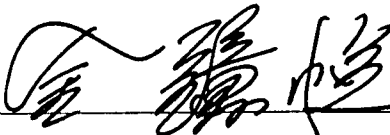



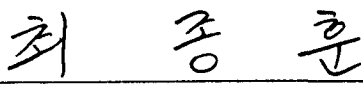

DNA

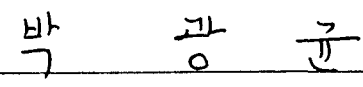

DNA



2001 6

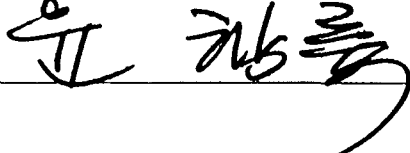

안종모의 박사 학위논문을 인준함

심사위원  

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심사위원  

심사위원  

연세대학교 대학원

2001년 6월 일

가

가

, 가

	
	
	
•	1
•	5
1.	5
2.	5
가. DNA	5
• DNA	6
• PCR	7
• DNA	7
•	9
1.	1	9
2.	DNA	9
•	15
•	20
	21
	28

Table 1. Ratio of analysis of mtDNA from the teeth on various heating temperature and exposure time	10
Table 2. Sequence polymorphisms in hypervariable control region (HV1) of 30 Korean teeth	12
Table 3. Distribution of sequence polymorphisms in mtDNA hypervariable control region (HV1)	13

Fig. 1. The teeth on various condition of heating temperature
and exposure time 10

Fig. 2. Amplified products of mtDNA hypervariable control
region (HV1) on 2% agarose gel 11

가 , 가

가 ,

가 , DNA

가 ,

가 33 100 , 150 , 200 , 250 , 300 1 ,

2 350 1 , DNA

DNA 1

Anderson

.

1. 33 300 2 30
DNA 가 .

2. 30 29 .

3. 1 가 45
39 , 6 .

4. 16,223 가 C T, 16,362 가 T C
가 가 .

,

가 DNA 가 ,
DNA가

DNA

.

DNA

()

•

가 ,
가 가

· ,
가
가 .

가

DNA(nuclear DNA) (DNA typing)

DNA(mitochondrial DNA, mtDNA) mtDNA

. (nuclear genome)

가 (molecular anatomy) 가

3.000 (polymorphic genetic

marker)가

(hypervariable minisatellite locus) variable

number of tandem repeat (VNTR locus)

(multilocus probe) (single

locus probe) ^{3,12,30,49,50)} (polymerase chain reaction, PCR) PCR VNTR (amplified fragment length polymorphisms, AMP-FLPs) short tandem repeats (STRs) 가 ^{13,29,38)} DNA DNA 가 mtDNA 가 mtDNA 가 15-50% , (mitochondrial DNA, mtDNA)가 가 200-1,700 가 , 16,569 base pair(bp) circular-duplex chromosome 10 . mtDNA (coding region) (non-coding region) small(12S) large(16S) rRNA, 22 tRNA, 13 (NADH dehydrogenase subunit 7, ubiquinol-cytochrome c oxidoreductase subunit 1, cytochrome c oxidase subunit 3, ATP synthase subunit 2)가 90% . tRNA proline tRNA phenylalanine 1,122bp D-loop(displacement loop) DNA H가 (H strand) (origin of replication) H L가 (L strand) (origin of transcription) (hypervariable region) , 가 5-10 ^{5,7)} 16,024 16,430 1 (hypervariable control region , HV1) 20 410 2 (hypervariable control region , HV2) ^{4,57,59)} .

