Paternity Testing by PCR-Based STR Analysis

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Abstract

We present application of polymerase chain reaction (PCR) - based short tandem repeat (STR) system for use in paternity testing. The process involves a single tube multiplex PCR of 9 STR loci on different chromosomes, in conjunction with Amelogenin sex test and internal size standards, followed by using an automated DNA sequencer to detect amplified products. The results showed that this system provided unambiguously reliable results. In addition, the method is useful for routine use in that it is robust and reproducible and provides a reliable means of paternity testing.

Key word : Paternity Testing, PCR, STR

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A polymerase chain reaction (PCR)-based short tandem repeat (STR) system has recently been developed for use in routine forensic identity testing[1]. The methodology involves the simultaneous amplification of alleles at many loci on different chromosome[2]. The use of fluorescence detection technology in conjunction with PCR-STR analysis allows for efficient automation. STR loci are found in large numbers throughout the human genome and are usually approximately every 6-10 kb[3]. Since STR loci have small allele sizes (generally less than 300 bp), they can be amplified easily

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by PCR and degraded samples are more amenable to analysis. PCR -based STR analysis for DNA profiling can be completed in a few days rather than several weeks as necessitated in RFLP (restriction fragment length polymorphism) analysis[4]. In addition, the increasing use of automation enhances the prospects of computer aided systems to perform the identification of STR alleles[5].

The purpose of this paper was to demonstrate the analysis of paternity testing using PCR-based STR method.

MATERIAL AND METHOD

Samples

EDTA-blood samples from individuals who requested paternity testing were collected and sent for DNA typing at Human Genetics Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital.

Amplification of sample DNA

Genomic DNAs were extracted from EDTA-blood samples as described previously[6]. Then, we performed multiplex PCR of 9 STR loci and the Amelogenin sex test using commercially available kits, either Profiler kit or Profiler Plus kit (Perkin Elmer, Applied Biosystem, USA). These STR loci in the first kit includes D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820. The STR loci in the second kit consisted of the same loci idicated in the first kit except loci D8D1179, D21S11 and D18S51 instead of TH01, TPOX and CSF1PO. Amplifications were carried out in thin-walled MicroAmp tubes (Perkin Elmer) in a geneAmp PCR System 2400 (Perkin Elmer) using the following conditions: 95°C for 13 min, 94°C for 1 min, 59°C for 1 min, 72°C for 1 min, for 28 cycles and followed by 60°C for 45 min. The amplified products were electrophoresed on automated DNA sequencer model 310 (Perkin Elmer). Fragment sizes were determined automatically using Genescan software (Perkin Elmer) and allelic designations were made by reference to allelic ladders.

RESULTS

Simple paternity testing

STR loci are routinely employed for individual identification. We examined the performance and reproducibility of a highly informative co-amplification system of a tetranucleotide STR loci in conjunction with the Amelogenin sex test. In a heterozygote, each heterozygous locus consists of 2 alleles and the peak areas are automatically calculated by Genescan software. The simple paternity testing of the first family consisted of a father, a mother and 2 sons (Fig. 1A-C). These 2 sons inherited each allele from either the father or mother and the result was inclusion. When a child does not inherit his alleles from an alleged father, it means that the alleged father is not the biologlical father of that child and the result of paternity testing is exclusion. The second family requested paternity testing of an alleged father, a mother and 2 children, a son and a daughter. The children shared one allele with the mother but the other allele was not shared with the alleged father. For the son, the genotypic alleles were different from the alleged father for 5 loci, D3S1358, vWA, D5S818, D13S317 and D7S820 (Fig. 2A-C). For the daughter, the genoty-

