SEX DETERMINATION IN FORENSIC SAMPLES BY PCR: A COMPARISON OF TWO ALTERNATIVE (AMGL/AMG AND DYZ3/DXZ1) MARKERS.

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INTRODUCTION

Sex determination in forensic samples is of crucial importance in some cases. Recently, two sex-determination methods that exploit the PCR technique has been reported. The first is based on amplification of the repetitive DYZ3/DXZ1 sequences [1] while in the second the simple copy homologous AMGL/AMG genes are amplified [2].
The purpose of this study is to compare the specificity, sensibility and reliability of these two alternative PCR methods for sex determination in forensic samples.

MATERIALS AND METHODS

Samples and DNA extraction

A total of 60 fresh blood samples, 48 bloodstains, 68 cigarette butts, 82 single hairs (41 puckled and 41 shed) and 9 paraffin embedded tissues has been analyzed (Table 1). The methods used for DNA extraction were a follows:

- Standard phenol-chloroform method [3]: bloods and bloodstains.
- Chelex resin extraction approach [4]: bloodstains, single hairs and cigarette butts.
- Phenol-chloroform extraction and ultrafiltration by Centricon-100 concentrators:single hairs, cigarette butts and paraffin embedded tissues.

Amplification and electrophoresis

Amplification of AMGL/AMG [2] and DYZ3/DXZ1 [1] was performed with the followig primers secuences and conditions:

AMGL/AMG	DYZ3/DXZ1
5´-CTG ATG GTT GGC CTC AAG CCT GTG-3' 5´-TAA AGA GAT TCA TTA ACT TGA CTG-3'	X1: 5'-AAT CAT CAA ATG GAG ATT TG-3' X2: 5'-GTT CAG CTC TGT GAG TGA AA-3 Y1: 5'-ATG ATA GAA CGG AAA TAT G-3' Y2: 5'-AGT AGA ATG CAA AGG GCT CC-3'
Preincubation: 94ºC (7 min.)/72ºC (6 min.) Cycles: 30 Temp: 94/55/72ºC Time: 1 2 2 min. Aditional extension: 72ºC (10 min.)	Cycles: 30 Temp: 94/55/72ºC Time: 1 1 2 min.

After amplification the samples were electrophoresed in 2% agarose (NuSieve 3:1) (AMGL/AMG) or 3.5% agarose (NuSieve 3:1) (DYZ3/DXZ1) gel containing ethidium bromide. Alternately amplified samples were separated by semi-dry discontinuous gel electrophoresis (8%T, 3%C for AMGL/AMG and 12%T, 3%C for DYZ3/DXZ1) using the Tris-chloride/Tris-glycine buffer system followed by silver stain [5].

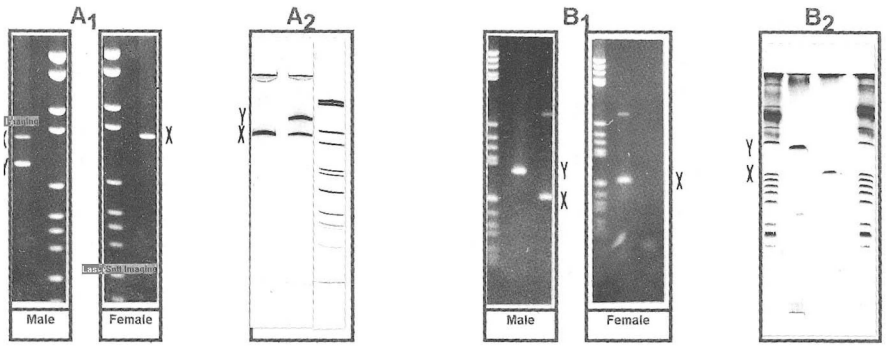

Fig. 1. Specificity of two PCR sex-determination methods. A: Electrophoretic analysis of a 788 bp PCR-amplified fragment (AMG) and a 977 bp PCR-amplified fragment (AMGL). B: Electrophoretic analysis of a 130 bp PCR-amplified fragment (DXZ1) and a 170 bp PCR-amplified fragment (DYZ3). A_1 and B_1 agarose gel electrophoresis and ethidium bromide staining. A_2 and B_2 discontinuous polyacrylamide gel electrophoresis and silver stain.

