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Short Communication

SEX IDENTIFICATION BY POLYMERASE CHAIN REACTION USING A Y-AUTOSOME HOMOLOGOUS PRIMER SET

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Summary We have developed a one-step polymerase chain reaction (PCR) technique which detects a sequence on the human Y chromosome and an autosomal sequence in one reaction. The method is very reliable for the sex determination, as the detection of the autosome-specific signal ensures the presence of DNA in the specimen even in the absence of the Y-specific signal.
Key Words polymerase chain reaction, PCR, sex identification, sequence tagged site, STS

Sex identification of clinical and forensic samples has been performed by both cytogenetic (ISCN, 1985) and DNA analysis (Kobayashi *et al.*, 1988). Recently, the polymerase chain reaction (PCR) has become popular since it requires minimal amount of samples, no isotope and a very short period of time (Witt and Erickson, 1989; Kogan *et al.*, 1987). The PCR technique depends its judgments on the presence or absence of a Y-specific signal. Therefore, a failure of technique, or degraded samples could result in an erroneous conclusion.

Previously, we reported a method of sex identification by PCR using a pair of primers from the X-Y homologous region coding the amelogenin gene (Nakahori *et al.*, 1991). The most advantageous point of the method was the success of monitoring by the presence of the X-chromosome signal since both the X (977 bp=base pairs) and the Y (788 bp) sequences were amplified simultaneously. In the field of archeology, forensic or sports medicine using buccal smear, the use of primers which yield shorter PCR products is preferable because the sample DNA

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is usually degraded and a short DNA segment has a higher chance to be intact.

In the course of studying Y chromosome, we have determined partial nucleotide sequences of the cosmid p20/21 (Wolfe *et al.*, 1984) located on the Y chromosome long arm. A pair of primers were designed for a sequence tagged site (STS) were:

p20/21 B2-1; 5′=ATCTCAGGACCCAACACCCG=3′

p20/21 B2-2; 5′=GATATGTCCCAGTGTCCCCAT=3′

Genomic DNA was prepared from the peripheral blood leukocytes of normal males and females. PCR procedures were described earlier (Nagafuchi *et al.*, 1992). Cycling conditions are; 25 cycles of 94°C for 1 min, 64°C for 2 min, and 72°C for 2 min. The final extension time was increased to 12 min at 72°C.

The expected PCR product for the Y-chromosome is 120 bp. Examples of electrophoresed PCR products using the above primers are shown in the Fig. 1. The presence of a 270 bp autosomal product in all the lanes could be a monitor for the success of the procedures. We have tested a total of 100 Japanese and Caucasian samples (50 males and 50 females), and attained to the correct sex diagnosis in all the samples.

We reported a method of sex identification using a novel primer pair, with which the Y chromosome and an autosomal sequences are amplified simultaneously. Since the procedure reveals both the Y-specific and an autosome sequence of different size, the method is dependable in the field handling a very small amount of or degraded DNA samples. In addition, the present technique amplifies short stretches of DNA fragments and thus should be resistant to the degradation of DNA samples.

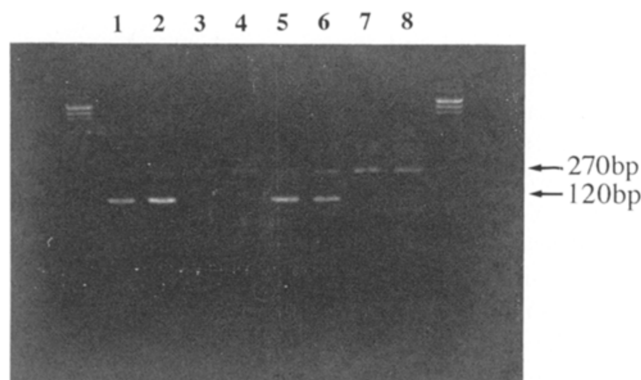

Fig. 1. Photograph of electrophoresed PCR product. Lanes 1 and 2, Caucasian males; lanes 3 and 4, Caucasian females; lanes 5 and 6, Japanese males; and lanes 7 and 8, Japanese females. In addition to the 120 bp band from the Y chromosome, a discrete 270 bp band always appears in both male and female samples of the same density, which indicates the 270 bp band was derived from an autosome.

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