

# DNA Typing for the Identification of Korean War Victims

---

Kyoung-Jin Shin, D.D.S., Ph.D.  
Department of Forensic Medicine  
Yonsei University College of Medicine

## Korean War (1950-1953)

---

- Military conflict between North Korea and South Korea regimes lasting from June 1950 until the armistice on July 1953
- Devastating casualties and losses
  - 1,500,000 civilians
  - **170,000 South Korean troops**
  - 400,000 troops of North Korean and China
- MAKRI (The MND Agency for Killed in Action Recovery and Identification)
  - DNA analysis since 2000



## DNA from Old Skeletal Remains

---

- Low template (LT) DNA
  - Highly degraded DNA
  - Presence of PCR inhibitors
- 

## Presentation Overview

---

- DNA Extraction
    - Complete demineralization
    - DNA recovery using silica-based column
  - mtDNA Analysis
    - Modified midi- and mini-primers
    - Quality analysis based on mtDNA phylogeny
  - STR Genotyping
    - Autosomal-STR and Y-STR
    - Size-reduced mini-STR
    - Redundant approach to data generation and analysis
-

# DNA Extraction and Quantification

---

## DNA Extraction from Old Skeletal Remains

---

### □ Complete demineralization



### □ DNA extraction using silica columns

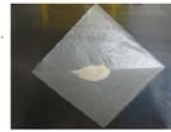


## Sample Preparation

- The surface of each bone sample was removed using a dental drill, and the sample was cut into small slices using a dental diamond disk.

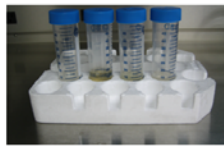


*Irradiation with UV light*



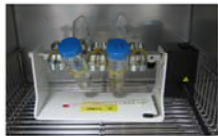
- Cleaned bone sample was powdered using 6750 Spex CertiPrep Freezer/Mill (SPEX CertiPrep, NJ)

## Complete Demineralization



### □ Demineralization solution

- 0.5 g bone power
- 15 ml of 0.5 M EDTA and 0.5% SDS
- 3 mg Proteinase K

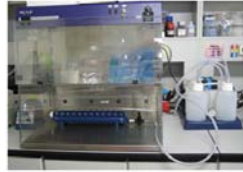


### □ Incubation

- 48 hours at 56°C in dry incubator
- 1 hour after additional treatment of 3 mg of Proteinase K

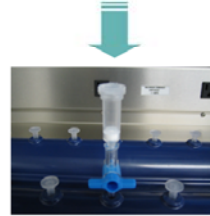


## DNA Recovery Using Silica-based Column



2 ml of DNA extract

- QIAamp® Blood DNA Maxi column
- Buffers from QIAquick® PCR purification kit



- Concentration of DNA extract
  - QIAamp® DNA Mini column
  - Buffers from QIAquick® PCR purification kit



50 µl of DNA extract

## Quantification Using Real-time qPCR

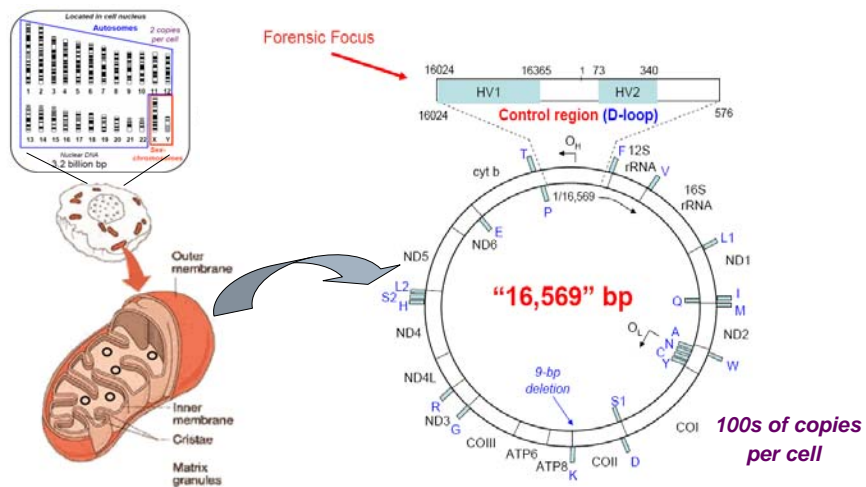
- Quantifiler® Human DNA Quantification kit (Applied Biosystems)
- Applied Biosystems 7500 Real-time PCR system

