

## Sex determination in bloodstains and single hairs

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### INTRODUCTION

Several methods for sex determination of human DNA have been developed, focusing on detection of the Y-specific chromosomal sequences (Gosden 1984; Fukushima 1988; Nagai 1991). The major component is a repeated sequence family characterised by the presence of Hae III sites spaced at 3.5kb. Various DNA extraction methods and DNA analysis were studied, with the aim of performing sex determination of minute human materials (McCabe 1987)

As we reported earlier, DNA was extracted from 10 µl one-year-old dried blood spots on paper, digested with restriction enzyme, then subjected to electrophoresis, transfer and hybridization. The human male Y-specific probe was hybridized with Y-specific 3.5kb repetitive-DNA.

The polymerase chain reaction (PCR) is an extremely useful method for primer-directed enzymatic amplification of Y-specific chromosomal sequences (Witt 1989). In order to compare with DNA microextraction methods for the PCR template, we have prepared many DNA samples from bloodstains and hairs using various methods, such as Method I (phenol-Centricon 30), Method II (phenol-ethanol precipitation), Method III (Tween 20-treatment) and Method IV (boiling).

### MATERIALS AND METHODS

Bloodstain materials were prepared by aliquoting known quantities of blood onto Whatman 3MM filter paper. DNA was extracted by the four methods described below.

Method I: In general, bloodstains were cut into pieces. These samples (bloodstains or hairs) were rinsed with cell lysis buffer (0.32M sucrose, 1% Triton X-100, 5mM MgCl<sub>2</sub>, 10mM Tris-HCl, pH7.5). Samples were resuspended in digestive buffer (10mM Tris-HCl, pH8.0, 10mM EDTA, 0.1M NaCl) containing 1% SDS and proteinase K (20µg/ml). The mixture was incubated for over 3 hours at 50°C. After the solution was extracted with water-saturated phenol, TE buffer was added. The solution was concentrated on a Centricon 30 microconcentrator (Amicon). After addition of distilled water and a second concentration, the solution was used for the DNA templates.

Method II: After rinsing with cell lysis buffer and centrifugation, bloodstains (hairs) were incubated in digestive buffer. DNA was purified by one phenol/chloroform extraction and one ethanol precipitation.

Method III: After treatment with cell lysis buffer, samples were suspended in another digestive buffer (50 mM Tris-HCl, pH8.5, 1mM EDTA and 0.5% Tween 20) containing Proteinase K. The samples were incubated for over 3 hours at 50°C, then subjected to centrifugation. These solutions were again incubated at 95°C for 10 minutes to inactivate the protease. The supernatant was used for PCR after centrifugation.

**Method IV** : Bloodstains were rehydrated in 3 ml of 0.85% NaCl for 2 hours. After centrifugation, the filter pieces were washed with 0.85% NaCl again and the elutant was spun down. The pellet was resuspended in 50 $\mu$ l of sterile water and boiled for 10 min. The solution was used as a template.

PCR was carried out at 94 $^{\circ}$ , 55 $^{\circ}$  and 72 $^{\circ}$  for 30 cycles using two pairs of primers(Y1,Y2 and X1,X2)(Witt 1989). Each product was analyzed on 12% polyacrylamide gel in a minigel apparatus.

## RESULTS AND DISCUSSION

The 170-bp amplification product was detected in male DNA from 0.5-1 $\mu$ l of three-year-old bloodstains extracted by either the phenol-Centricon-30 method or phenol-ethanol precipitation method. Although the same patterns of PCR product were obtained for 1 $\mu$ l of 6-month-old male bloodstain extracted by Tween 20 -treatment, amplified DNA was not detected in one-year-old or older bloodstains. In contrast, a relatively large amount of bloodstain was required for sex determination of DNA extracted by the boiling method. Amplified results were obtained from DNA of hair roots or the hair shaft(2-3cm) extracted by both the phenol-Centricon-30 and Tween 20-treatment methods.