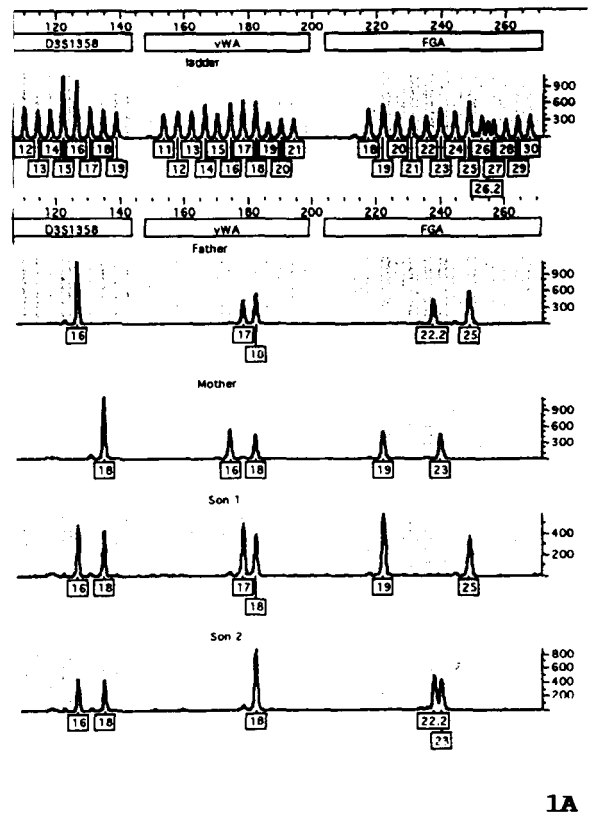
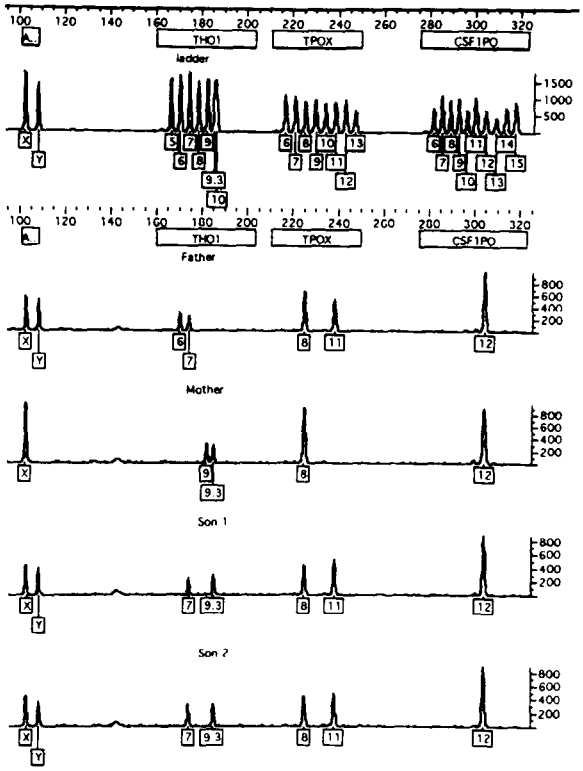
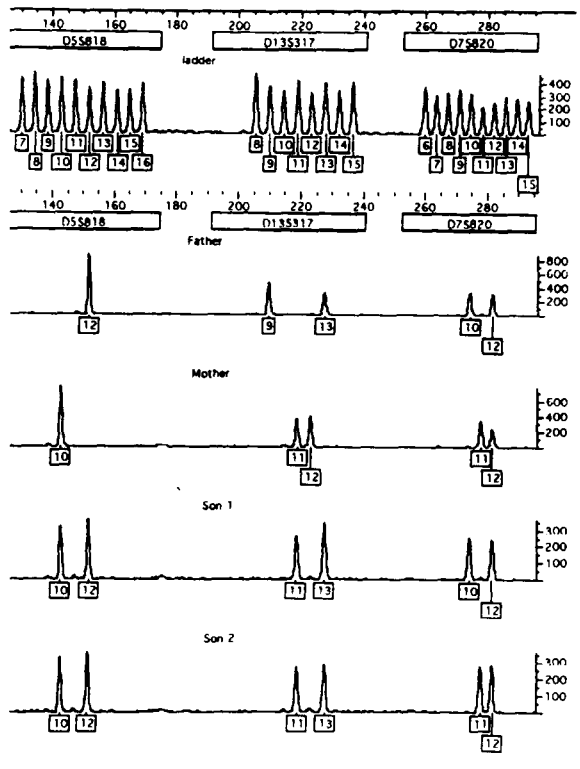


Fig. 1A. DNA profile of family 1. A) alleles of STR loci D3S1358, vWA and FGA.



1B

Fig. 1B. DNA profile of family 1. B) alleles of Amelogenin and 3 STR loci, TH01, TPOX and CSF1PO.