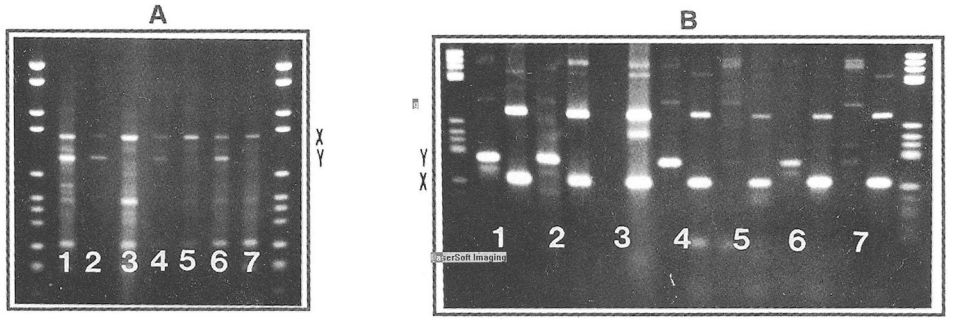

Fig. 2. Analysis of AMG/AMGL (A) and DYZ3/DXZ1 (B) from different forensic samples: 1. Male fresh blood; 2. Male bloodstain; 3. Female bloodstain; 4. Male hair; 5. Female hair; 6. Cigarette butt (smoked by a male); 7. Cigarette butt (smoked by a female).

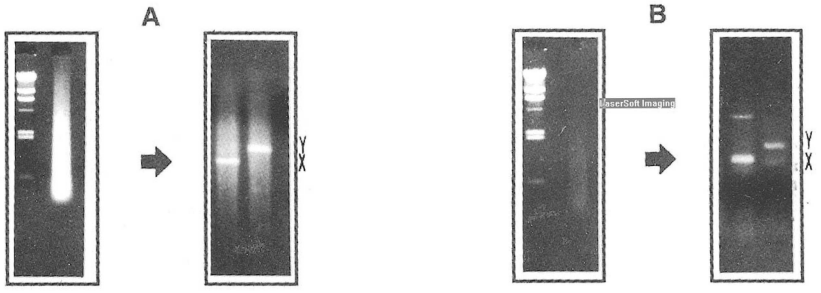

Fig. 3. Analysis of DYZ3/DXZ1 from degraded DNA samples that were unsuccessfull for AMG/AMGL amplification. (A): degraded DNA extracted from a paraffin embedded tissue. (B): degraded DNA from a ten years old bloodstain.

RESULTS AND DISCUSSION

Both the AMGL Y-specific fragment (788 bp) and the AMG X-specific fragment (977 bp) or the DYZ3 Y-specific fragment (170 bp) and the DXZ1 X-specific fragment (130 bp) were detected when male DNA was analyzed, while only the X-specific fragment (AMG or DXZ1) was observed from female samples (See Figure 1).

Both methods were employed in parallel for sex determination in different forensic samples (bloods, bloodstains, single hairs, cigarette butts and paraffin embedded tissues) and were found to be reliables (See table 1 and Fig. 2). However, the DYZ3/DXZ1 PCR-method was found to be a more sensitive method than the AMGL/AMG PCR-method as can be deduced from the rate of success obtained in critical samples (single shed hairs and cigarette butts). On the other hand, the amplification of the DYZ3/DXZ1 region was less susceptible to DNA degradation than the amplification of the amelogenin gen (See Fig. 3).

Table 1. Successful rate in the amplification of AMG/AMGL or DYZ3/DXZ1 from different forensic samples.

	AMGL/AMG			DYZ3/DXZ1		
	N		Successful	N		Successful
SAMPLES	Males	Females	rate (%)	Males	Females	rate (%)
Fresh blood	35	6	100	50	10	100
Bloodstains	29	19	100	25	15	100
Cigarette butts	21	21	47.6	40	28	95.6
Puckled single hairs	16	16	87	16	25	100
Shed single hairs	10	9	0	25	16	73.2
Paraffin embedded tissues	4	3	44.4	5	4	72.7

In conclusion, although both methods are specifics and reliables, it is recommended the use of the DYZ3/DXZ1 method for sex determination in critical and degraded samples.

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