	Yang et al. (1998)*	New method
Sample	Concentration (pg/µl)	Concentration (pg/µl)
1	12.7 ± 0.08	74.1 ± 05.64
2	108.1 ± 9.59	518.5 ± 82.13
3	39.5 ± 6.69	361.4 ± 09.90
4	38.9 ± 4.52	60.6 ± 18.80
5	29.4 ± 19.66	106.1 ± 05.49

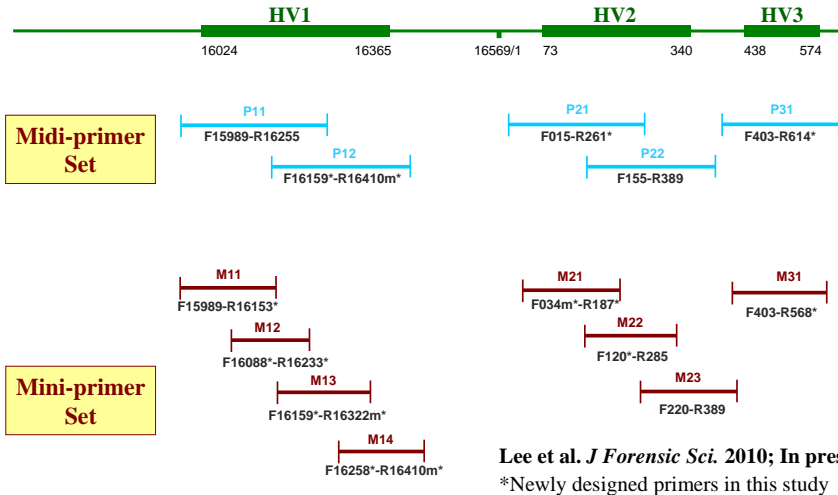
\*DNA was extracted using the QIAquick® PCR purification kit after incomplete demineralization of bone powder by small-volume high-concentration of EDTA.

# Sequence and Quality Analyses of mtDNA from Old Skeletal Remains

## Mitochondrial DNA (mtDNA)



# Primers for Amplification of mtDNA



Lee et al. *J Forensic Sci.* 2010; In press  
 \*Newly designed primers in this study

The screenshot shows the mtDNAManager web application interface. The browser address bar displays <http://mtmanager.yonsei.ac.kr>. The page title is "mtDNAManager" and the user is logged in as "Welcome, Colleague". The interface includes a navigation menu with "Sample", "Match", "Query", and "Home" tabs. Below the navigation, there are "Add", "Edit", and "Delete" buttons for the "Sample List". The main content area displays a table of samples with columns for Name, Description, Sample ID, Expected HG, Estimated HG, and various haplogroup markers (np 16024-16569, np 001-437, np 438-576). The table lists 20 samples (K001-K020) with their respective haplogroups and marker values. A "Group Information" sidebar on the left shows details for the "Demo-Asian" group, including its description, subpopulation (Korean), and control region status.