mtDNA 1981 Anderson ⁴⁾ mtDNA
 mtDNA (polymorphism)
 가 , Stoneking ⁴¹⁾, Cann ⁹⁾,
 Tikichinski ⁴⁴⁾ (restriction enzyme)
 (Restriction Fragment Length Polymorphism,
 RFLP)
 . Vigilant ⁴⁵⁾, Rienzo Wilson ³⁷⁾ Pfeiffer ³³⁾
 (DNA sequencing)
 RFLP
 . ⁵⁶⁾
 DNA 가 ⁶⁰⁾
 DNA
 , ⁵⁹⁾ DNA
 100 .
 가 Yamada ⁵¹⁾
 DNA RFLPs , Yamamoto
⁵²⁾ DNA Y-
 , Schwarzthz ⁴⁰⁾
 DNA , ⁵⁸⁾ 10
 DNA
 , ⁶²⁾ D1S80 DNA
 , ⁶¹⁾ STR
 .
 가
 .
 DNA 가 가
 , mtDNA

가

가

24 DNA가 mtDNA

가 ¹⁹⁾, mtDNA

DNA 가 ⁶⁰⁾.

가

DNA

DNA , mtDNA

가

가

mtDNA

DNA 1 ,

mtDNA

•

1.

가 2

33 , 3 11 (100 , 150 , 200 , 250 , 300
 1 , 2 350 1) ,
 electronics burnout furnace(Model-108, Jae myung
 co., Seoul, Korea) (Fig. 1).

2.

DNA
 1 (hypervariable control region , HV1) PCR

가. DNA

5.25% NaOCl 20 20
 95% ethanol ultraviolet(UV) light 20
 43). 1.5Ml eppendorf tube 720μl
 extraction buffer(10mM Tris, pH 8.0; 100mM NaCl; 50mM EDTA, pH 8.0; 0.5%
 SDS) 40μl proteinase K(10μg/μl) 56 24 .
 720μl phenol/chloroform/isoamylalcohol(25:24:1)

2 13,000rpm
 eppendorf tube . ,
 720 μ l 1-butanol 13,000rpm 2
 Centricon- 100 concentrator(Amicon, U.S.A.) 1M TE buffer
 20 3,000rpm . Column eppendorf
 tube - 20 ^{58,62)} .

DNA

1 2 (primer)
²⁷⁾ .

F(forward direction) 15989 5'-CCC AAA GCT AAG ATT CTA AT-3'

R(reverse direction) 16251 5'-GGA GTT GCA GTT GAT GTG TG-3'

F(forward direction) 16190 5'-CCC CAT GCT TAC AAG CAA GT-3'

R(reverse direction) 16410 5'-GAG GAT GGT GGT CAA GGG AC-3'

PCR buffer(10mM Tris-HCl(pH 8.3), 50mM KCl), 1.5mM
 MgCl₂, 10pmole, dNTP 250 μ M, DNA (DNA template) 100ng,
 Taq polymerase 1 unit(Bioneer, Seoul, Korea)가

20 μ l . PCR MinicyclerTM(MJ Research, U.S.A.)
 95 5 가 95 20 , 56 20 72
 30 30 72 5 가 .
 PCR 2 μ l PCR 2%
 agarose gel (Fig. 2). negative
 control , positive control mtDNA

. PCR

PCR	QIAquick PCR
Purification kit(Qiagene, Germany)	(purification)
kit	buffer PB PCR (15 μ l) 5 가
QIAquick spin column	13,000rpm 1
0.75M buffer PE	13,000rpm 1
column	buffer PE가
QIAquick spin column	1.5M eppendorf tube
Buffer EB(10mM Tris-Cl, pH 8.5) 30 μ l	가 13,000rpm 1

DNA

Cycling Termination Reaction Thermo Sequenase Cy5.5 dye terminator cycle sequencing kit(Amersham phamacia biotech, U.S.A.)

master reaction mix [PCR 5 μ l, reaction buffer 3.5 μ l, primer(10pmol/ μ l) 0.5 μ l, polymerase 2 μ l] 7 μ l dye terminator ('A', 'C', 'G', 'T') 1 μ l mt DNA thermal cycle PCR
 . Sequencing primer ²⁷⁾ .

