TECHNICAL NOTE

David A. Gangitano,[1] M.Sc.; Marisa G. Garófalo,[2]; Guillermo J. Juvenal,[2] Ph.D.; Bruce Budowle,[3] Ph.D.; and Ricardo A. Padula,[1] M.Sc.

Typing of the Locus DYS19 from DNA Derived from Fingernail Clippings Using PCR Concert™ Rapid Purification System

REFERENCE: Gangitano DA, Garófalo MG, Juvenal GJ, Budowle B, Padula RA. Typing of the locus DYS19 from DNA derived from fingernail clippings using PCR Concert™ rapid purification system J Forensic Sci 2002;47(1):175–177.

ABSTRACT: DNA extracted from the fingernails of female victims of a violent or aggressive act may assist in the identification of the male. Sometimes with the current autosomal STR loci, however, the victim's profile may mask the perpetrator's DNA profile or the perpetrator's DNA may be substantially lower in quantity than that of the victim's DNA. Thus, under these conditions, no characterization is possible. In this paper, an alternative DNA extraction procedure was employed, and the application of an STR locus residing on the Y chromosome DYS19 was typed to allow for genetic characterization of the perpetrator in such cases.

KEYWORDS: forensic science, DNA typing, fingernail clippings, polymerase chain reaction, short tandem repeats, Y chromosome, DYS19

During a sexual assault or homicide, the victim may try to defend herself. In such cases, fingernail clippings from the victim are sent to the forensic laboratory for DNA analysis. DNA extracted from these clippings, which could possibly originate from contact with the perpetrator's scratched skin, is a possible source of DNA from the perpetrator. Wiegand et al. (1) reported that a foreign profile can occasionally be obtained from debris scraped from underneath nails. In contrast, Oz et al. (2), studying the DNA profiles received from fingernail clippings typing after scraping, found that this profile typically matched that of the donor's nails; their study suggests that victim's nails often would not contribute essential information in forensic casework. This type of biological evidence in many cases may be of such a small quantity that in order not to lose any foreign material no preliminary study should be carried out to try to identify specific sources of DNA. In addition, Y chromosome microsatellites are genetic markers that may be informative in cases of male/female DNA mixtures and have been successful for typing samples in ratios up to 1:2000 (male:female) or 400 pg male DNA in 800 ng female DNA (3). In this work, an alternative DNA extraction procedure for fingernail clippings after scratching was optimized.

[1] DNA Section, Chemical Laboratory Division, Federal Police, Argentina.
[2] Nuclear Biochemistry Division, CNEA-CONICET, Argentina.
[3] FBI, 935 Pennsylvania Ave., NW, Washington, DC 20535.
Received 6 Jan. 2001; in revised form 16 March 2001; accepted 22 May 2001.

Materials and Methods

Eleven pairs of female volunteers scratched known male volunteers at which time one to four fingernails were clipped and preserved for DNA analysis. In addition, a buccal swab and blood sample was taken from each volunteer as a reference control and for the comparison of the typing results obtained from the clippings. In order to simulate a forensic situation, the volunteers were paired, and the female aggressively scratched the arm of the man without bleeding. Immediately the nails were cut, collected, and stored at room temperature for two weeks before DNA extraction. The DNA mass for two and four nail samples was pooled into a single extract.

Sample Preparation

The nail clippings received no treatment before extraction, which was performed according to the original protocol of Tahir et al. (4), with a simple modification consistent of a purification step with PCR Concert™ Rapid Purification System (GIBCO-BRL, Gaithersburg, MD) after the enzymatic digestion with Proteinase K. Briefly, 400 μL Binding Solution was added to 100 μL of extract into a spin cartridge, centrifuged 12 000 *g* for 1 min, and the flow-through was discarded. Then 700 μL of Wash Buffer was added, centrifuged 12 000 *g* for 1 min twice, and finally 50 μL of TE Buffer was added and centrifuged for 2 min. The quantity of DNA was estimated using Human DNA Quantiblot (Perkin-Elmer, Foster City, CA). Sample DNA recoveries were estimated to be between 0.25 and 1 ng/μL.

DNA Typing

DNA Samples (1 to 5 ng) were amplified and typed for DYS19 locus (GenBank ID: GOO-121-409) according to Santos et al. (5), with the modification of the addition of 4 μg of BSA to the PCR mixture. The amplification product was evaluated in 2% agarose gels. PCR products were separated on 0.4 mm 6% denaturing polyacrylamide gels. Products were visualized using the silver stain method according to Budowle et al. (6).

