samples produce a 500 bp segment. This is consistent with the idea that DNA from shed hair is not only present in small amount, but is also degraded (Higuchi *et al.*, 1988).

Samples successfully extracted using the buffer digest method were evaluated for the ability to amplify some nuclear microsatellite loci (LL1, PLA2A, FABP, vWF and FESPS) previously used in genotyping of chimpanzees (Gagneux *et al.*, 1999). As expected, these amplifications had a lower success rate due to the lower copy number of the nuclear targets. Approximately one-third of the samples could produce the expected 100 to 200 bp length products, and one locus (Pla2a) with small alleles typically ranging from 68 to 92 bp was amplified in about half of the attempts. For both the mtDNA and nuclear amplifications the results presented represent only one attempt with each sample; the percentage of successful samples could increase slightly with repeated testing. A positive PCR does not guarantee an accurate genotype, but the genotype and mtDNA segment sequence of one of the individuals had been previously determined using different methods and the same results were obtained using the methods described here (Gagneux, 1998).

Since the most significant problem presented by the hair samples is the low amount of DNA available, nonspecific amplification procedures to increase the amount of template available for subsequent analysis were tested. These DOP-PCR and PEP-PCR experiments followed the procedures described in the literature, and used 1 to 5 μl of DNA isolated from hair using the digestion in buffer method per reaction (Zhang *et al.*, 1992; Cheung and Nelson, 1996). One μl of the resulting product or the original hair extract was used as a template for conventional amplification of mtDNA and genomic DNA targets. In all cases where subsequent specific amplification from the preamplified sample was successful, an aliquot of the original sample diluted to the same original template concentration as in the preamplification reaction would also yield a product upon specific amplification. It may be that the fragmentary nature of the hair DNA makes nonspecific amplification of the genome unlikely.

Due to the very low quantities of DNA present in shed hair ($<$10 ng total), reliability of genotypes must be demonstrated by use of a multiple tubes approach to guard against errors such as stochastic sampling, false alleles and sporadic contamination (Taberlet *et al.*, 1996; Gagneux *et al.*, 1997). The use of a minimum of 3 positive PCRs per locus in multilocus genotyping can quickly exhaust the available sample and necessitate extraction of many additional hairs. Previous studies have used from 1/20th to 1/100th of the sample per PCR (Gagneux *et al.*, 1997; Taberlet *et al.*, 1997). Only 1/200th of the sample prepared by the buffer digest method is needed per reaction, thus permitting many PCR attempts from a single hair.

In conclusion, the use of shed hair samples for population studies, and in particular to examine nuclear rather than mtDNA, demands persistence, as some amplification failure is inevitable. The use of a procedure in which the hair root is digested in a PCR-compatible buffer allows the processing of the hair with no chance of loss of any sample material, and allowed in this small study the amplification of a mtDNA target from almost all samples. It remains to be seen whether alternate storage methods, such as the use of silica beads as was recently applied to preserve fecal samples, may be used to ward off degradation and enhance the ability to analyze DNA from hair (Wasser *et al.*, 1997).

Acknowledgements

I am grateful to P. Gagneux and C. Boesch for materials, information, and discussion. I also thank P. Morin, S. Pääbo, M. Stoneking, and D. Woodruff for helpful discussion.

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Received July 21, 1999; accepted September 29, 1999