F(forward direction) 15989 5'-CCC AAA GCT AAG ATT CTA AT-3'

R(reverse direction) 16251 5'-GGA GTT GCA GTT GAT GTG TG-3'

F(forward direction) 16190 5'-CCC CAT GCT TAC AAG CAA GT-3'

R(reverse direction) 16410 5'-GAG GAT GGT GGT CAA GGG AC-3'

PCR tube 7.5M ammonium acetate 2 μ l 100% EtOH 30 μ l

20 . 4 , 12,000rpm 20-30
 pellet 70% EtOH 200 μ l 가
 4 , 12,000rpm 5 .
 formamide 6 μ l pellet 70 3
 (denaturation) polyacrylamide gel 2 μ loading .
 Automated DNA sequencer SEQ 4 \times 4 personal sequencing system
 (Amersham Pharmacia Biotech, U.S.A.) , 30mA, 1500V,
 50 30 . 1 Anderson
 4) mtDNA .

•

1. 1

가 33
 genomic DNA 300
 2 30 1 가

(Table 1). DNA

PCR

(Fig. 2).

2. DNA

1 가 PCR 400
 (16,001 16,400) Anderson 4)
 44 (polymorphic nucleotide) 가 (Table

2).

30 400 (nucleotide substitution)
 45 , 39 (nucleotide transition)
 , 6 (nucleotide transversion) (Table 3).

가 Anderson 16,223
 (cytosine, C) 30 25 (83%) (thymine, T) ,
 16,362 16 (53%) . ,
 29 30 28 가
 2 .

Table 1. Ratio of analysis of mtDNA from the teeth on various heating temperature and exposure time.

Exposure time \ Temperature	100	150	200	250	300	350
	1 hour	3/3	3/3	3/3	3/3	3/3
2 hours	3/3	3/3	3/3	3/3	3/3	—

: analysed No/total No.

— : no test



100 , 1 hour

100 , 2 hours

150 , 1 hour

150 , 2 hours

200 , 1 hour

200 , 2 hours

250 , 1 hour

250 , 2 hours

300 , 1 hour

300 , 2 hours

350 , 1 hour

Fig. 1. The teeth on various condition of heating temperature and exposure time.



Lane 1 : 100bp ladder
Lane 2 : negative control
Lane 3-5: amplified products
Lane 6 : positive control

Fig. 2. Amplified products of mtDNA hypervariable control region (HV1) on 2% agarose gel.

Table 3. Distribution of sequence polymorphisms in mtDNA hyper-variable control region (HV1)

	HV1
Transition	39
Transversion	6
Total	45

가

가 .

(genetic marker)

southern-hybridization ^{15,18,21-24)} ^{1,2,36)}

(polymerase chain reaction;PCR), PCR minisatellite variant repeat-PCR ²⁰⁾ (MVR-PCR), mitochondrial DNA sequencing .

RFLP-VNTR southern hybridization DNA

DNA southern-hybridization

(RFLP) DNA가 가 .

ng DNA

2Kbp VNTR 400bp STR

(short tandem repeat locus) (amplified fragment length polymorphisms, AMP-FLPs)

가 .

, DNA RFLPs

⁵¹⁾ , DNA Y- ⁵²⁾ ,

DNA X-Y homologous amelogenin gene D1S80

⁵⁸⁾ , amelogenin gene STR F13A01,

LPL ⁵⁵⁾ . 가 DNA

伊東 勵 ⁵⁴⁾ ,

PCR amelogenin locus

250 15 가 DNA가

, ⁶¹⁾ 150 200 45 , 90 , 120

HumTH01 HumCD4 가 250 45

band가
DNA
300 2
DNA가
DNA
DNA
DNA (odontoblast) (process) 가 가
DNA 가 가
가
가
가 mtDNA
Hagelberg¹⁶⁾, Paabo³¹⁾ Paabo³²⁾ DNA
mtDNA
mtDNA 가 , ,
가^{16,19,27,31,32,48)} , DNA
, mtDNA (haploid)
(crossover)가
가^{5,7,14,47)}
, mtDNA DNA 5 10
mtDNA
^{17,35,42)}
mtDNA
mtDNA가 DNA ,

