Short Tandem Repeat Typing for the Identification of Skeletal Remains from Korean War Victims

Na Young Kim[1], Hwan Young Lee[1], Myung Jin Park[1], Jeong Eun Sim[1], Eun Jin Lim[1], Chang-Lyuk Yoon[2], Woo Ick Yang[1], Kyoung-Jin Shin[1]

[1]Department of Forensic Medicine and Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea
[2]Department of Oral Medicine and Forensic Odontology, Chosun University College of Dentistry, Gwangju, Korea

Introduction

In order to identify the remains of service members missing in the Korean War (1950-1953), a population-based, DNA-led identification has been performed since 2000 on the initiative of Korean Ministry of National Defense (MND) Agency for Killed in Action Recovery and Identification (MAKRI). Some of the missing casualties have been identified and returned to their families on the basis of circumstantial evidence and matching results of mitochondrial DNA (mtDNA) genetic profiles. However, since mtDNA testing is not so effective to provide positive identification, we need to analyze additional genetic markers to confirm the alleged relationship between missing casualties and their corresponding relatives with greater exactitude.

Therefore, we selected 21 skeletal remains which showed a match with alleged biological relatives from mtDNA typing results, employed recent efficient DNA extraction method and generated autosomal STR (AS-STR) and Y-chromosomal STR (Y-STR) results using three commercial STR systems (AmpFℓSTR® Identifiler®, AmpFℓSTR® MiniFiler™ and AmpFℓSTR® Yfiler™) and two in-house mini-STR systems (miniplex NC01 plus and Y-miniplex plus).

Materials and Methods

Sample

Long bones were used for the genetic identification of skeletal remains of 21 Korean War (1950-1953) victims. Based on circumstantial evidences and/or mtDNA sequence matching results, blood samples from 24 biological relatives of supposed victims were also analyzed.

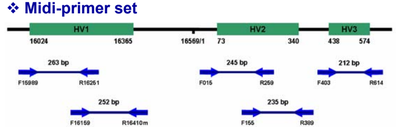
DNA Extraction

DNA from skeletal remain samples were extracted using the QIAamp® Blood Maxi spin column (Qiagen, Hilden, Germany) and PB/PE buffers from the QIAquick® PCR purification kit (Qiagen) after complete demineralization procedure by incubating samples in extraction buffer (0.5 M EDTA, 0.5% SDS, 3 mg of Proteinase K) at 56℃ for 48 hours. For DNA concentration, QIAamp® Mini spin column (Qiagen) and PB/PE buffers were used, and the final volume was 50 μL. The DNA extraction procedure was performed at least twice in each skeletal remain sample. DNA from blood samples were extracted using QIAamp® DNA Mini Kit (Qiagen) according to the manufacture's protocol.

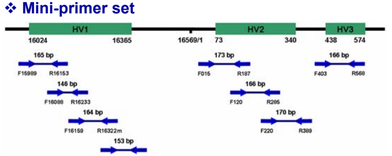
Amplification and Sequencing of mtDNA

To amplify mitochondrial HV1, HV2 and HV3 region, PCR amplification was first conducted using five pairs of midi-primers. For samples too degraded to be analyzed using these primers, mini-primers were used. Primers used for sequencing analysis were the same as used in PCR amplification.

❖ Midi-primer set



❖ Mini-primer set



Detailed protocol is available at http://forensic.yonsei.ac.kr.

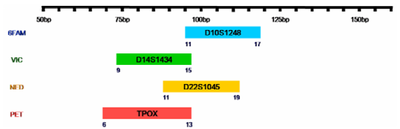
In order to ensure sequencing data quality, duplicate amplifications were sequenced in both the forward and reverse directions and the resultant consensus sequences were used for further analyses. In addition, to confirm the absence of artificial recombination or other errors, estimation of the most probable mtDNA haplogroups was carried out using the program, mtDNAmanager (http://mtmanager.yonsei.ac.kr).

Amplification and Genotying of STRs

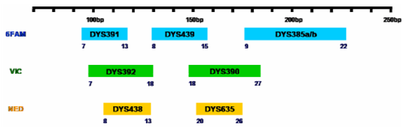
Three commercial STR systems (AmpFℓSTR® Identifiler®, AmpFℓSTR® MiniFiler™ and AmpFℓSTR® Yfiler™) and two in-house STR systems (miniplex NC01 plus and Y-miniplex plus) were used for DNA amplification. The commercial kits were used according to the manufacture's instructions with slight modifications. The protocols for in-house STR systems are available at http://forensic.yonsei.ac.kr.

