2005년 8월
박사학위논문

Analysis of 16 STRs in teeth

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치아에서 16 STRs(short tandem repeats) 분석

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지도교수 윤 창 륙

이 논문을 치의학 박사학위 신청 논문으로 제출함.

*2005*년 *7* 월 일

조선대학교 대학원

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김 남 리

김 남 리의 박사학위 논문을 인준함.

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치아에서 16 STRs(short tandem repeats) 분석

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서로 상관관계가 없는 10명의 사람으로부터 발거한 10개의 치아에서 D5S818, D13S317, D7S820, D16S539, HUMTH01, HUMVWA, D8S1179, HUMTPOX, HUMFGA, HUMCSF1PO, D21S11, D18S51, D2S1338, D19S433, D3S1358 그리고 amelogenin gene 등 16개의 STR유전자를 동시에 증폭시켜 개인식별에 응용할 수 있는지의 여부를 알아보고자, 각각의 치아에서 DNA를 추출하고 염기서열을 검사한 후 16개 STR유전자의 유전자형을 분석하여 다음과 같은 결과를 얻었다.

10개의 치아 중 8개의 치아에서는 16개 유전자 증폭이 모두 가능하였으나 1개의 치아에서는 HUMFGA를 제외한 15개 유전자의 증폭이 가능하였으며 또 다른 1개의 치아에서는 amelogenin gene만 가능하였다.

이 결과는 범죄사건이나 대량재해에서 법의학적 개인식별시 STR유전자를 이용하더라도 치아가 다른 어떤 장기 보다 효용성이 높다는 것을 말해준다.

핵심어 : short tandem repeats, amelogenin gene, 개인식별, 치아

Ⅰ. Introduction

Genetic analysis of short tandem repeat (STR) loci is currently the most powerful and widely used method to identify the contributor of biological samples collected in the context of criminal investigations, and the identification of human remains.[1] The successful application includes a variety of substrates such as blood, saliva, epithelial cells, hair roots and even compact bone samples. However, when more problematic samples such as old stains or decomposed tissue are subjected to STR typing, failure to obtain reproducible results may occur due to degradation of high molecular weight DNA.[2,3]

Human STR genotyping for population and forensic studies is nowadays mainly performed using commercial polimerization chain reaction(PCR) amplification kits available from different manufacturers. The most recent ones amplify more than 10 loci, forensically very informative and well characterised by the scientific community. Although from different manufacturers, these multiplex kits share most of the STR loci now commonly used, which are amplified, however, with distinct primer sets. If a primer is unable to anneal to the DNA template due to a polymorphism in its annealing region, then false homozygotes most likely appear.

The PowerPlex 16 system is a multiplex STR system for use in DNA typing, including mass disaster cases, paternity testing, forensic DNA analysis, human identity testing and tissue culture strain identification. The System allows the coamplification and three-color detection of sixteen loci (15 STR loci and amelogenin). The Powerplex16 System contains the loci Penta E, D18S51, D21S11,

TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. All sixteen loci are amplified simultaneously in a single tube and analyzed in a single injection or gel lane.

The loci included in the Powerplex16 System have been selected because they satisfy the needs of several major standardization bodies throughout the world, including the European police network, INTERPOL, the European Network of Forensic Science Institutes (ENFSI), GITAD (Grupo Iberoamericano de Trabajo en Analysis de DNA) and the United States Federal Bureau of Investigation (FBI). The system has undergone extensive validation efforts, including external CODIS validation for database and forensic casework, as well as internal validation. The PowerPlex 16 System also contains two new low-stutter, highly polymorphic pentanucleotide repeat loci, Penta E and Penta D. These loci add significantly to the discrimination power of the system, making the PowerPlex 16 System a single amplification system with a power of exclusion sufficient to resolve paternity disputes definitively. In addition, the extremely low stutter seen with Penta E and Penta D, makes them ideal loci for evaluation of DNA mixtures often encountered in forensic casework. Finally, the Amelogenin locus is included in the PowerPlex.

The aim of the present study was to type 16 STR loci(Allele frequency distributions for D5S818, D13S317, D7S820, D16S539, HUMTH01, HUMVWA, D8S1179, HUMTPOX, HUMFGA, HUMCSF1PO, D21S11, D18S51, D2S1338, D19S433, D3S1358 and amelogenin gene) at the same time in teeth.

