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Personal identification of the excavated ancient human bone
through molecular - biological methods

徐民錫 · 李奎植 · 鄭容在 · 李命熹*

Min Seok Seo, Kyu Shik Lee, Yong Jae Chung, Myeong Hui Lee *

ABSTRACT

DNA typing is often used to determine identity from human remains. Recently, the molecular-biological analysis of ancient deposits has become possible since methods for the recovery of DNA conserved in bones or teeth from archaeological remains have been developed. In the field of archaeology, one of the most promising approaches is to identify the individuals present in a mass burial site.

We performed nuclear DNA typing and mitochondrial DNA sequencing analysis based on PCR from a Korea ancient human remain excavated from Sa-chon Nuk-island and civilian access control line(CACL). A femur bone were collected and successfully subjected to DNA extraction, quantification, PCR amplification, and subsequently typed for several short tandem repeat(STR) loci. 4 types of STR systems used in this study were CTT multiplex(CSF1P0, TPOX, TH01), FFv multiplex(F13A01, FESFPS, vWA), Silver STR multiplex(D16S539, D7S820, D13S317), and amelogenin for sex determination.

This studies are primarily concerned with the extraction, amplification, and DNA typing of ancient human bone DNA samples. Also, it is suggestive of importance about closely relationship between both fields of archaeology and molecular biology.

*

(The Korean National Maritime Museum)

가 DNA() DNA가 가 가 가 . DNA (nucleotide) (pentose), 가 (deoxyribose) DNA() RNA() DNA (adenine:A) · (guanine:G) · (cytosine:C) · (thymine:T) 가 가 , DNA A 가 , G 가 , C 가 , T 가 4 가 . 4 가 DNA 4 DNA가 (Lee *et al* ., 1999). 가

가 DNA 95% , , (HGP: human genome project) 가 (tandem repeat) ,

(tandem repeat sequence)가 ,
 1
 가 . 14-70 bp VNTR
 (variable number of tandem repeats), 2-7 bp STR (short tandem
 repeats) . , VNTR STR (core
 sequence) (tandem repeat)
 (repetitive sequence) 가
 (Jeffeys *et al* , 1985).
 가 DNA
 가
 (Mendel) .
 ,
 .
 (DNA Typing) ,
 DNA 가
 () (PCR: polymerase chain
 reaction)
 ,
 ,
 가 . DNA
 , DNA
 , 가
 , () , ,
 가 , DNA
 ,
 () , (GMO) .
 ,

‘ DNA ’

404

99

(Lee *et al.*, 1999).

가

2

가

가

가

1.

30

(Clean bench) UV

(Fig. 1).

30 5x2cm
 50ml
 가
 3 3 ,
 vortexing 가
 (compact bone)가
 DNA 가

2. (Decalcification)

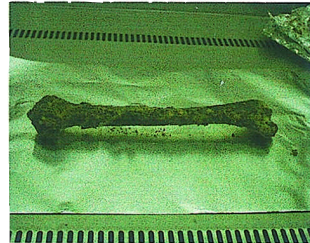
1.5ml
 , 0.5M EDTA 1ml 가 10 15
 . EDTA (Ca²⁺)
 DNA
 , EDTA
 , 가 , EDTA
 , DNA가 DNA
 , EDTA DNA
 DNA가 , EDTA ,

Fig. 1.
Photographs of
samples examined



(A)

A left femur bone from
Nuk-island



(B)

A femur bone from
Civilian access control line(CACL)

Fig. 1. Photographs of samples examined

3 EDTA DNA가

, EDTA ,

DNA가 () DNA () . 15

가 3

3. DNA (DNA Extraction)

가 DNA

(GeneClean for ancient DNA kit, Bio101)

(Fastprep FP120, Bio101) DNA

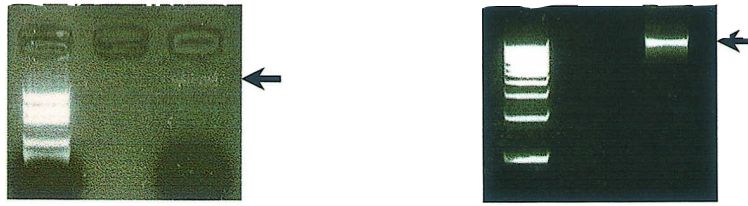
. DNA 1% agarose gel 100volt

, EtBr(ethidium bromide) UV DNA

(Fig. 2). DNA

, DNA 가 .

DNA (-20)



(A)
Electrophoresis photograph of extracted DNA from
Nuk-island human bone

(B)
Electrophoresis photograph of extracted DNA from
Civilian access control line(CACL)

Fig. 2.
Electrophoresis of DNAs
extracted from ancient
human bone.