Name	Description	Sample ID	Expected HG	Estimated HG	np 16024-16569	np 001-437	np 438-576
Demo-African	FSt: Genet (2008) 2:e45-e4	K001	D5a2	D5a2	16164 16172 16182T 16183C 16189 16223 162	73 150 263 309.1C 309.2C 315.1C	489 523d 524d
Demo-Asian	Int J Legal Med (2006) 120:5-14	K002	N9a1	N9a1	16111 16129 16223 16257A 16261 16296	73 150 263 315.1C	489
Demo-Casework	Skeletal Remains of Korean	K003	D4IG	D4IG	16223 16224 16362 16519	73 263 309.1C 315.1C	489
Demo-European	EMPOP (www.empop.org)	K004	M7b2	M7b2	16129 16189 16223 16257 16297 16298	73 150 199 263 315.1C	489
Demo-Hispanic	FSt: Genet (2008) 2:e45-e4	K005	D5b	D5b	16189 16223 16362 16519	73 146 150 252 263 309.1C 309.2C 31...	456 489
		K006	D4a	D4a	16086 16129 16223 16362 16519	73 152 263 315.1C	489
		K007	M7b2	M7b2	16129 16189 16223 16242 16297 16298	73 150 199 263 309.1C 315.1C	489
		K008	B5b	B5b	16140 16183C 16189 16243 16355 16519	73 103 263 309.1C 309.2C 315.1C	523d 524d
		K009	M10b	M10b	16066 16223 16311	73 263 315.1C	489 573.1C 573.2
		K010	A4c	A4c	16223 16290 16319 16362	73 200 235 263 309.1C 315.1C	523d 524d 573.1C
		K011	G2a1	G2a1	16183 16223 16227 16278 16362	73 146 207 263 315.1C	489
		K012	M10a	M10a	16129 16148 16193 16223 16311 16357 16497	73 146 152 263 309.1C 315.1C	489 523d 524d 573.1C
		K013	N9a1	N9a1	16111 16129 16223 16257A 16261	73 150 195 263 309.1C 309.2C 315.1C	489
		K014	B4	B4	16182C 16183C 16189 16217 16295	73 150 195 263 309.1C 315.1C	489
		K015	G1a1	G1a1	16075 16223 16325 16362 16519	73 150 263 315.1C	489
		K016	B4b1	B4b1	16136 16175 16183C 16189 16217 16218 16519	56d 58A 71.1G 73 263 309.1C 309.2C...	499
		K017	A5a	A5a	16187 16223 16290 16319	73 235 263 315.1C	523d 524d
		K018	B4c1a	B4c1a	16183C 16189 16217 16311 16519	73 263 309.1C 315.1C	489
		K019	N9a3	N9a3	16129 16223 16257A 16261	73 150 263 309.1C 315.1C	489
		K020	M9a	M9a	16223 16234 16316 16362 16519	73 263 309.1C 309.2C 315.1C	489

## STR Genotyping of DNA from Old Skeletal Remains

---

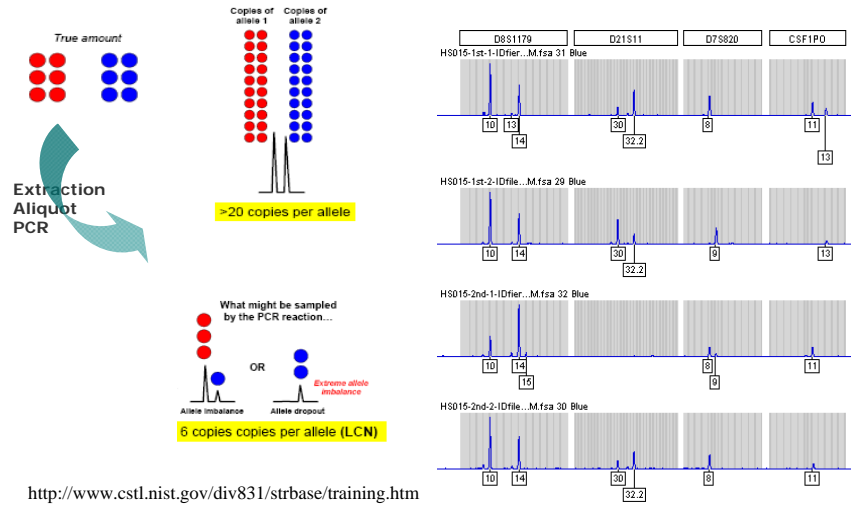
### PCR amplification for STR genotyping

---

- Basic PCR system
    - AmpFISTR Identifiler Kit
    - AmpFISTR Yfiler Kit
  
  - Modified PCR protocol
    - 10ul of reduced-scale reaction
    - Delayed ramping of thermal cycling
-



# Stochastic Effect on Autosomal STR Typing



## Suggestions to Optimal Results

- ❑ Extremely sterile environments is required for PCR setup to avoid contamination from laboratory personnel or other sources
- ❑ At least two DNA extracts from the old skeletal remains
- ❑ At least two PCR amplifications from the same DNA extract (if enough DNA is present, do more amplification)



“LT DNA interpretation rule”