Ⅱ. Materials and methods

A total of 10 teeth from unrelated Koreans were obtained from dentists after extraction and stored immediately at −20℃ until used. After mechanical cleaning of the surfaces the teeth were washed in 25ml sterile, distilled water followed by 10% commercial bleach and 95% ethanol. Using a diamond cutting disc the crowns were separated from the roots. The roots were then cut through the midline and the pulp tissue was collected using a spoon excavator and placed into a clean labelled microcentrifuge tube. Cementum and pulp chamber walls were removed using a dental bur. The isolated root dentin was washed in 10% commercial bleach, dried and crushed into a powder

Treatment of teeth prior to DNA extraction

Teeth are crushed to segment and decalcified by incubation for 48 h in 20mL EDTA(0.5 M, pH 8.0). The decalcified teeth is then washed 3 times in distilled water.

DNA extraction

Approximately 0.1~0.3 mg of teeth fragment is placed in a 1.5mL tube. Teeth is digested using 450 μL of DNA lysis buffer(10 mM Tris−HCl, pH 8.0, 100mM NaCl, 10 mM EDTA), SDS (50 μL of 10%), DTT(10 μL of 100 mM) and Proteinase K(20 μL of 10mg/mL). The solution is incubated overnight in a water bath at 56℃ with agitation.

Phenol:chloroform:isoamyl alcohol (25:24:1) extraction is performed with an equal volume, and separation of aqueous and organic layers is

achieved by centrifugation. The upper aqueous layers is recovered and the extraction repeated twice. The DNA is concentrated using a Microcon YM-100(Millipore, U.S.A.) Spin-rinse devices with distilled water and insert Microcon sample reservoir into vial. Pipette solution into sample reservoir without touching the membrane with the pipette tip. Seal with attached cap. Place assembly in a compatible centrifuge (400 rpm for 12 min) and remove

assembly from centrifuge, wash the membrane twice with distilled water prior to sample recovery. Place sample reservoir upside down in a new vial, then spin 3 min at 13000rpm to transfer concentrate to vial. The DNA is further purified by the QIAquickTM purification Method (Qiagen, Germany).

DNA quantitation

General information and protocols for using E-Gel pre-cast agarose gels Amount of DNA ; Use 20-100 ng DNA per band for samples containing one unique band, or up to 500 ng per lane for samples containing multiple bands.

Amplification of DNA and fragment analysis

Powerplex16 kits were used strictly as recommended by the manufacturers. The amplification for DNA isolated from almost teeth samples was performed in a GeneAmp PCR system 9700(Applied Biosystems, USA)

Sequencing and STR typing

Sequencing and STR typing was performed by ABI Prism 3100 Genetic

Analyzer and Sequencing Analysis Software 3.4.(Applied Biosystems, USA)

Ⅲ. Result

DNA were extracted successfully in all 10 teeth. It was possible to type 16 STR loci(Allele frequency distributions for D5S818, D13S317, D7S820, D16S539, HUMTH01, HUMVWA, D8S1179, HUMTPOX, HUMFGA, HUMCSF1PO, D21S11, D18S51, D2S1338, D19S433 and D3S1358) in the 8 teeth. But 15 STR loci except HUMFGA were typed in 1 tooth and amelogenin gene only typed in 1 tooth. The correct typing results for the DNA samples are listed in Table 1 for all STR loci in the Powerplex 16 multiplexes.(Table 1.) The multiplex typing results based on the recorded peak heights (in rfu) are depicted for the three most commonly used multiplexes.(Fig. 2, 3)

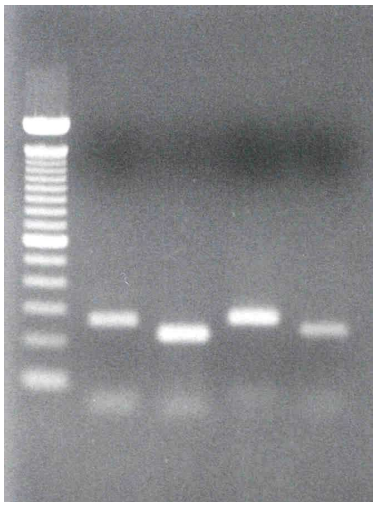

Fig 1. Electrophoretic pattern of PCR amplification products of teeth. Amplified products were separated in 1% agarose gel and direct visualization with ethidium bromide under UV light.
M : 100bp ladder, 1, 2, 3, 4 : Samples of PCR amplification

