TECHNICAL NOTE

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Typing of DNA HLA-DQα Alleles Extracted from Human Nail Material Using Polymerase Chain Reaction

REFERENCE: Tahir, M. A. and Watson, N., "**Typing of DNA HLA-DQα Alleles Extracted from Human Nail Material Using Polymerase Chain Reaction.**" *Journal of Forensic Sciences,* JFSCA, Vol. 40, No. 4, July 1995 pp. 634–636.

ABSTRACT: The deoxyribonucleic acid (DNA) typing of human Leukocyte Antigen (HLA) DQα from human fingernails is described. HLA-DQα genotypes can be accurately determined from clipped fingernails. We have typed 26 nails accurately, while one did not give any type since that one sample did not amplify due to the low quantity of DNA. The cut off limit for the digested material to be amplified is approximately 9 mgs of nail material.

KEYWORDS: pathology and biology, deoxyribonucleic acid, DNA polymorphism, genetic typing, HLA-DQα, locus, polymerase chain reaction, fingernails

Recent advances in recombinant deoxyribonucleic acid (DNA) techniques using variable number tandem repeat probes, or minisatellite probes, have changed current forensic methodologies and given new applications to practicing forensic science. These include positive individual identifications [*1–6*] and paternity testing [*7–9*], for example. In order to obtain accurate data in such investigations, large amounts of high molecular weight DNA is needed. This inevitable condition precludes the analysis of forensic samples, such as an aged small bloodstain, semen stain, hair or nail material.

Saiki et al. in 1985, described the PCR procedure which allowed the enzymatic amplification of a specific segment of human genomic DNA in vitro [*10*]. The PCR procedure has significantly increased our ability to detect genetic variability, and has been applied to forensic identifications.

The analysis of body fluids and tissues other than those of blood and semen to identify the donor of the tissue has an obvious role in forensic science. For example the analysis of DNA in hair by gene amplification was reported by Higuchi et al. [*11*]. Westwood et al. also reported successfully typing HLA DQα from single hairs [*12*]. Uchich et al. reported successfully typing DQα from single hairs [*13*]. Kaneshige et al., reported the amplification and typing of fingernails for the D-Loop region in MtDNA, the second exon of HLA-DPB1 gene and MCT118 locus in nuclear DNA [*14*]. Nail tissue is one which has not previously received attention concerning genetic analysis for forensics. It is conceivable that nail clippings or torn nail fragments may be recovered in the course of the collection of trace evidence samples in violent crimes such as rapes, sexual assaults and battery, etc. These nails constitute robust tissue which is likely to be resistant to degradation. In the present study we have described a reliable technique to type HLA DQα from human nails.

Received for publication 10 June 1994; revised manuscript received 24 Oct. 1994; accepted for publication 26 Oct. 1994.

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This article was presented at the Third International Symposium on Human Identification sponsored by Promega 1992.

Materials and Methods

Sample Collection

Sample nail clippings were collected from volunteers, along with a small blood sample, taken by finger prick from each donor and preserved as a dried bloodstain on clean cotton cloth. This stain acted as a positive control for comparison with any amplification product generated from the materials from the nail clipping of the corresponding donor.

Sample Treatment

DNA was extracted from the nail clippings by a modification of the protocol described by the FBI for the isolation of DNA from hair [*15,16*]. The nail was first boiled for five minutes in distilled water and then chopped into fine pieces with a scalpel. In some instances, it was possible to shave a soft layer of nail tissue from the underside of the nail. This manipulation was done to help the nails digest faster. In case of harder nails it is very important to shave nails.

The nail material was then placed in a microcentrifuge tube with 0.5 mL of digest buffer. This was composed of 10 mM EDTA, 50 mM NaC1 and 2% SDS (w/v). To the 0.5 mL of reaction mixture, 20 μL of a 1 M DTT solution and 15 μL of a 20 mg/mL solution of proteinase K were added.

The dry weight of the nail before digestion was recorded as was the weight of the dried solid residue remaining in the tubes following digestion. The difference is given in the Table 1 as the "Total nail mass digested."

The reaction mixture was incubated at 56°C for six hours, or overnight, in a water bath. The DTT and the proteinase K were then replenished by adding 20 and 15 μL of each, respectively, and incubated for a further six hours, or overnight.

The digestion products were filtered using Centricon 100 microconcentrators and the retentate was washed three times in TBE buffer (that is, 1 M Tris-base, 1 M anhydrous Boric Acid and 20 mM $Na_2EDTA.H_2O$). The DNA was quantitated by slot blot method (Table 2) to find out the quantity of the DNA to use for amplification.

Reference DNA was collected from the comparison bloodstain by placing a 2 mm^2 cutting into a microcentrifuge tube. One mL of sterile water was pipetted into the tube, and this was incubated at room temperature for 30 minutes and mixed occasionally by vortexing. The tube was then centrifuged for three minutes at 10,000–15,000 rpms, and the supernatant decanted leaving approximately 30 μL. The cutting remained in the tube with the pellet.

A 5% suspension of Chelex was added to bring to a volume of 200 μL, and the tube incubated at 56°C for 15 to 30 minutes. Following this, the tube was vortexed vigorously for ten seconds and then incubated in a boiling water bath for eight minutes [*17*]. Finally, the tube was vortexed for ten seconds at high speed and centrifuged for three minutes at 14,000 rpm. A 20 μL aliquot was added to a PCR Reaction mix.

