SEX IDENTIFICATION OF FORENSIC SAMPLES USING PCR ANALYSIS FOR THE PRESENCE OF Y-CHROMOSOME SPECIFIC DNA SEQUENCES

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INTRODUCTION

Analysis for Y-chromosome specific DNA sequences can be used to determine the sex of donors of biological samples containing nuclear DNA. In a case in which a child had died in a fire accident, and the abdomen was charred beyond recognition of the sex, we analysed DNA from an intact vertebra for the presence of the following Y-specific sequences, using PCR: alpha satellite repeat (DYZ3), part of the testis determining gene (SRY), and part of the Y-specific zinc-finger protein gene (ZFY).

MATERIALS AND METHODS

A large yield of high molecular weight DNA was obtained from the vertebra by routine methods. Control samples were either isolated DNA or stabilized whole blood from normal adults.
For all three systems PCR was carried out on a programmable thermoblock (Hybaid) in 100 µl, containing 200 µM of each dNTP and 2.5 units of Taq DNA polymerase (Promega). The PCR products were analysed by agarose gel electrophoresis (2 % Nusieve + 1 % Bio-Rad) with HinfI digested φX174 as size marker.
DYZ3. Primers: 5'-ATGATAGAACGGAAATATG-3' and 5'-AGTAGAATGCAAAGGGCTCC-3' (PCR product 170 bp; Witt and Erickson 1989). Isolated DNA from normal adults (two males and a female) served as controls. Primers 0.4 µM each; DNA either 1.0 or 0.1 µg.
35 cycles were performed as follows: 1 min at 90°C (3 min in the first cycle), 1 min at 50°C, and 1.5 min at 70°C (6.5 min in the last cycle).
SRY. Primers: 5'-GAATATTCCCGCTCTCCGGAG-3' and 5'-ACCTGTTGTCCAGTTGCACT-3' (PCR product 418 bp; Sinclair et al. 1990). Blood samples from normal adults (a male and a female) served as controls. A 321 bp fragment of the Factor IX gene (F9) was amplified as an internal control. Primers: 5'-AACATAGGTGAAAGTCAATTAAG-3' and 5'-TTCTCAATCACAGTACCAGTAAT-3' (Yoshitake et al. 1985).
Primers 0.1 µM each; DNA per reaction was 0.1 µg from the forensic sample, and 2 µl whole blood from each of the controls. With the forensic sample 35 cycles were performed as described for DYZ3. With control blood samples the first PCR cycle was skipped, and the reaction mixture for the remaining 34 cycles was prepared as follows (Schwartz 1991): 2 µl blood were incubated with Taq polymerase buffer (Promega) for 1 h at room tp. followed by 10 min denaturation at 99°C, and subsequent addition of primers, dNTPs, and Taq polymerase.

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ZFY. Primers: 5'-CATCCTTTGACTGTCTATCCTTG-3' and 5'-CATTATGTGC-TGGTTCTTTTCTG-3' (PCR product 1131 bp; Schneider-Gädicke et al. 1989, Palsbøll et al. 1991).
PCR was carried out as described for SRY except that annealing was at 60°C, and elongation was 4 min per cycle (14 min in the last cycle).
After amplification 5 µl reaction mixture were incubated with 10 units TaqI restriction enzyme in 20 µl at 65°C for 1.5 h.

RESULTS AND DISCUSSION

Photographs of the three PCR gels are shown in Fig.s 1 and 2.

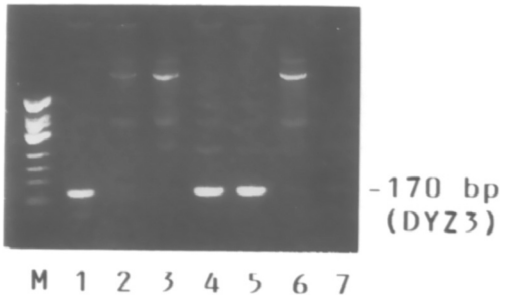

Figure 1. Analysis for Y-specific alpha repeat (DYZ3). M: size marker (φX174/HinfI). Lane 1: previously amplified 170 bp alpha repeat fragment (normal male). Lanes 2 and 3: female control. Lanes 4 and 5: male control. Lanes 6 and 7: forensic sample. Even no.s: 1.0 µg DNA. Odd no.s: 0.1 µg DNA

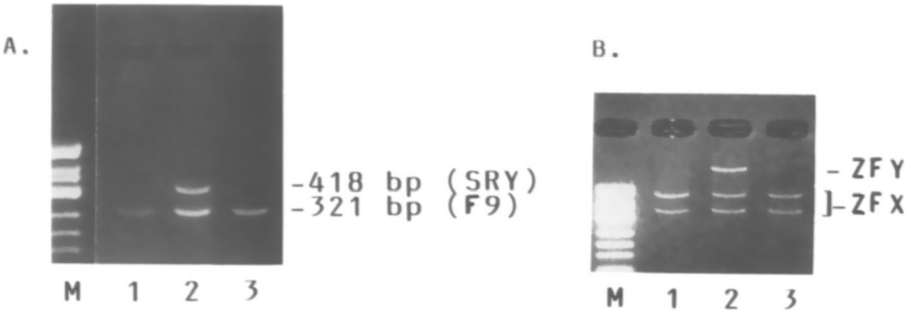

Figure 2. Panel A: Analysis for SRY, with F9 fragment as internal control. Panel B: Analysis for ZFY. M: size marker (φX174/HinfI). Lanes no. 1: forensic sample, 0.1 µg DNA. Lanes no. 2: male control (2 µl blood). Lanes no. 3: female control (2 µl blood)

DYZ3 (Fig. 1). DNA from the male controls gave a high yield of the expected 170 bp repeat fragment (lanes 1, 4 and 5), whereas both the female control (lanes 2 and 3) and the forensic sample gave weaker and larger fragments, which served as convenient internal controls.

SRY (Fig. 2A). The F9 fragment was amplified in all three reactions, but only the male control (lane 2) gave the SRY-specific 418 bp fragment.

ZFY (Fig. 2B). The primers used in these reactions amplify fragments of identical size from homologous genes on the X (ZFX) and Y (ZFY) chromosomes. The present analysis exploits the fact, that the ZFX fragment has a TaqI site (bp no. 670-673), which is absent in the ZFY fragment. Another TaqI site (bp no. 49-52) is present in both fragments. TaqI thus cleaves off a 50 bp fragment from both PCR products and cuts the ZFX product once more giving two larger fragments of approx. 620 and 460 bp, while leaving the corresponding 1080 bp ZFY fragment intact.
In the present analysis only the male control (lane 2) gave PCR products both with and without the TaqI site in question.

It is evident from these results, that the DNA from the forensic sample did not contain any of the three Y-chromosome specific DNA sequences, in agreement with the police report of a missing two-year old girl.

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