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PCR에 의한 X,Y-Specific Alphoid Repeat Sequences의 분석

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Gender Determination of X and Y-Specific Alphoid Repeat Sequences by PCR

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요 약: 사람에게서 유래된 DNA시료의 X,Y 특이의 alphoid gene을 PCR법으로 분석하면 성별을 확인할 수 가 있다. PCR법으로 alphoid gene을 분석한바 매우 예민도가 높아 genomic DNA 약 60pg까지 성별을 분석할 수 있었다. 그리고 성별이 혼합되어 있는 DNA에서 female DNA의 1/10비 까지는 male DNA를 분석할 수 있었다. 따라서 이 결과는 혼합된 DNA에서 X,Y 특이의 alphoid gene을 분석하는데 기준으로 활용할 수가 있다.

Abstract: Recently, it has been possible to the alphoid gene, which has X and Y specificity, and determine the sex from human physical evidence using PCR methods. Samples from single sources, PCR method applied to the alphoid gene results in highly sensitive and accurate results even when only 60 pg of the genomic DNA was available for sex determination. Even for samples containing DNA from more than one gender source where the female DNA was present in the amount 10 times than that of the male, sex determination was possible. Therefore, this result suggests that alphoid gene, which has X and Y specificity, could be used effectively for sex determination in case of mixed DNA samples from biological evidence.

Key words: gender analysis, alphoid gene, PCR, human DNA

1. INTRODUCTION

Gender analysis by polymerase chain reaction (PCR) can be a valuable tool not only after selective lysis of mixed stains but also in the analysis of pure stains. Since in many forensic cases persons of both sexes are involved, a rapid and simple sex determination assay may give valuable information on the origin of the stain by identifying the sex of an unknown person involved in the crime.

Several methods for sex determination with PCR have been described which combine PCR with subsequent restriction digest followed by agarose gel electrophoresis[1,2] or with dot blot hybridization with Y-specific probes.[3,8] The amplified regions of the X and Y chromosomes are the alphoid repeat regions,[10] the zinc finger protein ZFX/ZFY,[1] DYZ1/DXS424,[7] or the amelogenin gene.[5,9] However the most rapid methods require only electrophoresis of the amplified products in an agarose gel.[5,7,10]

Mixtures of blood and sweat or saliva on clothing and mixtures of vaginal fluid and semen may originate from 2 different persons. A problem in the analysis of mixed stains is DNA competition which presumably occurs in VNTR (variable number of tandem repeats) and STR (short tandem repeats) system during PCR.

We report on the sex determination of mixed

DNAs by amplification of X and Y-specific DNA alphoid repeat regions using pair of two different primer as described by Witt and Erickson.[10]

2. MATERIAL AND METHODS

K562 DNA (Promega) and Human genomic DNA (Promega) were used as the quantified DNAs of female DNA and male DNA, respectively. Selective lysis from mixed stain in a case of sexual assault was done in the TEN buffer (40 mM Tris-Cl; pH 7.5, 1 mM EDTA; pH 8.0, 150 mM NaCl) containing proteinase K and DTT (dithiothreitol).

The amplification of X and Y-specific DNA fragments was performed in 2 parallel assays with primers described by Witt and Erickson[10] in 15 μl reactions by thermal cycler (Perkin-Elmer Cetus 9600, *Table* 1). After 30 cycles (30 sec at 94°C, 30 sec at 55°C, 40 sec at 72°C) the amplified X-specific (131 base pair; bp) and Y-specific (170 bp) products were separated in a 3% agarose gel and visualized by EtBr (ethidium bromide) staining.

3. RESULTS AND DISCUSSION

PCR products from female genomic DNA and male genomic DNA are shown in *Fig*. 1.

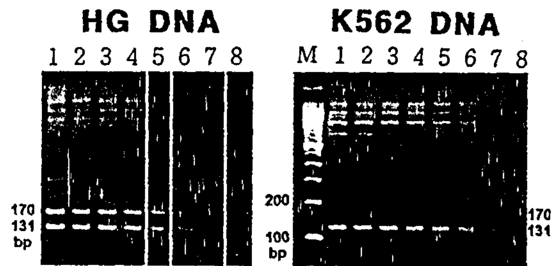

Fig. 1. Gender analysis of the diluted DNAs. HG DNA: Human genomic DNA (Promega), M: 100 bp DNA ladder (Gibco-BRL), 1; 10 ng, 2; 5 ng, 3; 2 ng, 4; 1 ng, 5; 0.5 ng, 6; 0.25 ng, 7; 0.125 ng, 8; 0.0625 ng.