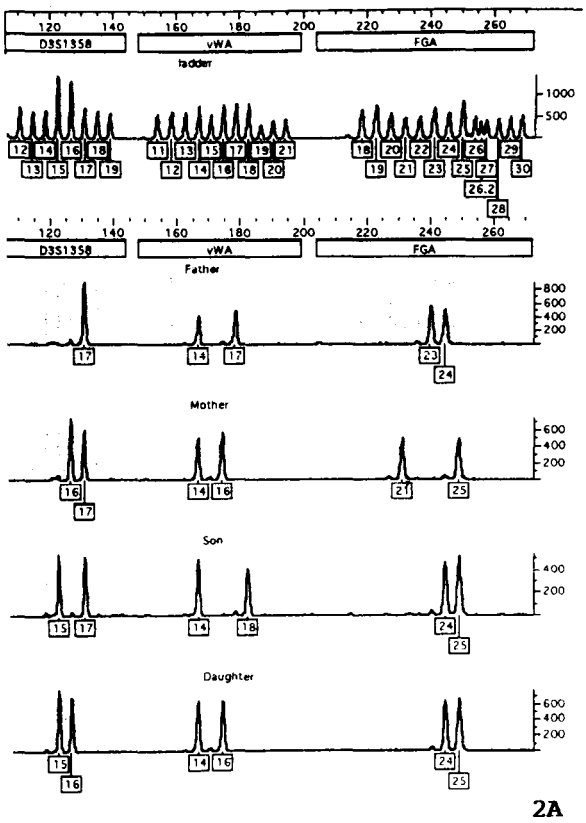
1C

Fig. 1C. DNA profile of family 1. C) alleles of STR loci D5S818, D13S317 and D7S820, respectively. Each row represents DNA profile of each individual. The results are inclusion.

pic alleles were different from the alleged father for 3 loci, D3S1358, D5S818 and D7S820 (Fig. 2A-C). However, if the assay comprised only genetic markers of TH01, TPOX and CSF1PO, misinterpretation of this family would happen.

Paternity testing of deceased father

Identification of a deceased person can be investigated by using reference blood samples (collected from living relatives) which had been positively identified. Family 3 and 4 were examples of a deceased, alleged father's DNA firgerprint investigation. The deceased father had married mother 1 and had 4 children, son 1, son 2, daughter 1 and son 3, respectively (Family 3). Before he passed away, he had a second wife and then, had a daughter, daughter 2 (Family 4). After death, the second wife requested proof that her daughter was the child of this father. Therefore, all involved persons were subjected to DNA typing. First of all, DNA typing of family 4 was identified (Fig. 3). The parental genotypes of family 4 could be predicted. Then, the genotype of the alleged-deceased father was investigated from his family 3's children (Table 1). The deceased father's genotypes of 9 STR loci, D3S1358, vWA, FGA, TH01, TPOX, CSF10, D5S818, D13S317 and D7S820 were predicted to be 16 and 15 or 17, 18 and 19, 23 and 24, 6 and 10, 8 and 8 or 9, 12 and 10 or 12, 7 and 11 or 12, 10 and 12, and 9 and 8 or 9, respectively (Table 1). The genotypes from the alleged-deceased father, mother 2 and daughter 2 were compared and the



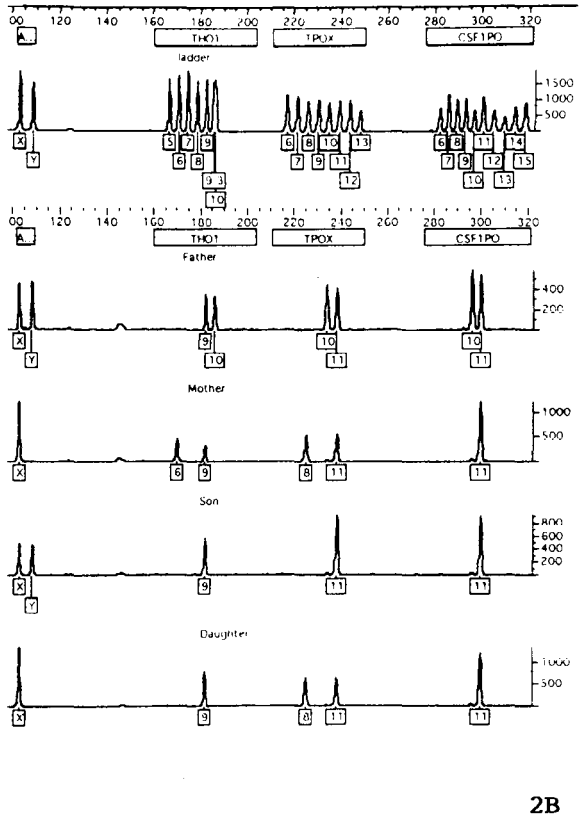


Fig. 2A. DNA profile of family 2. A) alleles of STR loci D3S1358, vWA and FGA.