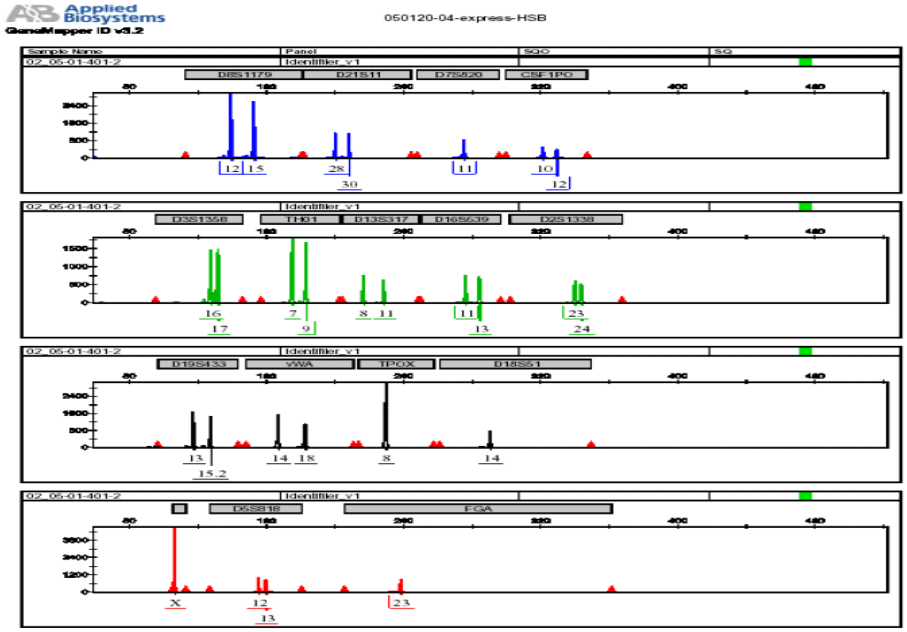

Fig 2. Sequence analysis of STR loci of tooth(S1)

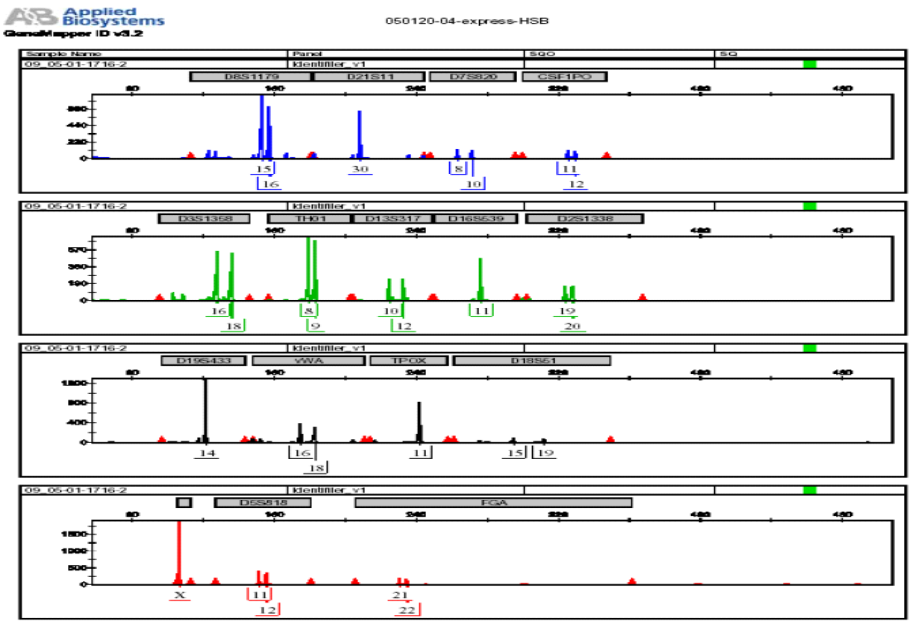

Fig 3. Sequence analysis of STR loci of tooth(S10)

