Design of novel oligonucleotide probes for sex determination and its forensic application

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INTRODUCTION

Sex determination of forensic samples is a very important element in the analysis of biological evidence submitted to forensic science laboratories. Although sex determination has been performed by Southern blotting (Gill 1987; Kobayashi 1988) or PCR (Kogan 1987; Witt 1989), we sometimes observed that the results obtained by PCR were different from those obtained by Southern blotting. Since the contamination of the samples found in criminal spots is usually unknown, PCR may sometimes give false results because of its high sensitivity. By contrast, although Southern blotting for sex determination is less sensitive and takes a long time, the findings obtained are reliable. The aim of our study was the design of the novel oligonucleotide probes for Southern blotting to lower the detection limit and to shorten the working time.

MATERIALS AND METHODS

Design of oligonucleotides

Two oligonucleotide probes (YJ1: TTC CAT TCC ATT CCA TT, Y1.3: TTC TAT TCC CTT CTA CTG CAT AC) were designed and synthesized. The region with 88.2% and higher homology with YJ1 is found in 45.8% of DYZ1 (Nakahori 1986). By contrast, the region with 80% and higher homology with Y1.3 is located in only 0.65% of DYZ1. These oligonucleotides were labeled with alkaline phosphatase by LIGHTSMITH II Luminescence Engineering System (Promega, WI).

Southern blotting

DNA was isolated from 123 bloodstains picked up in criminal spots as described elsewhere (Kobayashi 1988) and was digested with HaeIII or EcoRI. After electrophoresis of digested DNA on 0.8% agarose gel and transferring the fractionated DNA fragments onto a nylon membrane, the membrane was hybridized with each probe for 1 hour at 42°C. After

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hybridization, the membrane was washed three times with 6 x SSC for 5 min at 42°C. The probe-target hybrids was detected by development with NBT/BCIP. The DNA specimens were concluded to be male, when the 3.4-kb band was apparently observed.

PCR for sex determination

For comparison with Southern blotting, PCR was performed as described by Witt, M. et al. (1989). About 50 ng of DNA isolated from bloodstains was amplified using Y chromosome specific primers (Y1: ATG ATA GAA CGG AAA TAT G, Y2: AGT AGA ATG CAA AGG GCT CC) or X chromosome specific primers (X1: AAT CAT CAA ATG GAG ATT TG, X2: GTT CAG CTC TGT GAG TGA AA). PCR was run for 30 cycles of 94°C for 1 min, 65°C for 1 min, and 72°C for 2 min. After amplification, the PCR products were analyzed by electrophoresis on 2% agarose gels. The DNA specimens were concluded to be male, when the 170-bp band was apparently observed.

RESULTS AND DISCUSSION

1. In Southern blotting, the Y1.3 probe could determine the sex of specimens using 400 ng of DNA. By contrast, the YJ1 probe could also determine the sex using 25 ng of the DNA (Fig. 1).
2. The working time could be shortened from 23 hours to 3.5 hours by the use of the YJ1 or Y1.3 oligonucleotide probe.
3. Sexes were determined in 106/123 (86.2%) of bloodstains by Southern blotting with both probes (Table 1). Of the remaining 17 specimens, we could not determine the sex, because the yield of DNA isolated from these bloodstains was very low. However, no mistake in sex determination results occurred.
4. PCR was used to determine the sexes in 32/36 (88.9%) of bloodstains. The sex determined by PCR was different from that determined by Southern blotting in one case. In this case, the bloodstain spattered on men's underwear was used. Although the result of PCR demonstrated that the sex of specimen was male, the true sex of specimen was female. This indicates that PCR could detect not only a female's DNA isolated from bloodstains but also a man's slight DNA isolated from the man's underwear because of the high sensitivity of this method.
5. Although the sensitivity of Southern blotting is not higher than that of PCR, we did not obtained any false results by Southern blotting.
6. In conclusion, despite the lower sensitivity compared to PCR, Southern blotting using YJ1 or Y1.3 oligonucleotide as a probe is a suitable method for sex determination in forensic practical cases.

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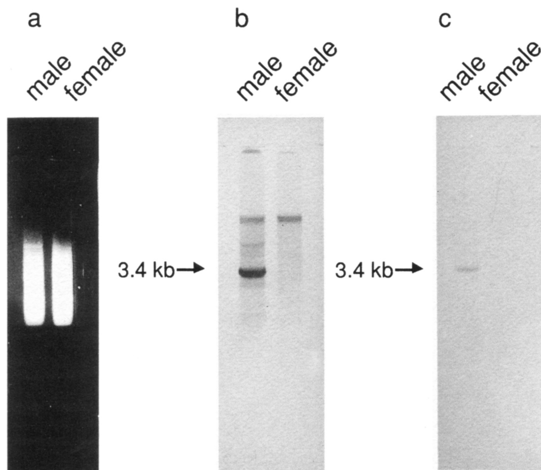

Fig. 1 Hybridization patterns of HaeIII-digested DNA. Four hundred nanograms of DNA was digested with HaeIII and fractionated on 0.8% agarose gel. After transferring DNA fragments onto a membrane, the membrane was hybridized with YJ1 or Y1.3 probe. a; ethidium bromide staining, b; hybridization with YJ1, c; hybridization with Y1.3

Table 1. The results of sex determination of bloodstains by Southern blotting with YJ1

Sex estimated prior to the test	Sex determined by Southern blotting: Case (%)			
	Male	Female	Undetermined	Total
Male	60 (89.6)	0 (0.0)	7 (10.4)	67 (100.0)
Female	0 (0.0)	40 (87.0)	6 (13.0)	46 (100.0)
Unknown	5 (50.0)	1 (10.0)	4 (40.0)	10 (100.0)
Total	65 (52.9)	41 (33.3)	17 (13.8)	123 (100.0)

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