Fig. 2B. DNA profile of family 2. B) alleles of Amelogenin and 3 STR loci, TH01, TPOX and CSF1PO.

Table 1. Identification of an alleged-deceased father.

STR locus	Genotypic alleles					
	mother 1	son 1	son 2	daughter 1	son 3	father*
D3S1358	15,17	16,17	16,17	16,17	15,17	16,15 or 17
vWA	16,19	16,18	16,19	16,18	19,19	18,19
FGA	22,22	22,23	22,23	22,23	22,24	23,24
TH01	7,9	6,7	7,10	9,10	7,10	6,10
TPOX	8,9	8,8	8,8	8,8	8,9	8,8 or 9
CSF1PO	10,10	10,12	10,12	10,12	10,12	12,10 or 12
D5S818	11,12	11,12	11,12	7,11	7,11	7,11 or 12
D13S317	8,11	10,11	8,10	11,12	8,12	10,12
D7S820	8,10	9,10	8,9	8,9	8,9	9,8 or 9

* predicted paternal genotype

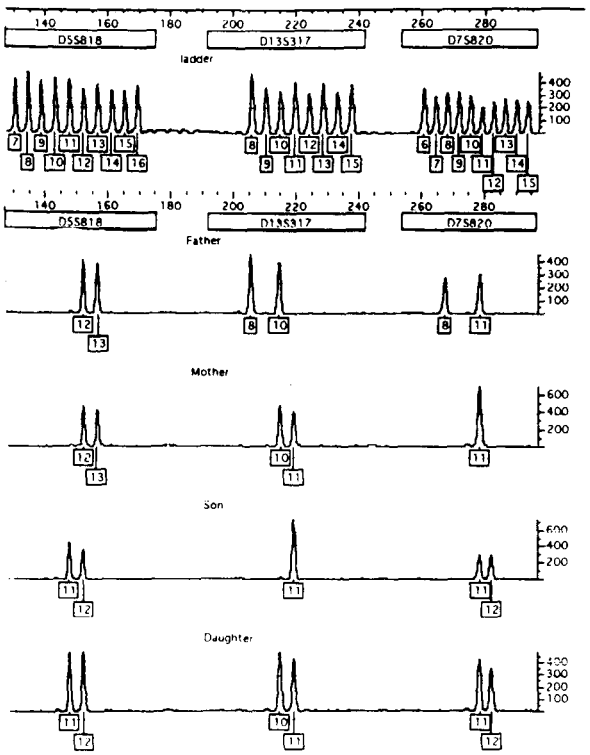


Fig. 2C. DNA profile of family 2. C) alleles of STR loci D5S818, D13S317 and D7S820, respectively. Each row represents DNA Profile of each individual. The results are exclusion.

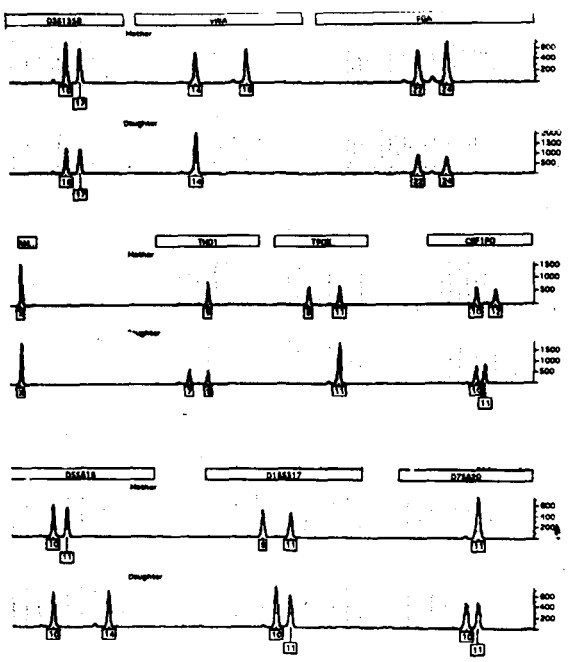


Fig. 3. DNA profile of family 4. The first and second rows are profiles from mother and daughter, respectively, on 3 STR loci, D3S1358, vWA and FGA. The third and fourth rows represent profiles from those mentioned, respectively, on Amelogenin and 3 STR loci, TH01, TPOX and CSF1PO. The Fifth and sixth rows indicate profile from those mentioned, respectively, on 3 STR loci, D5D818, D13S317 and D7S820.