Two separate PCR amplifications were performed for duplicate DNA extracts (1st and 2nd DNA extracts) of each skeletal remain sample. An allele was scored when peak height was above the interpretational threshold of 75 relative fluorescent units (RFUs). Alleles with below 15% of peak height of main allele were not scored in each locus. Then, consensus profile was obtained when an allele is present at least tree times in four replicate samples.

❖ NC01 plus



❖ Y-miniplex plus



Calculation of Likelihood Ratios

Cumulative likelihood ratios (LR) were obtained by multiplying LRs obtained from AS-STR, Y-STR and mtDNA analysis results. LRs for AS-STR were calculated using AS-STR manager program (Shin, Korea, unpublished) according to Brenner and Weir (*Theor Popul Biol* 2003;63:173-8). LRs for Y-STR and mtDNA were obtained from an inverse number of the frequencies of Y-STR and mtDNA calculated according to Balding and Nichols (*Forensic Sci Int* 1994;64:125-40).

Results

Table 1 Success rate of AS-STR genotype determination using the AmpFℓSTR® Identifiler® Kit, AmpFℓSTR® MiniFiler™ Kit and the NC01 plus from skeletal remains (n=21)

Locus	Size reduction	AmpFℓSTR® Identifiler® Kit (%)	AmpFℓSTR® MiniFiler™ Kit (%)	NC01 plus (%)
D3S1358	—	21 (100.0)	—	—
D5S818	—	19 (90.5)	—	—
D8S1179	—	21 (100.0)	—	—
TH01	—	19 (90.5)	—	—
VWA	—	16 (76.2)	—	—
D19S433	—	20 (95.2)	—	—
D2S1338	183 bp	9 (42.9)	20 (95.2)	—
FGA	87 bp	12 (57.1)	18 (85.7)	—
CSF1PO	201 bp	9 (42.9)	21 (100.0)	—
D7S820	129 bp	13 (61.9)	20 (95.2)	—
D13S317	99 bp	18 (85.7)	20 (95.2)	—
D16S539	157 bp	13 (61.9)	21 (100.0)	—
D18S51	168 bp	10 (47.6)	21 (100.0)	—
D21S11	33 bp	18 (85.7)	20 (95.2)	—
TPOX	148 bp	14 (66.7)	—	21 (100.0)
D10S1248	—	—	—	21 (100.0)
D14S1434	—	—	—	21 (100.0)
D22S1045	—	—	—	21 (100.0)

Table 2 Success rate of Y-STR genotype determination using the AmpFℓSTR® YFiler™ Kit and the Y-miniplex plus from skeletal remains (n=21)

Locus	Size reduction	AmpFℓSTR® YFiler™ Kit (%)	Y-miniplex plus (%)
DYS19	—	13 (61.9)	—
DYS389I	—	19 (90.5)	—
DYS389II	—	9 (42.9)	—
DYS393	—	21 (100.0)	—
DYS437	—	16 (76.2)	—
DYS448	—	12 (57.1)	—
DYS456	—	21 (100.0)	—
DYS458	—	21 (100.0)	—
GATA H4.1	—	19 (90.5)	—
DYS385	75 bp	14 (66.7)	15 (71.4)
DYS390	49 bp	16 (76.2)	18 (85.7)
DYS391	59 bp	19 (90.5)	21 (100.0)
DYS392	197 bp	9 (42.9)	20 (95.2)
DYS438	118 bp	15 (71.4)	20 (95.2)
DYS439	89 bp	16 (76.2)	21 (100.0)
DYS635	95 bp	12 (57.1)	19 (90.5)

Table 3 Mean number of loci, which were successfully genotyped with the AmpFℓSTR® Identifiler® Kit, AmpFℓSTR® MiniFiler™ Kit, NC01 plus, AmpFℓSTR® YFiler™ Kit and the Y-miniplex plus from skeletal remains (n=21)

Sample quality	Number of samples	15 AS-STR loci		18 AS-STR loci	17 Y-STR loci	
		AmpFℓSTR® Identifiler® Kit	AmpFℓSTR® MiniFiler™ Kit	NC01 plus	AmpFℓSTR® YFiler™ Kit	Y-miniplex plus
Low	7	6.7	12.1	15.7	8.7	11.6
Medium	8	12.0	14.6	18.0	12.4	14.8
High	6	14.8	15.0	18.0	16.5	17.0
Total	21	11.0	13.9	17.2	12.3	14.3