Replicate analyses with duplicate results prior to reporting alleles

## PCR Strategy for LT DNA

---

### Independent set up of PCR

#### ■ 1<sup>st</sup> amplification

PCR master mix I for 1<sup>st</sup> DNA extract

PCR master mix II for 2<sup>nd</sup> DNA extract

#### ■ 2<sup>nd</sup> amplification

PCR master mix III for 1<sup>st</sup> DNA extract

PCR master mix IV for 2<sup>nd</sup> DNA extract

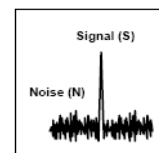
---

## Scoring of STR Alleles

---

### Peak detection threshold

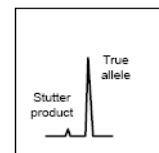
- 75 ~ 200 RFU



Signal > 3x sd of noise

### Cut-off minor allele

- Under 15% of peak height of major peak



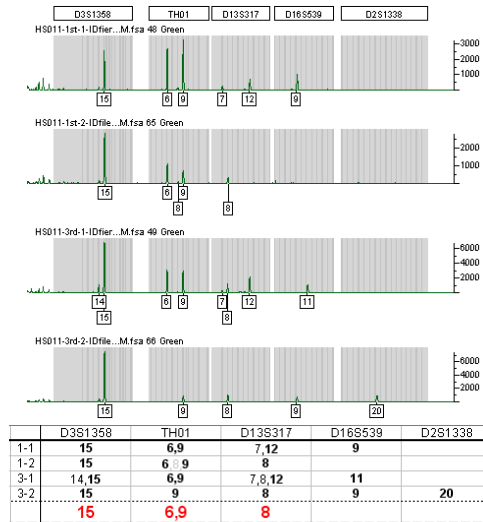
Stutter location above 15%

- ### An allele cannot be scored unless it is observed at least three times in four PCR reactions