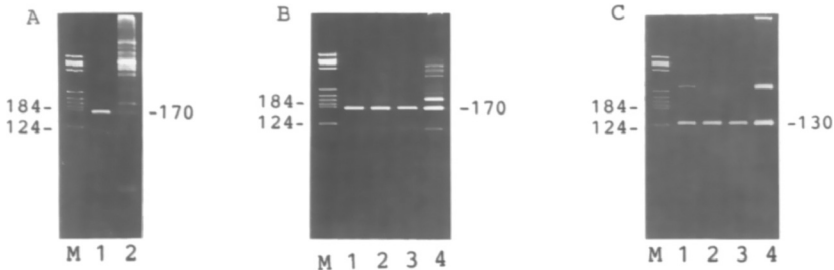


Fig.1 Effect of the Mg<sup>++</sup> concentration and the amount of template DNA  
 A:Y-specific band(170bp) B:Y-specific band(170bp)  
 M: pBR-322/Hae III C:X-specific band(130bp)  
 1: 1.5mM MgCl<sub>2</sub> 1: 1ng DNA 2: 10ng DNA  
 2: 2.5mM MgCl<sub>2</sub> 3: 50ng DNA 4: 100ng DNA

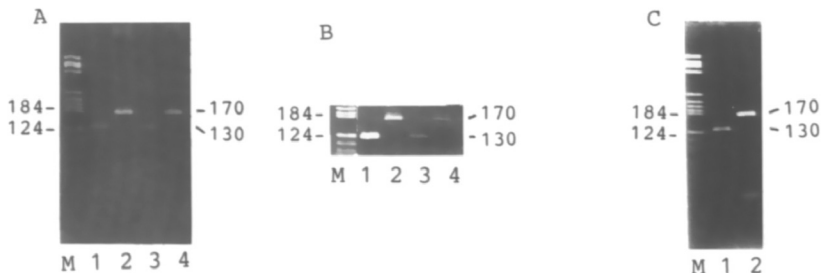


Fig.2 PCR analysis of DNA extracted from male bloodstains.  
 A(Method I): B(Method III): C(Method IV):  
 Y1,Y2 primers(2,4) Y1,Y2 primers(2,4) Y1,Y2 primers(2)  
 X1,X2 primers(1,3) X1,X2 primers(1,3) X1,X2 primers(1)  
 1,2:0.5 $\mu$ l bloodstains 1,2:1 $\mu$ l bloodstains 1,2:50 $\mu$ l  
 3,4:5 $\mu$ l bloodstains 3,4:5 $\mu$ l bloodstains bloodstains

These results indicate that this PCR method may be very useful in neonatal screening for some genetic diseases and in forensic research for the analysis of biological evidence.

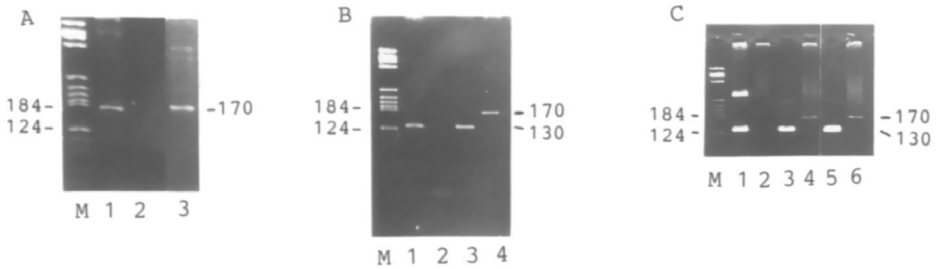


Fig.3 PCR analysis of DNA extracted from male hair shafts

A(Method I):	B(Method II):	C(Method III):
Y1,Y2 primers(1,2,3)	Y1,Y2: primers(2,4)	Y1,Y2 primers(2,4,6)
1:hair bulb	X1,X2: primers(1,3)	X1,X2 primers(1,3,5)
2:1 cm	1,2:2 cm	1,2:1 cm, 3,4:2 cm
3:2 cm	3,4:5 cm	5,6:5 cm

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