Table 1. Type of 16 STRs in the teeth

Samples / DNA profiles	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	S 10
HUMCSF1PO	10 12	10 11	9 14	9 14	10 11	9 13	10 13	11 14	–	11 12
HUMFGA	23 23	22.2 24.2	23 24	23 25	19 23.2	16 25	23.2 27	–	–	21 22
HUMTH01	7 9	6 6	7 7	6 10	6 6	9 10	6 11	7 8	–	8 9
HUMTPOX	8 8	8 11	8 8	8 14	9 12	8 12	11 12	9 13	–	11 11
HUMVWA	14 18	17 18	14 15	16 17	15 16	15 17	16 19	17 19	–	16 18
D2S1338	23 24	15 19	16 19	20 28	21 27	17 28	15 24	19 23	–	19 20
D3S1358	16 17	9 16	10 16	12 12	13 16	9 19	18 19	13 17	–	16 18
D5S818	12 13	9 13	7 8	8 12	9 12	14 15	11 15	9 13	–	11 12
D7S820	11 11	8 9.1	7 9.1	11 12	11 13	13 14	9.1 13	10 14	–	8 10
D8S1179	12 13	10 14	10 10	12 16	12 15	13 13	14 15	10 13	–	15 16
D13S317	8 11	7 9	8 9	10 14	9 13	8 13	7 10	8 10	–	10 12
D16S539	11 13	10 11	9 11	13 14	10 14	11 13	9 12	11 11	–	11 11
D18S51	14 14	13 26	17 26	18 23	17 17	15 22	13 13	16 22	–	15 19
D19S433	13 15.2	13 14.2	15 18	14.2 26	26 32	24 32	15 32	18 26	–	14 14
D21S11	28 30	29 32.2	28 29	30 30.3	28 32.2	30 32.2	28 31	29.2 32.2	–	31 33
Amelogenin gene	X	XY	XY	X	XY	X	X	XY	XY	X

Ⅳ. *Discussion*

Microsatellites (STRs) have become an important and widely used tool in forensic casework. With the use of these markers the successful extraction of high molecular weight DNA is no longer needed. Hence, PCR-based methods have been of great value in identification dealing with decomposed human remains including skeletons.[4,5)]

Forensic criminal casework may involve the need to compare the DNA profiles of stains from the scene of crime with the profile of a deceased suspect.

Short TandemRepeat (STR) markers have become a valuable tool for forensic DNA typing. Multiplex amplification of STR loci using the polymerase chain reaction (PCR) can generate typing results from meager amounts of starting material. However, in many forensic cases, DNA samples are highly degraded(e.g., mass disasters such as the World Trade Center collapse of September 11, 2001. If DNA is exposed to the elements or to fire for any length of time, degradation can occur due to bacterial, biochemical or oxidative processes. Additionally, environmental contaminants can also be commingled with the forensic evidence. In such specimens a loss of signal is typically observed with larger-sized STR products.This loss of signal may be the result of either particular PCR inhibitors present in the forensic evidence or a DNA template that has been fragmented into small sizes.[6-8)]

STRs are genetic loci containing tandemly repeated sequences of 2-6 base pairs in length. DNA profiling based on STRs is the most popular method of human identification due to the highly polymorphic nature of

STRs and the ease of their genotyping. In comparison to minisatellites, the small amplified fragment length of STRs facilitates its utility in the analysis of degraded DNA samples.

However, in situations where DNA is highly degraded, poor amplification of the larger sized loci (300-500 base pairs) in standard multiplex typing kits is common. As the sample decomposes, the DNA template can become highly fragmented, and the yield of complete target fragments is greatly reduced. Thus, in multiplex kits with a wide range of amplicon sizes, a "decay curve" is seen, in which the peak height is inversely proportional to the amplicon length. In this case, the larger amplicons often have lower sensitivity and fall below the detection threshold. This can result in a partial genetic profile. To solve this problem, redesigned primer sets were developed in which the primers were positioned as close as possible to the ends of the repeat to reduce the amplified product size. These reduced sized primer sets were called Miniplexes. The primer sequences were originally designed to be compatible with the use of matrix-assisted- laser-desorptionionization time-of-flight mass spectrometry, but have been modified to function with fluorescence based sequencers for the detection of degraded DNA.