DNA mtDNA가 DNA

Potter Newbold³⁴⁾ Brown⁶⁾ mtDNA RFLP , Denaro¹¹⁾

Cann Willson⁸⁾ 112 mtDNA RFLP mtDNA 9 14

95 mtDNA , Satoshi Matsunaga³⁹⁾ 116 mtDNA RFLP

(diversity) 가

Vigilant⁴⁵⁾ 가 (mtDNA type) 97% 92%

, Rienzo³⁷⁾ 가 117 1

620 79 88 mtDNA ,

Ward⁴⁶⁾ 63 1 360

95.4% 93.9%

, mtDNA Pfeiffer³³⁾

가 60 1 2

57 mtDNA ,⁵⁹⁾ 100

1 440 84 mtDNA 98%

,²⁶⁾ 100 1 2

97 mtDNA 99% 99%

. mtDNA

가 가

가 33

가 30 가 DNA 16,001

16,400 400 (nucleotid

substitution) 44 .

29	30	28		가
2				
	30	25 (83%)	16,223	(cytosine, C)
(thymine, T)			16 (53%)	16,362
(thymine, T)		16,189		7
(25%)		⁵⁹⁾ 100		DNA
1		100	74 (74%)	16,223
		16,189		
49 (49%),	16,362		21 (21%)	
가				
	mtDNA			
	mtDNA		mtDNA가	
	가	DNA		
mtDNA			1	
2		1,122		가
	가			
			가	
	DNA		mtDNA	DNA
	가			
	가			

•

가 , 가

가 ,

가 , DNA

가 ,

가 33 100 , 150 , 200 , 250 , 300 1 ,

2 350 1 , DNA

DNA 1

Anderson

1. 33 300 2 30
DNA 가 .
2. 30 29 .
3. 1 가 45
39 , 6 .
4. 16,223 가 C T, 16,362 가 T C
가 가 .

가 DNA 가 ,

DNA가 DNA

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- 1- 11
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ABSTRACT

Mitochondrial DNA Analysis for Individual Identification from Heated Teeth

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(Directed by Prof. **Chong-Youl Kim**, D.D.S., M.S.D., Ph.D.)

Teeth are relatively firm compare to other organs and stable to a variety of environment such as heat and putrefaction, therefore they are forensically significant samples in sex and age determination, blood typing and DNA typing.

The author conducted the experiment on heated teeth to detect mitochondrial DNA related to individual identification in high temperature.

The experiment was done on 33 teeth of unrelated Koreans consisted of heated ones to 100 , 150 , 200 , 250 , 300 for 1 hour and 2 hours respectively and to 350 for 1 hour. DNA was extracted from each tooth and amplified hypervariable region 1 of mitochondrial DNA by Polymerase Chain Reaction(PCR). Then mitochondrial DNA sequencing data was analysed according to the Anderson sequence and the followings are the results :

1. It was able to analyse mitochondrial DNA of 30 heated teeth to 300 for 2 hours.
2. It was confirmed 29 mitochondrial lineages from 30 teeth of unrelated

Koreans.

3. Among the sequences 45 nucleotide sites are polymorphic, 39 nucleotides of which is substituted by transition and 6 nucleotides by transversion.

4. The most frequent polymorphisms were found at the position 16,223(C to T transition) and 16,362(T to C transition)

As a result, it is possible to extract DNA from charred teeth, therefore teeth can be used as a source of mitochondrial DNA for individual identification especially when nuclear DNA is not extracted in charred body in high temperature.

Key words : charred body, teeth, individual identification, mitochondrial DNA, hypervariable region 1.