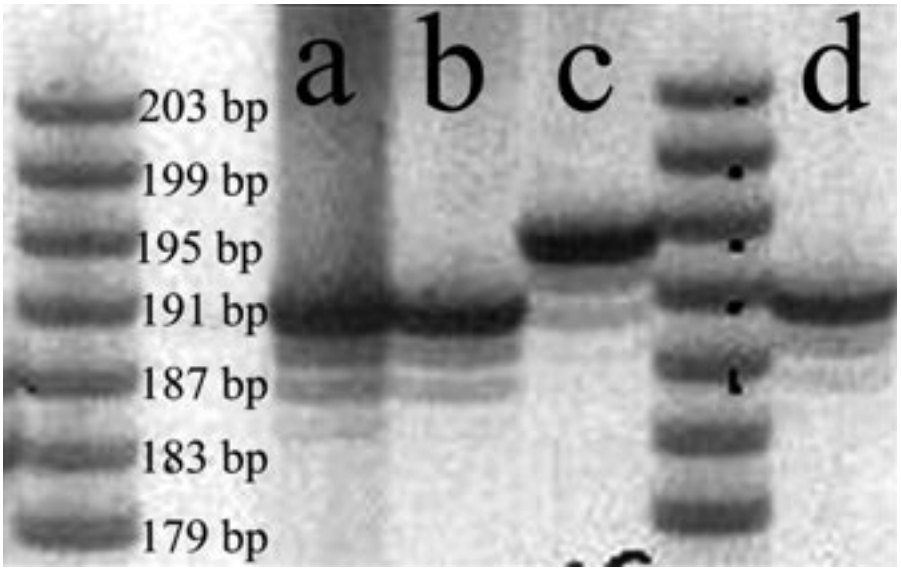

FIG. 1—*DYS19 profiles:* (a) *scratched man buccal swab,* (b) *scratched man blood,* (c) *control DNA,* (d) *scratcher nail clippings, Left: molecular weight marker.*

TABLE 1—*Typing locus DYS19 results.*

Number of Nails	Nails Mass* (mg)	Total DNA Extracted (ng)	Results	% Success	DNA Used for Amplification (ng)
1	14, 7	18, 7	−		2, 3
1	11, 3	7, 8	−	25	1, 0
1	20, 1	17, 1	−		2, 1
1	12, 1	18, 5	+		2, 3
2	9, 1	17, 3	−		2, 2
2	20, 3	14, 5	+	66, 7	1, 8
2	7, 8	9, 8	+		1, 2
4	20, 3	19, 8	+		2, 5
4	20, 4	11, 3	+	100	1, 4
4	21, 5	38, 3	+		4, 8
4	21, 5	12, 1	+		1, 5
			Mean	64	

* Mass for the two and four nail samples was pooled into a single extract.

Results and Discussion

The advantages of this method consist of: (a) the use of a nonorganic extraction technique that reduces purification-concentration time to 5 min and eliminates the use of hazardous solvents and expensive microconcentrators; and (b) the specific analysis of DYS19 marker allows the detection of the perpetrator's profile without the potential confusion of the female victim's profile. The results shown in Table 1 represent the data for locus DYS19 (probability of exclusion: 0.66) typing from fingernail clippings of the volunteers. In cases of male perpetrator and female victim the success rate of typing the suspect's profile is variable, ascending to 100% if there is sufficient DNA recovered from the nail material. As can be seen, there is no good correlation between the success ratio and the total DNA concentration and/or DNA used for amplification. It can be assumed that only a minor proportion of DNA extracted from nails belongs to the perpetrator, and, of course, it is increased with the number of nails. On the contrary, not all the nails from the victim contain the same amount of DNA as was demonstrated by Tahir et al. (4). A positive identification corresponding to one of the male DNA contributors (from four nails) is shown in Fig. 1.

Typically, the victim's nail (or skin tissue attached to the nail) will contain a considerable amount of DNA compared to the tissue transferred during minimal contact with the perpetrator. When typing low-quantity samples with autosomal STRs, the amplification process may be such that a minute amount of DNA from the epithelial cells of the perpetrator may not be detectable. This may be why the victim's profile was generally observed in the study described by Oz et al. (2).

Conclusion

In conclusion, the application of this method and the typing of more Y-STR markers may allow more definitive characterization of the perpetrator on victims' fingernail clippings, particularly in the situations where the perpetrator's contribution may be too minute or may be masked by the profile of the victim.

Acknowledgments

We want to thank the scientific advice of Dr. Leon Krawiec (CONICET) and Rocio Durán (Colombian Police DNA Analysis Laboratory). Thanks to Dr. Mario Pisarev, Marcos Agote, Alejandra Dagrosa, Pablo Noseda, Gabriela Beraldi, Mabel Viaggi, Lisa Thomasz, Guillermo Casas, and Don Santos Soria from Atomic Energy Commission for their cooperation in this work.

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Additional information and reprint requests:
David A. Gangitano
Radiobiology Unit
National Atomic Energy Commission
Av. del Libertador 8250
(1429) Buenos Aires
Argentina