The Y-specific (170 bp) band is absent from the female PCR products, the X-specific PCR products yield the X-specific (131 bp) band.[10] The X,Y-specific alphoid repeat sequences are very sensitive. About 60 pg of genomic DNA per 15 μl assay is sufficient to give clearly visible PCR products after 30 cycles of amplification (*Table* 2). Our studies revealed DNA competition during PCR when mixed DNAs were investigated for gendering by X,Y-specific alphoid repeat sequences. Competition can be a drawback in PCR analysis of mixed stains, since the cells from one of the individuals can be in

Table 1. Primer sequences and PCR conditions for X and Y-specific alphoid repeat sequences

Locus	Primer sequences (5'→3')	PCR conditions		
		denaturation	annealing	extention
Chromosome X-specific alphoid repeat sequences				
X1	dAATCATCAAATGGAGATTTG	94°C	55°C	72°C
X2	dGTTCAGCTCTGTGAGTGAAA	30 sec	30 sec	40 sec
Chromosome Y-specific alphoid repeat sequences				
Y1	dATGATAGAAACGGAAATATG	94°C	55°C	72°C
Y2	dAGTAGAATGCAAAGGGCTC	30 sec	30 sec	40 sec

Table 2. Gender analysis of the mixed DNAs

K562 DNA\HG DNA	10	5	2	1	0.5	0.25	0.125	0.063 (ng)
10	++	++	++	++	+	+	±	-
5	++	++	++	++	+	+	±	-
2	++	++	++	++	+	+	±	-
1	++	++	++	++	+	+	±	-
0.5	++	++	++	++	++	++	+	+
0.25	++	++	++	++	++	++	+	+
0.125	++	++	++	++	++	++	++	+
0.0625 (ng)	++	++	++	++	++	++	++	±

Relative intensities of the bands are indicated by the number of (+) signs, (±); faint bands, (-); no bands visible in the gel.

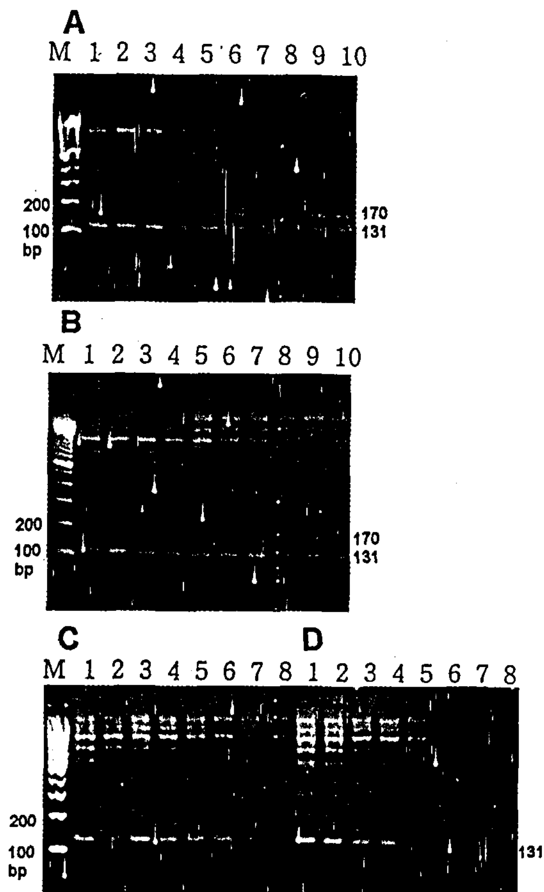

Fig. 2-1. Gender analysis of the mixed DNAs. A; HG DNA 0.5 ng, B; HG DNA 0.25 ng, C; HG DNA 0.125 ng, D; HG DNA 0.063 ng. M: 100 bp DNA ladder (Gibco-BRL), lane 1; 10 ng, lane 2; 5 ng, lane 3; 2 ng, lane 4; 1 ng, lane 5; 0.5 ng, lane 6; 0.25 ng, lane 7; 0.125 ng, lane 8; 0.063 ng of K562 DNA.