Table 4 Cumulative log likelihood ratios calculated from AS-STR, Y-STR and mtDNA analysis results

No.	Skeletal Remains	Family Sample	Family Relations	Log Likelihood Ratio						
				AmpFℓSTR® Identifiler®	AmpFℓSTR® MiniFiler™	NC01 plus	AmpFℓSTR® YFiler™	Y-miniplex plus	mtDNA	Final
1	SR0001	FS0011	brother	-1.6	1.5	0.4	1.8	2.8	5.1	5.1
2	SR0002	FS0001	brother	3.0	3.2	2.4	4.9	4.9	6.9	6.9
		FS0001-1	sister	6.0	6.9	6.5	—	—	8.4	8.4
3	SR0004	FS0016	sister	1.3	4.0	5.7	—	—	7.7	7.7
4	SR0008	FS0009	brother	7.7	7.7	7.0	5.8	5.8	8.1	8.1
5	SR0011	FS0016	sister	2.6	4.4	5.6	—	—	7.6	7.6
6	SR0012	FS0006	brother	1.4	1.2	2.7	4.7	5.0	7.3	7.3
7	SR0014	FS0015	brother	-2.4	-2.3	-3.6	-4.5	-4.3	-2.0	-2.0
8	SR0015	FS0012	brother	2.5	5.5	6.4	8.1	8.2	10.5	10.5
9	SR0016	FS0007	brother	0.4	4.3	6.1	8.5	8.5	10.8	10.8
10	SR0017	FS0003	brother	2.0	3.9	4.5	6.9	6.9	9.1	9.1
11	SR0018	FS0013	sister	3.6	3.9	4.4	—	—	6.7	6.7
12	SR0078	FS7221	brother	1.8	3.5	4.9	6.8	7.0	9.0	9.0
13	SR0079	FS7216	sister	3.3	5.0	6.5	—	—	8.7	8.7
		FS7217	nephew	2.7	3.0	2.8	5.4	5.4	—	5.4
14	SR0080	FS7222	brother	0.6	5.9	6.4	9.0	9.0	11.4	11.4
15	SR0081	FS7218	brother	1.9	4.6	5.0	7.6	7.6	10.1	10.1
16	SR0083	FS7219	son	2.7	3.1	3.7	5.6	6.2	—	6.2
		FS7220	daughter	2.4	4.0	5.0	—	—	—	5.0
17	SR0135	FS7205	brother	1.0	4.4	4.4	7.0	7.0	9.1	9.1
		FS7206	sister	1.2	3.1	3.5	—	—	5.7	5.7
18	SR0221	FS7227	brother	3.5	5.6	6.0	8.2	8.2	10.5	10.5
19	SR0315	FS0019	sister	1.3	1.3	3.1	—	—	5.4	5.4
20	SR4003	FS0020-1	son	3.3	3.3	3.8	6.3	6.3	—	6.3
21	SR7001	FS7001	sister	0.6	0.6	0.2	—	—	2.6	2.6

Discussion

- Optimization of DNA extraction step by employing large scale silica-based extraction method combined with complete demineralization could considerably enhance the genotyping success rate in old skeletal remains.
- By using reduced-size amplicons of the AmpFℓSTR® MiniFiler™ Kit and in-house miniplex NC01 plus as a complement to the AmpFℓSTR® Identifiler® kit, all 21 skeletal remain samples produced successful STR typing results with the mean success rate of 95.8% (17.2 from 18 loci). Based on AS-STR genotyping results, likelihood ratios for the alleged relationship were over 100 in almost all cases except for 3.
- By using reduced-size amplicons of in-house Y-miniplex plus as a complement to the AmpFℓSTR® YFiler™ Kit, Y-STR genotyping efficiency was significantly increased displaying the mean success rate of 84.1% (14.3 from 17 loci). In case of having paternal male relatives, Y-STR typing confirmed the alleged relationship by increasing the likelihood ratios.
- Our analyses emphasize the value of mini-STR and Y-STR systems as well as efficient DNA extraction method in the success and efficiency of forensic DNA testing for the identification of old skeletal remains.

Acknowledgement

This work was supported by grants from the Ministry of National Defense Agency for Killed In Action Recovery and Identification (MAKRI).