Table 2. Comparison of daughter with alleged-deceased father.

STR locus	Genotypic alleles		
	mother 2	daughter 2	father
D3S1358	16,17	16,17	16,15 or 17
vWA	14,18	14,14	18,19*
FGA	22,24	22,24	23,24
TH01	9,9	7,9	6,10*
TPOX	8,11	11,11	8,8 or 9*
CSF1PO	10,12	10,11	12,10 or 12*
D5S818	10,11	10,14	7,11 or 12*
D13S317	9,11	10,11	10,12
D7S820	11,11	10,11	9,8 or 9*

* excluded alleles

result showed that there were non-transmitted alleles from the alleged father for 5 STR loci in daughter 2's profile (Table 2). These excluded alleles were genotypes of STR loci vWA, TH01, TPOX, CSF1PO, D5S818 and D7S820.

Paternity testing of a uniparent

For the purpose of paternity testing, it is necessary to have samples from each parent and a child or children. However, in some cases, it is difficult or impossible to get the samples from both

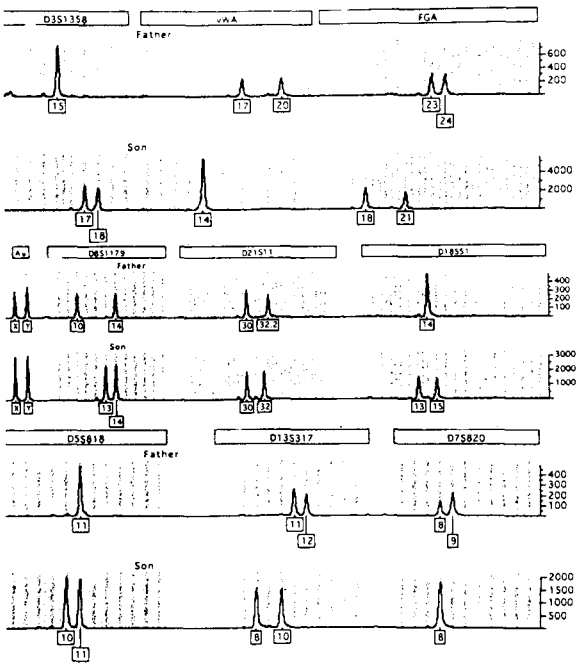


Fig. 4. DNA profile of family 5. The first and second rows are profile from alleged father and son, respectively, on 3 STR loci, D3S1358, vWA and FGA. The third and fourth rows represent profiles from mentioned individuals on Amelogenin and 3 STR loci, D8S1179, D21S11 and D18S51. The fifth and sixth rows indicate profiles from those mentioned individuals on STR loci D5S818, D13S317 and D7S820. The results are exclusion.

parents. Family 5 is an example of paternity testing based on the DNA samples from only a father and a child (Fig. 4). By comparing 9 STR alleles of the son with an alleged father, it is possible to evaluate the data precisely eventhough there is no information from maternal evidence. The non-transmitted alleles occured in 5 STR loci, D3S1358, vWA, FGA, D18S51 and D13S317. Therefore, the alleged father was not the biological father of this chid.

DISCUSSION

The STR loci exhibit length polymorphism among individuals due to variation in the number of repeat units[4]. A multiplex PCR system has been developed for routine identity testing in forensic casework[1]. The typing results are usually recorded as DNA fragment lengths or "alleles" indicating the number of core repeat elements for STR[7]. Clear and unambiguous results were obtained from all samples encountered in casework. DNA fingerprint which is completely specific to an individual can be applied directly to human identification, including parenthood testing[8]. From the fact that a child inherits his alleles from each biological parent, it is possible to investigate the relationship of all members of the family. However, there are 4 possible genetic recombinations of the parental genotypes at each locus[9]. In cases of personal identification by comparison with other family members, the situation is even more complicated.