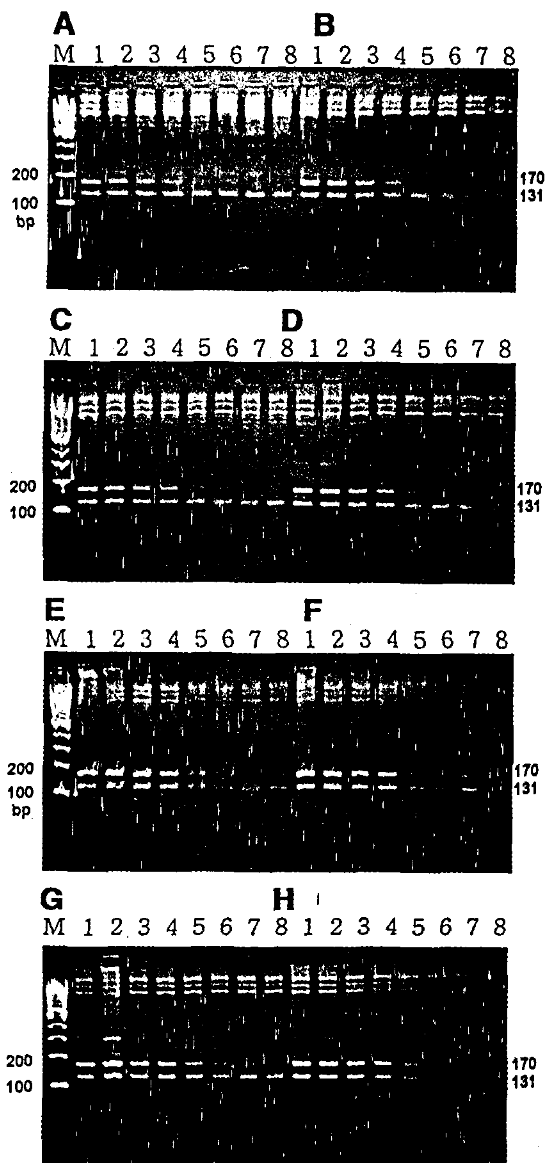

Fig. 2-2. Gender analysis of the mixed DNAs. A; K562 DNA 10 ng, B; K562 DNA 5 ng, C; K562 DNA 2 ng, D; K562 DNA 10 ng, E; K562 DNA 1 ng, F; K562 DNA 0.5 ng, G; K562 DNA 0.125 ng, H; K562 DNA 0.063 ng. M: 100 bp DNA ladder (Gibco-BRL), lane 1; 10 ng, lane 2; 5 ng, lane 3; 2 ng, lane 4; 1 ng, lane 5; 0.5 ng, lane 6; 0.25 ng, lane 7; 0.125 ng, lane 8; 0.063 ng of HG DNA.

high excess in the case of samples containing sperm and other cells. As such, depending on the quality of the specimen[8] selective lysis is not always 100% effective. We sought for a XY-PCR process keeping in mind its sensitivity and the competition which occurs when using DNA mixtures. In the presence of a 21-fold excess of DNA, corresponding to a 10-fold higher concentration of female DNA, Y-specific product of the male DNA was easily detected (*Fig*. 2-1 and 2-2).

Higher primer concentrations may be helpful to reduce the effect of competition, but this increases the risk of artefacts due to mispriming. Also silver staining of the gels instead of staining with EtBr or southern blotting, and subsequent hybridization with labeled probes will be helpful for a better detection of the poorly amplified alleles.

Amplification of both X and Y-specific PCR is a

rapid and easy method which could lead to successful investigation by enrichment of the sperm cells through preferential lysis in case of mixed samples containing both sperm and vaginal secretions. Because of its sensitivity, its apparent lack of competition effects and easy detection, we consider the method reported here rapid and valuable for sex determination in forensic stains.

REFERENCES

1. E. Aasen and J. F. Medrano, Amplification of the ZFX and ZFY genes for sex identification in humans, cattle, sheep and goats. *Biotechnology*, **8**, 1279 (1990).
2. R. E. Gaensslen, K. M. Berka, D. A. Grosso, G. Ruano, E. M. Pagliaro, D. Messina and H. C. Lee, A polymerase chain reaction (PCR) method for sex and species determination with novel controls for deoxyribonucleic acid (DNA) template length. *J. Forensic Sci.*, **37**, 6 (1992).
3. Z. H. He, X. H. Jiang, S. H. Lu, G. L. Wang, Y. W. Zhu, S. W. Wang, Y. Shen, Q. S. Gao, J. Z. Liu and G. Y. Wu, A study of sex identification of trace, dried bloodstains using a Y-chromosome specific deoxyribonucleic acid (DNA) probe. *J. Forensic Sci.*, **34**, 346 (1989).
4. J. Kreike and A. Lehner, Sex deternimation and DNA competition in the analysis of forensic mixed stains by PCR. *Int. J. Leg. Med.*, **107**, 235 (1995).
5. A. Mannucci, K. M. Sullivan, P. L. Ivanov and P. Gill, Forensic application of a rapid and quantitative DNA sex test by amplification of the X-Y homologous gene amelogenin. *Int. J. Leg. Med.*, **106**, 190 (1994).
6. S. Patri, L. Daheron and A. Kitzis, Chomel J. C. Evaluation of bone marrow transplanatation efficiency by competitive PCR on Y sequences. *PCR Methods Appl.*, **3**, 361 (1994).
7. H. Pfitzinger, B. Ludes and P. Mangin, Sex determination of forensic samples: co-amplification and simultaneous detection of a Y-specific and an X-specific DNA sequence. *Int. J. Leg. Med.*, **105**, 213 (1993).
8. L. Potsch, U. Meyer, S. Rothschild, P. M. Schneider and C. Rittner, Application of DNA techniques for identification using human dental pulp as a source of DNA. *Int. J. Leg. med.*, **105**, 139 (1993).
9. K. M. Sullivan and A. Mannucci, C. P. Kimpton and P. Gill. A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. *Biotechniques*, **15**, 636 (1993).
10. M. Witt and R.P. Erickson. A rapid method for detection of Y-chromosomal DNA from dried blood specimens by the polymerase chain reaction. *Hum genet.*, **82**, 271 (1989).