Fig. 2. Electrophoresis of DNAs extracted
from ancient human bone.

4. (Polymerase Chain Reaction, PCR)

DNA
(polymerase chain reaction, PCR)

DNA 가
STR(short tandem repeat) . STR Promega
CTT, FFv, STR , Amelogenin
(Technical manual, promega, 1985).
가
(Taq polymerase) (Gold STR Taq
polymerase) , cycle
(Table 1).
2% agarose gel 100volt , EtBr
UV (Fig. 3).

Table 1.
PCR condition for
each STR loci of
ancient human
bone DNA

	Amelogenin/CTT reaction	FFv/STR reaction
Initiation condition	95 11min	95 11min
initiationl denaturation	96 2min	96 2min
Denaturation(10cycles)	94 1min	94 1min
Annealing	64 1min	60 1min
Extension	70 1.5min	70 1.5min
Denaturation(20cycles)	90 1min	90 1min
Annealing	64 1min	60 1min
Extension	70 1.5min	70 1.5min 60 30min
Holding reaction	4 Holding forever	4 Holding forever

5. (Acrylamide - Urea Gel Electrophoresis)

(DNA Typing) , 6% acrylamide - urea sequencing gel 45watt
 1200volt sequencing . 4% gel 6% gel
 STR , gel
 6% gel sequencing . 6% gel 0.5M
 TBE(Trisma - base, Boric acid, EDTA) 30
 gel (loci
 ladder) 2
 (loading dye) 가 95 2
 . -4 0 . 30
 gel 가 50 가 ,
 0.5M TBE
 . 가 30cm gel 2/3
 gel .



(A)
Application system applied to DNA extracted from excavated ancient human bone, CTT multiples(lane 1), FFv multiples(lane 2)

(B)
Application product of DNA from Civilian access control line(CACL) bone, CTT multiplex (lane 1), amelogenin (lane 2), FFv multiplex (lane 3), STR (lane 4)

Fig. 3. Multiplex STR amplification system applied to DNA extracted from excavated ancient human bone.

Fig. 3.
Multiplex STR amplification system applied to DNA extracted from excavated ancient human bone.

6. Silver (Silver Staining Detection)

가 gel , gel 가 . , Silver 가 1 2 , (over staining) (acetic acid) Silver gel (over 10%) CTT FFv (Fig. 4), FFv, Amelogenin, STR (Fig. 5).

Fig. 4.
4.6% acrylamide-urea gel
electrophoresis of PCR products
from excavated Nuk - island
human bone

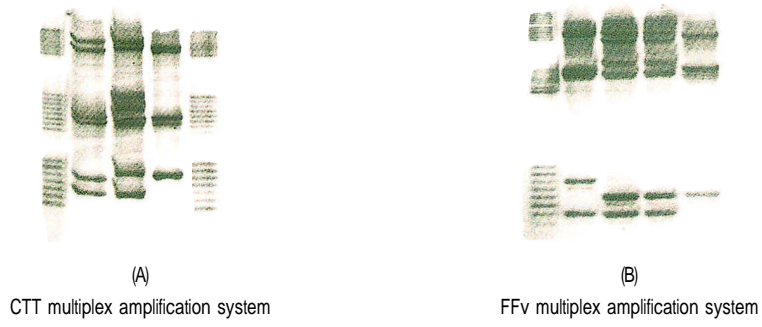


Fig. 4. 4.6% acrylamide - urea gel electrophoresis of PCR products from excavated Nuk - island human bone

Fig. 5.
6% acrylamide - urea gel
electrophoresis of PCR products
from excavated civilian access
control line(CACL) human bone.

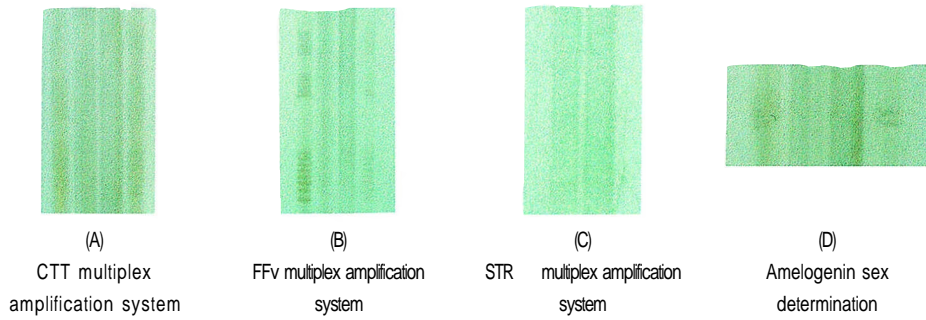


Fig. 5. 6% acrylamide - urea gel electrophoresis of PCR products from excavated civilian access control line(CACL) human bone.