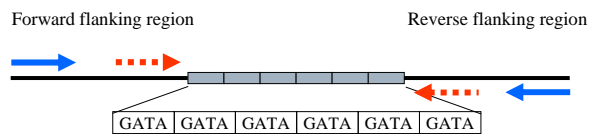
---

<http://www.cstl.nist.gov/div831/strbase/training.htm>

## Scoring of STR Alleles

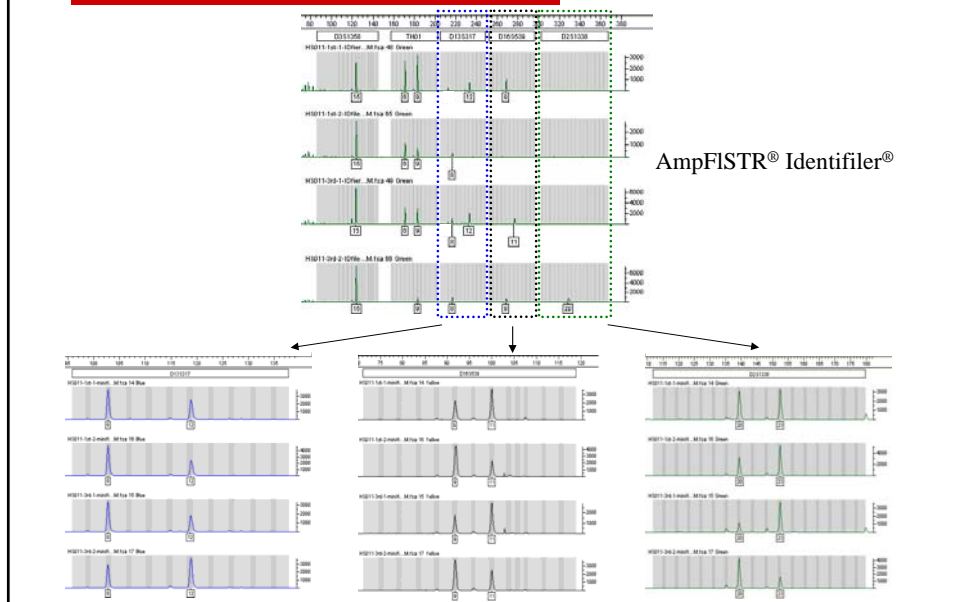


## Size-reduced Amplicon of mini-STRs

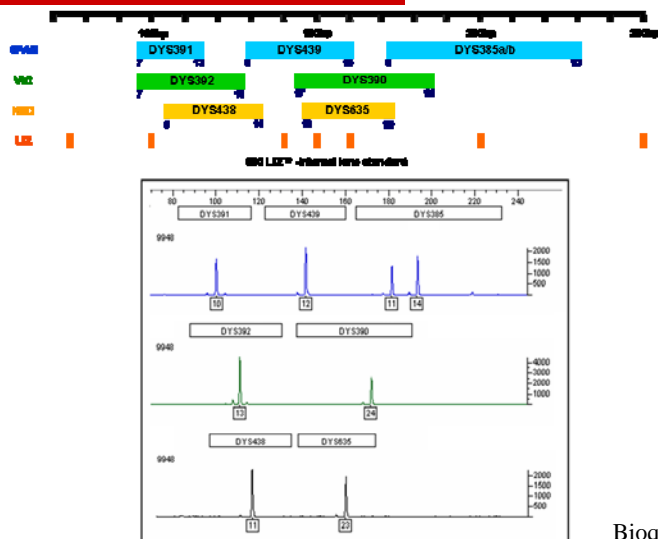


- Advantages of mini-STRs
  - Retains same marker information (database compatibility)
  - Uses highly polymorphic STR loci (high discriminatory power)
- AmpFISTR® MiniFiler™ was used as a complement to the AmpFISTR® Identifiler®
- Y-miniplex plus as a complement to the AmpFISTR® Yfiler™
- In-house miniplex NC01 plus was used to increase the discrimination capacity of the system

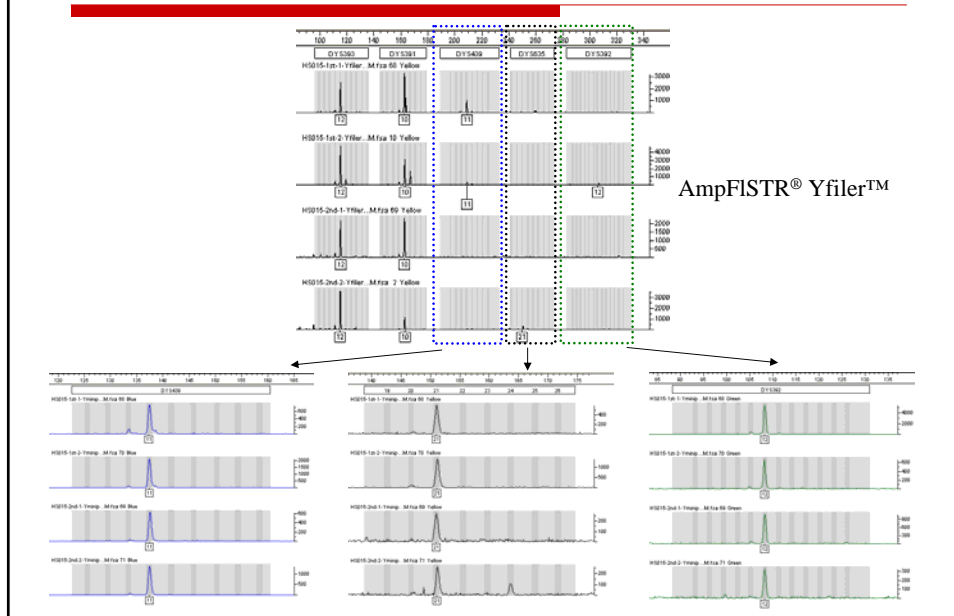
# AmpFISTR® MiniFiler™



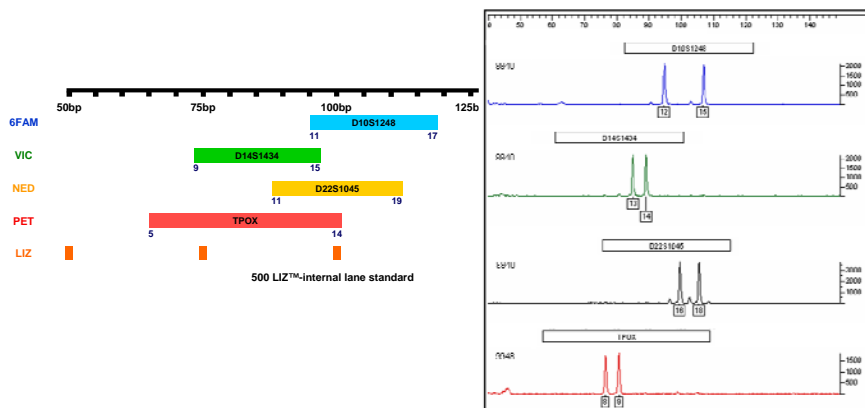
# Y-miniplex plus (In-house)



# Y-miniplex plus



# NC01 plus (In-house)



<http://forensic.yonsei.ac.kr/protocols.html>

## Improved STR Typing Results

- Mean number of successfully genotyped STR loci was calculated using AmpF $\ell$ STR<sup>®</sup> Identifiler<sup>®</sup> Kit, AmpF $\ell$ STR<sup>®</sup> MiniFiler<sup>™</sup> Kit, NC01plus, AmpF $\ell$ STR<sup>®</sup> YFiler<sup>™</sup> Kit and the Y-miniplex plus in skeletal remains obtained from Korean War victims (n = 21).