The breakdown of DNA into smaller fragments by chemical or physical means is always a special challenge for forensic scientists performing STR typing for the genetic identification of human remains or crime stains [1, 2, 3, 4 and 5]. The DNA degradation rate varies with light, humidity and temperature more than between individual DNA samples. Bacterial and fungal contaminations followed by the growth of these microorganisms may rapidly lead to physical, chemical and biochemical degradation of high molecular weight genomic DNA. Once the average

DNA fragment length is reduced to a size smaller than 300bp, a loss of genetic information occurs due to the lack of suitable template DNA for PCR and the subsequent failure of STR amplification.[9-13)]

I attribute this to the omittance of the decalcification step prior to DNA extraction which makes the extraction much easier and results in a greater yield of DNA, or in a decreased loss of DNA during extraction.

PCR inhibitors or DNA degradation typically produce a partial genetic profile with allele and/or locus dropout. The problem is further exacerbated when large multiplex PCR reactions are used due to the wide size range of PCR products generated. The current commercially available multiplex STR kits used in forensic DNA typing can generate amplicons in the size range of 100 bp to 450 bp. In situations where the samples are so badly degraded that STR analysis is not possible., the analysis of the mitochondrial DNA (mtDNA) hypervariable regions is typically used. However, mtDNA testing is a laborious and cost-prohibitive procedure for most forensic laboratories to utilize. Additionally, given the haploid and maternal-only transmission of mtDNA, the power of discrimination is not as powerful for identification as a full multilocus STR match. Some STR loci repeatedly exhibited problems. HUMFGA was affected by allelic dropout in the ABI multiplexes. This was more prominent using enhanced conditions for loci that did not amplify at all under standard conditions.[14)]

Some of the characteristics of STR typing results from degraded DNA samples from this study have also been observed under "low copy number" (LCN) conditions, where stochastic effects strongly influence the outcome of PCR amplification. Thus, some of the guidelines developed for interpretation of LCN STR profiles may also be applicable

when highly degraded DNA has to be analyzed.

The use ofstandardized degraded control DNA is helpful to understand and optimize the parameters affecting the success of STR typing. The availability of such a degraded control DNA in sufficient amounts would enable the forensic laboratories to simulate a situation of heavily decomposed DNA, and thus allow studies to enhance the efficiency of their typing methods.[15)]

The desire to gain more information from a sample, coupled with the need to limit consumption of a DNA sample where its availability may be limited (such as evidence from a crime scene) has led to the coamplification and typing of multiple STR systems. Multiplex PCR, which involves adding more than one set of PCR primers to the reaction in order to target multiple locations throughout the genome, is an ideal technique for DNA typing because the probability of identical alleles in two individuals decreases with an increase in the number of polymorphic loci examined. The advent of fluorescent labeling with multiple fluorescent detection permits the multiplexing of STR loci which may have alleles that fall in the same size range.[16-22)]

Multiplexing can save time and money, but difficulties may arise when coamplifying several loci. Primers for one locus can complex with those of other loci and completely inhibit amplification. This effect may be exhibited by dropout of a specific STR locus under certain conditions(e.g., sample mixtures). Finding the optimum PCR conditions, particularly the annealing temperature and the primer concentrations, can be challenging and time-consuming.

Another approach to analyzing multiple sites on the genome while

conserving limited DNA has been demonstrated by Lorente and coworkers. These studies involved recovering the original genomic DNA template following each PCR amplification and reusing it for the next reaction in what has been termed sequential multiplex amplification (SMA). Using SMA, less than 5 ng of template genomic DNA was needed to type five different PCR-amplified loci.

In conclusion, PCR-based STR analyses are suitable in human identification and forensic casework dealing with different tissues, especially teeth.

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저작물 이용 허락서

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본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

2005 년 8 월 일

저작자 : 김 남 리 (서명 또는 인)

조선대학교 총장 귀하