Amplification

The amplification was conducted by the procedure specified by the Cetus Amplitype HLA DQα Forensic DNA Amplification and Typing Kit [*18*]. Some 40 μL of washed nail digest retentate was added to a mixture of 50 μL of PCR "Reaction Mix" and 50 μL of 0.8 mM $MgCl_2$ in sterile water. The amplification was conducted in either a Perkin Elmer Thermal Cycler (Indiana) or a Hybaid Thermal Reactor (Strathclyde). In both cases, thirty-two cycles, as detailed below, were used, followed by a seven minute incubation to ensure the complete extension of all the amplification product.

TABLE 1—*DQα genotypes obtained from bloodstains and fingernails from twenty-seven individuals.*

Donor #	Bloodstain DQα Type	Nail DQα Type	Total Nail Mass Digested, mgs
1	2,3	2,3	14.4
2	1.2,4	1.2,4	9.5
3	1.1,1.3	-.-	6.0
4	1.1,1.3	1.1,1.3	17
5	4,4	4,4	10.6
6	1.1,4	1.1,4	11.5
7	1.2,2	1.2,2	7.4
8	2,3	2,3	12.1
9	1.1,1.1	1.1,1.1	10.8
10	1.1,1.1	1.1,1.1	9.8
11	2,3	2,3	9.2
12	1.1,4	1.1,4	13.4
13	1.2,3	1.2,3	15.1
14	4,4	4,4	10.6
15	4,4	4,4	11.8
16	1.3,4	1.3,4	11.6
17	1.1,4	1.1,4	14.2
18	1.3,4	1.3,4	9.4
19	1.2,4	1.2,4	8.9
20	1.2,4	1.2,4	9.7
21	2,3	2,3	12.7
22	1.1,4	1.1,4	10.9
23	4,4	4,4	9.8
24	1.2,4	1.2,4	13.3
25	1.2,4	1.2,4	14.1
26	1.2,3	1.2,3	13.6
27	1.1,1.3	1.1,1.3	12.3

TABLE 2—*Total DNA (ng) extracted from various fingernail samples and the quantity of DNA used for DQα amplifications.*

Donor #	Total Nail Mass Digested, mg	Total DNA Extracted (ng)	Amount of DNA Use For Amplification (ng)
1	14.4	12.1	5
2	9.5	9.2	4.6
3	6.0	0.9	0.42
4	17	11.9	5
5	10.6	9.7	4.3
6	11.5	8.6	4.6
7	7.4	4.7	2.8
8	12.1	11.2	3.9
9	10.8	6.7	2.4
10	9.8	5.9	2.3
11	9.2	8.3	3.4
12	13.4	7.6	3.2
13	15.1	11.8	4.9
14	10.6	6.9	2.8
15	11.8	8.7	2.9
16	11.6	9.3	3.21
17	14.2	10.9	5.2
18	9.4	8.7	2.7
19	8.9	5.9	2.2
20	9.7	6.2	2.5
21	12.7	7.3	3.1
22	10.9	6.1	2.4
23	9.8	5.8	2.1
24	13.3	8.9	4.1
25	14.1	11.7	4.21
26	13.6	10.4	4.4
27	12.3	7.9	2.7

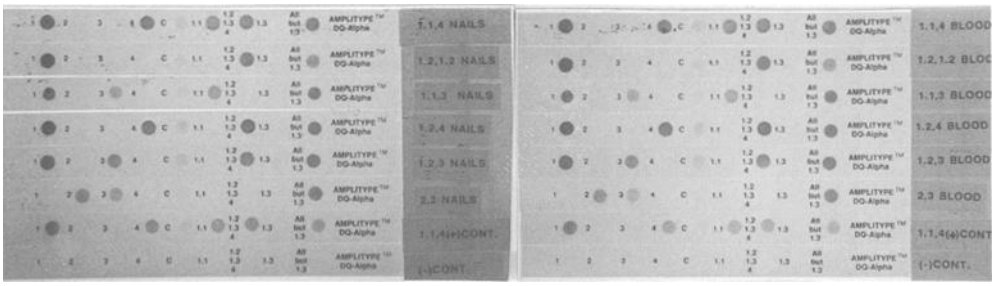
The cycle settings used were as follows: denaturation at 94°C for 60 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds.

The amplification products were subjected to post amplification product gels before committing time and reagents to DQα typing. The products were tested on a 3% NuSeive plus 1% Seakem gel, prepared and run in TBE buffer. The extracts were mixed with a bromophenol blue tracking dye solution and loaded into wells in the gel. The electrophoresis was conducted at 200 volts until the dye had run 2 cm. The DNA isolated from the dried bloodstains was not quantitated.

Results and Discussion

The results shown in Table 1 represents the data for the DQα typing from different individuals from fingernails and from the blood. In the case of donor 3, we failed to obtain any amplified product as determined by a NuSeive gel. This may be a consequence of the small quantity of nail material digested, however, we cannot confirm this at present. Sample #3 did not give us any results when checked. The total nail mass digested was 6.0 mg which was lowest of all the samples. We believe failure to type this donor might be due to the small quantity of the nail and ultimately less DNA as shown in Table 2.

These tests suggest that DNA analysis of fingernail clippings or fragments is a reliable and useful approach whenever needed. In some instances nails may be a preferred source tissue due to its relative resistance to putrification, and it may be preferred by some donors in lieu of having a blood sample taken. To date all the samples we have typed have given us the accurate type.



Acknowledgments

Thanks to Jeani Murphy for typing this manuscript and Tina Banks for graphing the tables. Thanks also due to Jim Hamby for providing the funds for this project and Sandy Sovinski and Joe Caruso for the technical assistance.

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