For investigation of a deceased person's DNA profile, the most straight forward situation, other than where the deceased has a surviving monozygotic twin, is where both mother and father have provided reference samples. In this situation, the deceased's genotype is predicted simply by considering all the possible genetic combinations, according to Mendelian principles, that could arise from a mating between the 2 individuals[10]. In family 3, DNA profile was obtained from the deceased's children compared to that of his wife. The information was gathered from a family tree.

Often, only a single parent is surviving. The sufficiently informative STR loci investigated could make possible interpretation of the result. Hence, family 5 which had no information of maternal DNA profile could be summarized unambiguously.

This report has demonstrated the utility and potential of PCR amplification of STR loci when applied to individual identification. Simultaneous amplification of all STR loci decreased time and the cost of genotyping and enabled addition of controls, including internal size standards to score alleles precisely. The separation of amplified products by an automated DNA sequencer was shown to be sensitive, high throughput and reproducible.

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REFERENCES

1. Kimpton CP, Gill P, Walton A, et al. Automated DNA profiling employing multiplex amplification of short tandem repeat loci. PCR Methods Appl 1993; 3: 13-22.
2. Anderson JF, Greenhalgh MJ, Butler HR, et al. Further validation of a multiplex STR system for use in routin forensic identity testing. Forensic Sci Int 1996; 78: 47-64.
3. Beckman JS, Weber JL. A survey of human and rat microsatellites. Genomics 1992; 12: 267-31.
4. Roy R, Steffens DL, Gartside B, et al. Producing STR locus patterns from bloodstains and other forensic samples using an infrared fluorescent automated DNA sequencer. J Forensic Sci 1996; 41: 418-24.
5. Gill P, Sparkes R, Kimpton C. Development of guidelines to designate alleles using an STR multiplex system. Forensic Sci Int 1997; 89: 185-97.
6. Scherczinger CA, Bourke MT, Ladd C, et al. DNA extraction from liquid blood using QI Aamp. J Forensic Sci 1997; 42: 893-6.
7. Schneider PM. Basic issues in forensic DNA typing. Forensic Sci Int 1997; 88: 17-22.
8. Jeffreys AJ, Wilson V, Thein SL. Individual-specific fingerprints of human DNA. Nature 1985; 316; 76-9.
9. Sasaki M, Shiono H, Fukushima T, et al. Human identification by genotyping of personal articles. Forensic Sci Int 1997; 90: 65-75.
10. Clayton TM, Whitaker JP, Maguire CN. Identification of bodies from the scene of a mass disaster using DNA amplification of short tandem repeat (STR) loci. Forensic Sci Int 1995; 76: 7-15.

การพิสูจน์ความเป็นพ่อโดยการวิเคราะห์สารพันธุกรรม STR ที่ถูกเพิ่มปริมาณในหลอดทดลอง

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รายงานนี้เกี่ยวกับการประยุกต์ใช้เทคนิคการเพิ่มปริมาณสารพันธุกรรมในหลอดทดลองเพื่อพิสูจน์ความเป็นพ่อ ขบวนการพิสูจน์เป็นการเพิ่มปริมาณสารพันธุกรรมที่มีลำดับซ้ำกัน 1-6 เบส เป็นจำนวนหลายชุดซึ่งเรียกว่า STR โดยจำนวน STR ที่ใช้มีทั้งสิ้น 9 ตำแหน่ง การเพิ่มปริมาณสารพันธุกรรมนี้สามารถทำได้พร้อมกันหลายชุดของบริเวณเป้าหมาย รวมทั้งตำแหน่ง Amelogenin ที่ใช้บอกเพศและขนาดโมเลกุลมาตรฐาน การแยกขนาดของสารพันธุกรรมที่เพิ่มปริมาณแล้วนั้น ทำโดยการใช้เครื่องวิเคราะห์ลำดับสารพันธุกรรมอัตโนมัติ ผลของการวิเคราะห์แสดงให้เห็นว่าวิธีการนี้ให้ผลที่ชัดเจนและน่าเชื่อถือ วิธีการนี้สามารถใช้ในการวิเคราะห์ตัวอย่างได้ครั้งละหลายตัวอย่างโดยให้ผลที่คงที่ ดังนั้นจึงเป็นวิธีการที่น่าเชื่อถือในการพิสูจน์ความเป็นพ่อ

คำสำคัญ : การพิสูจน์ความเป็นพ่อ, การเพิ่มปริมาณสารพันธุกรรมในหลอดทดลอง, การวิเคราะห์สารพันธุกรรม STR

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