가 , DNA
가 , amelogenin X, Y
가 ,

7. DNA(Mitochondrial DNA)
(Base Sequencing Analysis)

DNA 가 ,
 DNA mtDNA sequencing .
 DNA 5ul mtDNA
 , primer sequence mtDNA
 HV1(15971 16410) F15989/R16410 F15971/R16258
 . STR
 가 가
 . Qiagen PCR purification kit oligomer ,
 , Dye Terminator Cycle Sequencing Kit Perkin
 Elmer Prism 377 DNA Sequencer , Sequence
 Navigator (Fig. 6). DNA forward reverse sequence
 Anderson sequence mtDNA가 (Fig. 7).
 , DNA Anderson sequence
 DNA가 , DNA
 .
 . DNA , 99
 .
 (nuclear DNA Typing) mtDNA
 가 .

Fig. 6. MtDNA PCR product electrophoresis and sequence analysis as Perkin Elmer Prism 377 DNA Sequencer

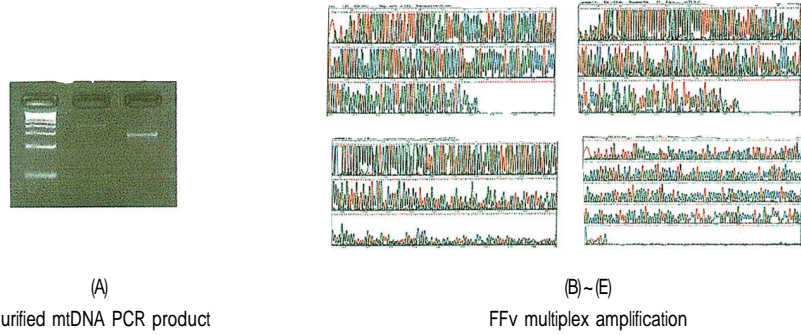


Fig. 6. MtDNA PCR product electrophoresis and sequence analysis as Perkin Elmer Prism 377 DNA Sequencer

Fig. 7. Sequence homologous analysis between human mtDNA and civilian access control line(CACL) human bone mt DNA as DNAsar Editseq sequence analysis software

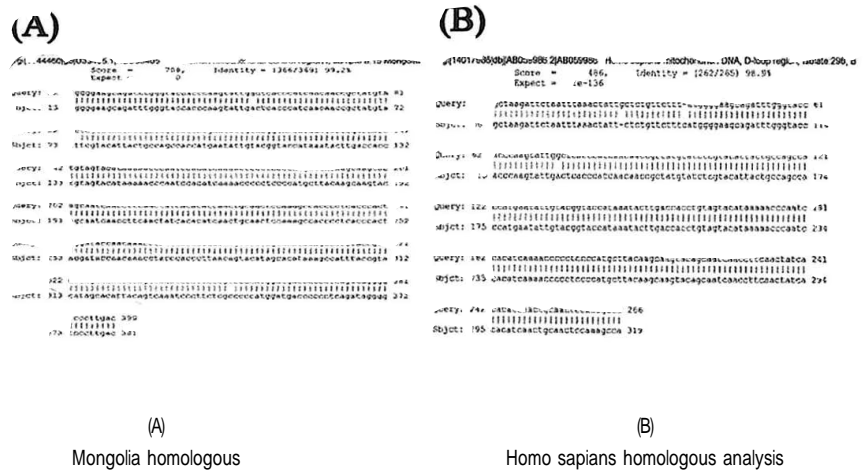


Fig. 7. Sequence homologous analysis between human mtDNA and civilian access control line(CACL) human bone mt DNA as DNAsar Editseq sequence analysis software

DNA 가 , DNA , DNA가 STR

가 , STR amelogenin
 . mtDNA HV1 HV2
 HV1 primer ,
 (automatic sequencing analysis) .
 DNA
 가 .
 DNA .
 DNA
 . 가 ,
 DNA 1980 .
 DNA (PCR) .
 DNA 가
 가 ,
 , BT(Bio - Technology)
 , BT 가 가
 , 가
 가
 DNA
 가 .

1. Jeffeys AJ., Wilson V., and Thein. SL. Nature 1985; 314; 67 - 73
2. Technical Manual. GenePrint STR Systems. Promega. 1985
3. Lee KS, Chung YJ, Han SH, Lee MH, Han MS, and Choi DH. Conservation Studies 1999; 20; 5 - 19
4. Chung YB. DNA Typing. Inje university press. 1996
5. Holland MM. and Parsons TJ. Mitochondrial DNA sequencing analysis - Validation and use for forensic casework. Central police university press. 1999