Sample quality	Number of samples	15 AS-STR loci		18 AS-STR loci	17 Y-STR loci	
		AmpF $\ell$ STR <sup>®</sup> Identifiler <sup>®</sup> Kit	AmpF $\ell$ STR <sup>®</sup> MiniFiler <sup>™</sup> Kit	NC01 plus	AmpF $\ell$ STR <sup>®</sup> YFiler <sup>™</sup> 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

## Excavation on the Finding Spot



## Mitochondrial DNA Control Region Sequence of a Victim and the Alleged Brother

---

Sample	HV1	HV2	HV3	Haplo group
Victim	16223T-16362C	73G-194T-263G-309.1C-315.1C	489C-523d-524d	D4b2b
Brother	16223T-16362C	73G-194T-263G-309.1C-315.1C	489C-523d-524d	D4b2b

Match Probability = 1/144

---

## Y-STR Genotypes of a Victim and the Alleged Brother

---

STR locus	Allele		Shared Allele
	Victim	Brother	
DYS19	-	17	-
DYS389I	12	12	12
DYS389II	-	28	-
DYS390	25	25	25
DYS391	10	10	10
DYS392	14	14	14
DYS393	12	12	12
DYS385a/b	12	12-20	12
DYS437	15	15	15
DYS438	11	11	11
DYS439	12	12	12
DYS448	-	20	-
DYS456	15	15	15
DYS458	15	15	15
DYS635	20	20	20
GATA H4.1	21	21	21

Match Probability = 1/133

---

## Likelihood Ratio (LR) Calculation for Full Sibling with Probabilities of Dropout : $D = 0, \frac{1}{4}$ and $\frac{1}{2}$

STR Locus	Genotype		LR (Sibling Index)		
	Victim	Brother	$D = 0$	$D = \frac{1}{4}$	$D = \frac{1}{2}$
D8S1179	11-15	11-15	11.962	11.962	11.962
<b>D21S11</b>	<b>30</b>	29-30	<b>0.924</b>	<b>1.084</b>	<b>1.135</b>
D7S820	11-12	8-12	0.729	0.729	0.729
CSF1PO	10-12	10-11	0.750	0.750	0.750
D3S1358	16-17	16-17	3.514	3.514	3.514
<b>TH01</b>	<b>9</b>	7-9	<b>0.733</b>	<b>0.873</b>	<b>0.931</b>
D13S317	9-10	9-10	8.854	8.854	8.854
D16S539	12-13	9-10	0.250	0.250	0.250
<b>D2S1338</b>	<b>22</b>	22	<b>336.985</b>	<b>31.200</b>	<b>22.756</b>
D19S433	13.2-14	15.2-16.2	0.250	0.250	0.250
<b>vWA</b>	<b>19</b>	15-19	<b>3.124</b>	<b>3.345</b>	<b>3.363</b>
TPOX	-	8-11	-	-	-
D18S51	11-15	13-15	0.922	0.922	0.922
<b>D5S818</b>	<b>11</b>	9-10	<b>0.250</b>	<b>0.420</b>	<b>0.466</b>
<b>FGA</b>	<b>23</b>	23-25	<b>1.435</b>	<b>1.625</b>	<b>1.661</b>
Cumulative LR			2,996.3	791.3	734.3

The STR loci and alleles which have possibility of allele dropout, and their LR's are represented in bold italic.

## Summary

- ❑ DNA was extracted using a large-scale silica-based extraction method combined with complete demineralization.
- ❑ The analysis of mitochondrial DNA control region sequences was carried out using modified midi- and mini-primer sets.
- ❑ Three commercial STR systems (AmpFISTR® Identifier®, AmpFISTR® MiniFiler™ and AmpFISTR® Yfiler™) and two in-house STR systems (miniplex NC01 plus and Y-miniplex plus) were used for DNA amplification.
- ❑ To ensure data quality from degraded samples, a redundant approach to data generation and analysis was employed.



# Thank you for your attention!

---

[kjshin@yuhs.ac](mailto:kjshin@yuhs.ac)

<http://forensic.yonsei.ac.kr>

